Synergistic action of Fibroblast Growth Factor Receptor and N-cadherin Adhesion Receptor in the control of cell adhesion and migration

Hoang Trinh Thao Nguyen

Université Pierre et Marie Curie

Ecole doctorale Complexité du Vivant, ED 515

Institut Jacques Monod

Equipe Cell Adhesion and Mechanics

Synergistic action of Fibroblast Growth Factor Receptor and N-cadherin Adhesion Receptor in the control of cell adhesion and migration

Par Hoang Trinh Thao NGUYEN

Thèse de doctorat de Biologie Cellulaire

Dirigée par Dr. René-Marc Mège

Présentée et soutenue publiquement le 20 Septembre 2016

Devant un jury composé de

Pr. Vania Braga	Rapportrice
Pr. Elisabetta Ada Cavalcanti Adam	Rapportrice
Pr. Jean-Loup Duband	Examinateur
Pr. Hervé Enslen	Examinateur
Pr. Claire Fournier-Thibault	Examinateur
Pr. Benoit Ladoux	Examinateur

Abstract

Embryogenesis, immunity, wound healing, metastasis rely on cell migration. This process results from changes in expression profile of adhesion molecules such as cadherins and transmembrane receptors. Literature suggestS that various cell type specific cadherin/tyrosine kinase growth factor receptor crosstalks may have more general impact during physiological and pathological processes. For example, N-cadherin and FGFR were shown to be upregulated and mutually lead to increased cancer cell migration. Synergistic actions between N-cadherin and FGFR stimulate neurite outgrowth or increase viability of gonadic cells. Interaction between VE-cadherin and VEGFR were reported to control endothelium permeability. In intestinal epithelium, disruption of E-cadherin mediated junctions involved EGFR ensuring proper enterocyte apoptosis. The underling mechanisms of these two families crosstalk remain unknown.

In this work, we studied the combined effects of N-cadherin and FGFR1 in the regulation of cell migration related to cell-cell junctions. We showed, in the first part, that FGFR1 strengthened N-cadherin-mediated cell-cell contacts. This increases adhesion of N-cadherin expressing cell to the N-cadherin coated-surface, leading to decrease of single cell migration. The strength of cell-cell contacts is controlled by the prevalence of adhesive protein at the cell surface. We showed a negative regulation of FGFR1 on the N-cadherin endocytosis.

In the second part of our work, we inquired on the effects of strong N-cadherin-mediated cell-cell adhesion induced by FGFR1 on collective cell migration. FGFR1 decreases the fluidity of N-cadherin expressing cell sheets by enhancing cohesion between cells, especially at the front of migration. FGFR1 / N-cadherin front cells show large protrusion at the free edge and formed robust cell-cell junctions with the followers and align the actin fibers in the direction of migration. FGFR1 expression confers a leader role to N-cadherin expressing cells. Altogether, FGFR1 seals cells in the N-cadherin expressing monolayer, reorganizes their cytoskeleton and guide the whole cell sheet to move persistently and efficiently.

Taken together, we conclude that FGFR1 specifically strengthens N-cadherin-mediated cell-cell contacts leading to two opposite effects on two modes of migration: decreased migration of individual N-cadherin expressing cell on a N-cadherin coated surface while increased the collective migration of N-cadherin expressing cell monolayer. Our work suggests that experimements aiming for changing migratory capacity of cells, by affecting cadherins-mediated cell-cell contacts, should take in consideration the mode of cell migration, such as in the case of cancer treatment.

Table of content

1 ABREVIATION

AJs	Adheren Junctions
CAMs	Cell Adhesion Molecules
CBD	C-terminal Binding Domain
CHD	CAM Homology Domain
CNCs	Cephalic Neural Crest cells
CNS	Central Nervous System
DN	Dominant Negatif
ECM	ExtraCellular Matrix
ECs	Endothelial Cells
ECs	Extracellular Cadherins
EGFR	Epithelial Growth Factor Receptor
EMT	Epithelial to Mesenchymal Transion
FAs	Focal Adhesions
FGF	Fibroblast Growth Factor
FGFR	Fibroblast Growth Factor Receptor
FRS2	FGFR Substrat 2
GCs	Granulosa Cells
HS	Heparan Sulfate
HUVECs	HUman Vessel Endothelial Cells
ICD	InterCalated Discs
JMD	Juxta Membrane Domain
LVFM	Longitudinal Visceral Muscular Fiber
mEpiSC	mouse Epiblast Stem Cell
NCCs	Neural Crest Cells
NEs	NeuroEpithelial Cells
NGFR	Neuronal Growth Factor Receptor
NPCs	Neural Progenitor Cells
NSCs	Neural Stems Cells
PDMS	Poly-Di-Methyl-Siloxan
ROSEs	Ovarian Surface Epithelial Cells
RTK	Tyrosin Kinase Receptor
siARN	small interfering ARN
SMCs	Smooth Muscular Cells
VSMCs	Vascular Smooth Muscular Cells
WT	Wild Type

2 INTRODUCTION

2.1 Cadherins and cell-cell adhesion

2.1.1 Cell-cell adhesion generalities

The ability of cells to adhere to one another is critical for the development and function of multicellular organisms. Cell-cell interaction allows cells to communicate with each other in responses to changes in their surrounding microenvironment. Each cell is capable of sending/receiving mecano-chemical signals to/from other cells. This allowes a coordinanted function of cells in a tissue, tissues in an organ, organs in a system and system in the body. The loss of cell-cell adhesion can result in uncontrolled cell growth and cancer. Some cell-cell interactions are transient such as interactions between immune cells and endothelial cells in inflammation. In contrast, most cells are in physical contacts with other cells at all times, usually as members of organized tissues such as those in the skin, gut or heart… These cells form stable junctions that are critical for tissues maintenances, shape and functions.

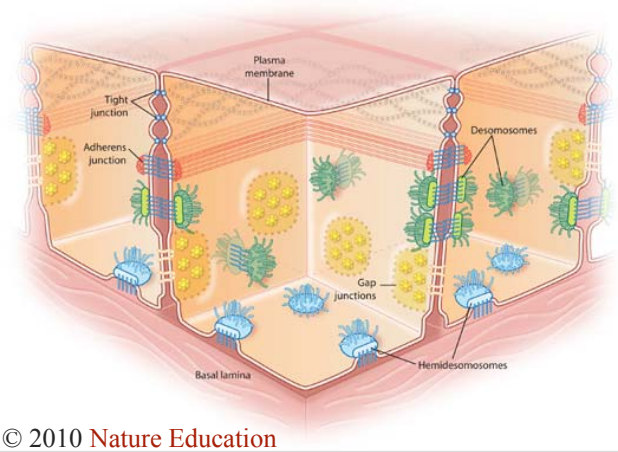


Figure 1: The different types of cell juntions. Tight junctions (blue dots) between cells are connected areas of the plasma membrane that stitch cells together. Adherens junctions (red dots) join the actin filaments of neighboring cells together. Desmosomes are even stronger connections that join the intermediate filaments of neighboring cells. Hemidesmosomes (light blue) connect intermediate filaments of a cell to the basal lamina, a combination of extracellular molecules on other cell surfaces. Gap junctions (yellow) are clusters of channels that form tunnels of aqueous connectivity between cells.

Cell-cell junctions are formed by multiprotein complexes, composed of various cell adhesion molecules (CAMs), which seal apposed cell membranes together. There are three main types of cell junctions: gap junctions, tight junctions and *adherens junctions* (Figure 1). Gap junctions mediate the diffusion of small molecules between adjacent cells. Tight junctions prevent plasma membranes proteins and lipid to diffuse, and small extracellular molecules from leaking, from one side of the junction to the other. *Adherens junctions* (AJs) mechanically attach cells to their neighbors by forming interconnected lateral bridges that link the actin cytoskeleton of neighboring cells. This coupling is especially important for cells to coordinate collective movements. All three types of cell junctions and appropriate CAMs are important to control the physical and chemical interactions between cells. However, AJs are particularly important for their multiple functions including stabilization of cell-cell adhesion, regulation of actin cytoskeleton, intracellular signaling and transcriptional regulation. The

core components of AJs are clusters of cadherins and catenins. Together, these proteins control the formation, maintenance and function of AJs.

2.1.2 Cadherin family

Cadherins were first identified as cell surface proteins responsible for Ca^{2+}-dependent homophilic cell-cell adhesion during morula compaction in the pre-implantated mouse embryo. To date, it has come clear that the role of cadherins is not limited to physical adhesion between cells. Rather, cadherin function extends to biochemical signaling and mechano-transduction, in particular at AJs where they are most concentrated. This results in multiple biological aspects ranging from cell recognition, sorting, morphogenetic movement, and physical homeostasis of mature tissues. Consistent with these roles, expression of cadherin is highly spatial-temporo regulated in response to external/internal cues. Logically, unproper expression of cadherin alters the normal cellular cohesion and viability that leads to unorganized tissues, cells dispersion, pathologic migration and metastasis….

2.1.2.1 Structure of cadherins

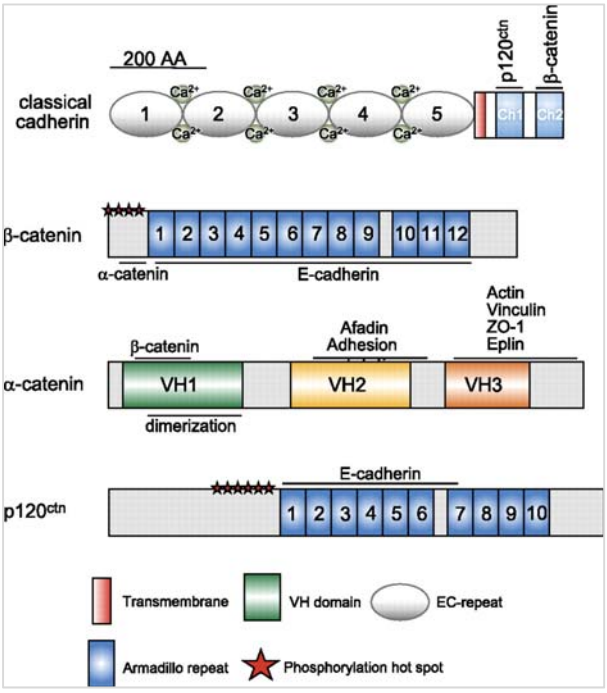


Figure 2: Cadherins structures and partner binding sites. Domain structures of the cadherin-catenin complex components. Schematic overview of the domain structure of a classical cadherin and its three associated catenins: β-catenin, α-catenin, and p120-ctn (C. M. Niessen et al., 2011).

Cadherins are composed of an extracellular domain responsible for homophilic interactions between cadherins molecules, a transmembrane domain and a cytoplasmic tail (Figure 2). The extracellular domains primary structure allows the subdivision of the superfamily into multiple subfamilies based on the number, conservation and arrangement of EC domains. The most studied and best understood of these subfamilies is the vertebrate classical cadherins, comprised of 6 major type I and 13 type II cadherins. The extracellular part constitutes of five or more Extracellular Cadherin (EC) repeated domains, which are structurally related to immunoglobulin (Ig) domains and responsible for Ca2+ binding. Type I cadherins are named according to tissue in which they are identified initialy: E (Epithelial)-, N (Neuronal)-, P (Placental)- and R (Retinal)-cadherins and the closely related VE (Vascular/Endothelial)-cadherin. In contrast, this motif is lacking in type II classical cadherins, named by numbers 6-12 (Angst et al., 2001). Type I cadherins are weak binders but confers strong adhesion

through cooperativity. Whereas type II establish large contacts interfaces but weak adhesions (Thiery et al., 2012). Type I cadherins typically have broad distributions that is segregated by tissue type whereas type II cadherins display more condensed distribution that is often overlapping (Patel et al., 2006)

The ability of cadherins to function as adhesion molecules and resist against detachment forces depends, ultimately, on the intrinsic binding properties of their ectodomains. The conformation of the cadherin molecule is stable only in the presence of Ca2+, whose binding site are highly conserved and located between neighbouring EC repeats. The extracellular domain (EC1) allows homophilic recognition between two identical cadherins molecules from opposing cells. To note, within type I subfamily, some cadherins exhibit heterophilic binding properties. N-cadherin and R-cadherin have been shown to form heterocomplexes which are recruited at the cell-cell contacts when co-expressed in L cells. In the same study, E-cadherin can not participate in these complexes (Shan et al., 2000). Other EC domains of each individual family members contribute to the interaction with others binding partners, for example EC4 domains of N-cadherin binds fibroblast growth factor receptor (FGFR) (Williams et al., 2001).

Cadherins cytoplasmic domains bind to catetins serving as adaptator to cytoskeleton, required for cell-cell adhesion strengthening and resistance against disruption (Y. S. Chu et al., 2004). The cytoplasmic domain is highly conserved between the different classical cadherins and unstructured in the absence of binding partners (Huber et al., 2001). It contains two important domains, a Juxta Membrane Domain (JMD) binding to p120-catenin (p120) and a C-terminal Binding Domain (CBD) binding to β-catenin. Cadherins lacking p120 or β-catenin binding sites exhibit loss of adhesion, suggesting that the cytoplasmic machinery is required for cadherin clustering and the initial assembly of AJs (Nagafuchi and Takeichi, 1988).

2.1.2.2 Cadherin mediated cell-cell adhesion

2.1.2.2.1 Cadherin and partners

The best-known cadherin cytoplasmic partners are the catenins α-, β-, p120- catenin. α-, β-catenin serve as intermediate linkers between the cadherins and actin filaments (Nelson, 2008) whereas p120 regulates cadherins turnover and modulates actin assembly (Reynolds and Carnahan, 2004).

β-catenin and α-catenin were the first binding partners to be co-immunoprecipitated stochiometrically with E-cadherin (McCrea and Gumbiner, 1991; Nagafuchi and Takeichi, 1988). β-catenin directly binds to the cytoplasmic cadherin fragment and serve as a intermediate for subsequent α-catenin attachment. Besides, β-catenin plays an important role in the protection of cadherins against degradation. Indeed, impaired binding of β-catenin to cadherin leads to proteosomal degradation of the

latter (Chen et al., 1999) because β-catenin fixation masks a cadherin binding site for ubitquitine ligase (Huber et al., 2001). Concerning α-catenin, it can exist either as α-β-catenin heterodimers or as homodimers unable to bind β-catenin (Pokutta and Weis, 2000). α-catenin ability to bind to both β-catenin and F-actin led to the conclusion that α-catenin links the complexe cadherin/β-catenin to actin. But in 2005, the Nelson and Weis groups contradicted this notion by showing that α-catenin that is bound to β-catenin cannot bind F-actin directly. Indeed, the ternary complex formed from the mixture of E-cadherin cytoplasmic tail, α-catenin, and β-catenin did not bind actin filaments in an actin-pelleting assay (Drees et al., 2005). In the last few years, the controversial ideas about direct binding cadherin/catenin complex and actin have been unravelled: α-catenin in the cadherin/catenin complex binds to F-actin but under tension (Buckley et al., 2014). Therefore, it is clear that β-catenin and α-catenin are central to link cadherins to F-actin, directly under force or indirectly through additional adaptators such as vinculin. In addition to direct binding to actin under force, α-catenin binds several actin-binding proteins that could provide multiple ways of indirect linkage and regulation between cadherins and actin network. For example, α-catenin recruits formin-1, a nucleator of unbrached actin cable to cell-cell contacts (Kobielak and Fuchs, 2004). Its homodimers inhibit binding of the actin-nucleating Arp 2/3 complex to F-actin and thereby suppress actin polymerization (Drees et al., 2005). Thus, α-catenin could coordinate the suppression of Arp 2/3-mediated lamellipodia activity, formin-1 recruitment and formation of unbranched actin bundles at cell-cell contacts to regulate the formation of these contacts.

p120 was first identified in a screen for substrate of the Src family protein tyrosine kinases [12], and was only later discovered to co-immunoprecipitate with classical cadherins (Daniel and Reynolds, 1995; Reynolds et al., 1994). The relative weak interaction between cadherins and p120 in detergent cell lysates suggests that roughly, 5-20% of total cellular p120 is in complex with cadherins (Ohkubo and Ozawa, 1999). Contrarily to β- and α-catenin, p120 is metabolically stable in cadherins lacking cells (A431D or L cells), but localizes almost exclusively in the cytosol instead of being recruited at the membrane as in the case of cadherin expressing cells (MDA 231 cells) (Thoreson et al., 2000). These results support the role of cadherins as p120 anchors at the plasma membrane. Moreover, A431D cells expressing E-cadherin lacking p120 binding display weak cohesion in the aggregates contrarily to those expressing WT E-cadherin (Thoreson et al., 2000). Consequently, the actin cytoskeleton failed to insert properly into peripherical E-cadherin plaques, resulting in disparition of the circumferential cortical ring of actin as habitually seen in epithelial type cells (Thoreson et al., 2000). Therefore, cadherins are both necessary and sufficient for recruitment of p120 to membranes and this recruitment is required for transition from loose to tight E-cadherin-mediated cell-cell adhesions, involving actin reorganization.

Although catenins are core partners of cadherins, it is important to emphasize that other effectors can bind the cadherin directly or indirectly. These include other adhesion molecules like neural cell adhesion molecule NCAM (Hansen et al., 2008), cell signaling molecules such as tyrosine kinases and protein tyrosine phosphatases (Piedra et al., 2003; Takahashi et al., 2014), actin cytoskeleton regulators and myosin motors (Mège et al., 2006). For example, Rho family GTPases (including RhoA, Rac, cdc42) that mediate cytoskeletal dynamics have emerged as crucial regulators of cadherin-dependent adhesion (McCormack et al., 2013). Cadherins ligation and clustering is thought to activate Rac1 and cdc42, which enhances actin polymerization thus membrane protrusion (Kovacs et al., 2002) by activating Arp2/3.

2.1.2.2.2 Formation of cadherin-mediated cell-cell contacts

Nascent cell-cell contacts are initiated by formation of adhesive homophilic trans-bond originating from EC1 of cadherins of the opposing cells (Boggon et al., 2002). This process requires the binding of Ca^{2+}. This binding results in a curvature of the cadherins extracellular part favoring the trans-interaction (Boggon et al., 2002), which has been shown to be essential for cis-interactions (between cadherins from the same cell) formation. In contrast, cis-interractions are not required for trans-interaction. However, without subsequent cis-interractions, trans-dimers are unable to cluster to form ordered structures (Zhang et al., 2009). These results suggest strongly that trans-bonds were firstly formed during contact initiation subsequently triggering cis-bonds interactions (Figure 3). This increases cadherin prevalence allowing contact expansion via ziperring of the edge by diffusion trapping. The accumulation of cadherins at the cell-cell interface stimulates events at the cytoplasmic side of the adhesion sites like the recruitment of adaptor proteins in which catenins family are the flagship.

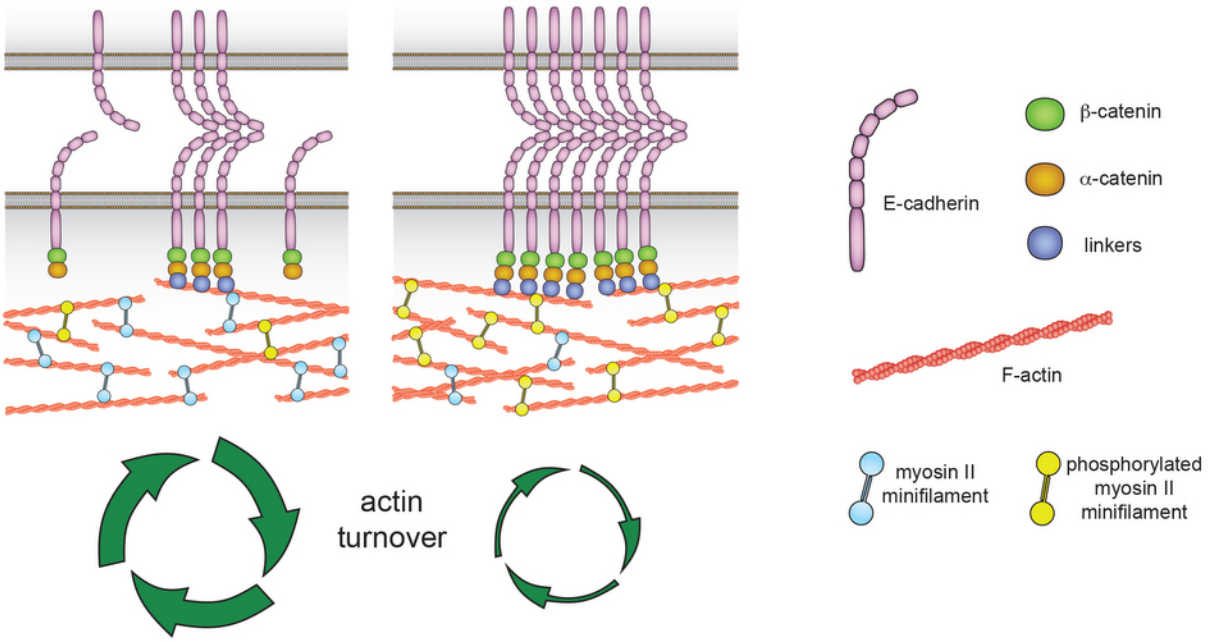


Figure 3: Schematics cadherin-based cell-cell contacts formation. Initiation of cell-cell contacts is induced by trans-interaction of cadherins of opposing cell membrane, followed by oligomerization of cadherins within the plasma membrane of a single cell. Stabilization of cadherin-mediated cell-cell contacts is regulated by the turnover dynamics of the underlying actin cortex. In turn the turnover dynamics is modulated by the tension of the cortex generated by the actomyosin contractility. This mechanism provides a regulatory pathway for enhanced adhesion in cortex under tension (Engl et al., 2014).

Cell-cell contacts are initiated by cadherin ectodomain interaction and strenghthen over a time scale of minutes thanks to rearrangement of cytoskeleton near the developing junction (Y. S. Chu et al., 2004). Firstly, highly dynamic lamellipodia extensions from opposing cells form transient contacts containing cell-surface cadherin and catenin (Vaezi et al., 2002). Cadherin-catenin complexes then cluster into punctate structures where actin bundles emanating from underlying cortical actin belt anchor (Huveneers et al., 2012). Futhermore, F-actin was shown to concentrate at cadherin adhesion sites, for example in circumferential apical ring parallel to AJs in epithelia (Kovacs et al., 2002). As the contacts mature, cadherins become concentrated between opposed cells surfaces, lamellipodial movements slow down and eventually cease as a stable cell-cell contact is established (Vaezi et al., 2002) and actin filaments adjacent to mature AJs become organized into unbranched bundles parallel to the membrane (Hirokawa et al., 1983). Interestingly, while cortical actomyosin cytosketon is usually reduced at the contact during initial cell-cell contacts formation and expansion (Yamada and Nelon, 2007), junctional myosin appears to be essential for epithelial contact maturation by controlling cadherin clustering and actin dynamics (Shewan, 2005). In sum, cadherins regulate the turn over of actin by regulating its polymerization and anchoring tensile actomyosin network at the cell cortex. In turn, the mechanical state of actin regulates cadherin turnover by exerting forces driving remodeling of cell-cell contacts and probably endocytosis.

2.1.2.2.3 Regulation of cadherin-mediated cell-cell contact

Cadherin-mediated cell-cell junctions are dynamic structures that are regulated by the prevalence of cadherin on the cell surface [71]. The biosynthesis regulation involves changes in transcription factors expression or activities. To note, it is unlikely that transcriptional regulation alone contributes to rapid and dynamic changes in cadherins function since the metabolic half-life of E-cadherin was estimated about 5-10 hours in cell cultures (Shore and Nelson, 1991). The total level of cadherins expressed at the cell surface is determined by the balance between biosynthesis and degradation. Thus, the plasma membrane levels of cadherins are the result of the trafficking process including endocytosis, exocytosis. Thus, the junctional integrity can be compromised when membrane trafficking is disrupted.

Endocytosis of cadherins remains as a crucial factor in the dynamic control of cadherins-based cell-cell contacts. Cadherin internalization employes both clathrin-dependant and -independent pathways involving a range of adaptor proteins (Kowalczyk and Nanes, 2012). Naturally, catenins family drew much of attention in the regulation of cadherin internalization. p120 plays a key role as an inhibitor of cadherin turnover. Loss of p120 results in cadherin endocytosis (Xiao, 2003). Knockdown of p120 using siRNA and competitive expression of other cadherins subtypes induce dowregulation and endocytosis of cadherins (Maeda et al., 2006). These results raise the interesting possibility that p120 might serve as a master regulator of cadherin levels in cells; and that p120 increases the retention of cadherins at the plasma membrane by preventing cadherin internalization. Multiple regulations of p120 in cadherins turnover have been proposed. Binding to p120 has also a direct stabilizing function because it masks a conserved dileucine motif in N-cadherin juxtamembrane domain that is necessary for endocytosis (Nanes et al., 2012). Kowalczyks' lab proposed that binding of p120 to

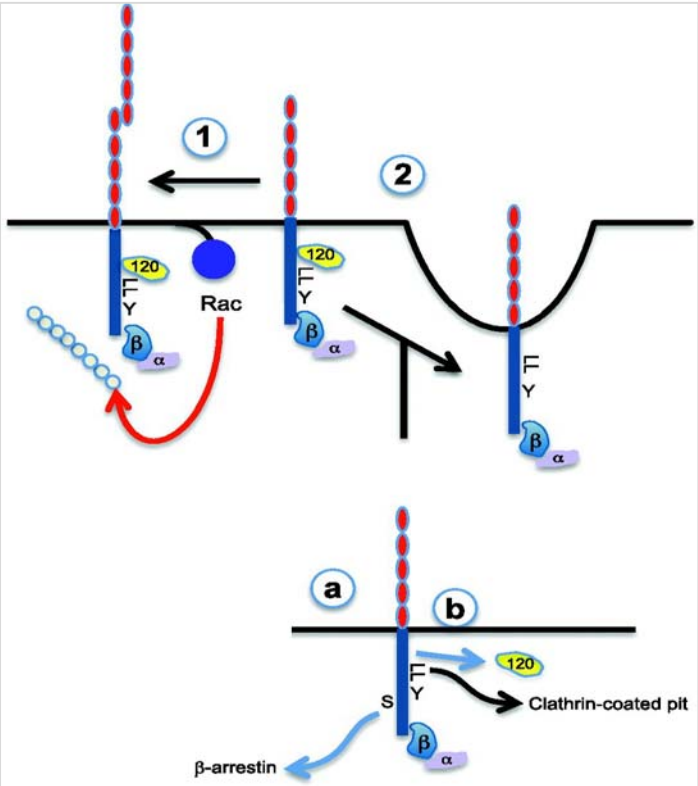


Figure 4 Regulation of cadherin endocytosis in control of surface expression. Surface cadherins can be fated either for stabilization on the surface (1) or for endocytic uptake (2). 1: Stabilization, so that cadherins are not internalized, is promoted by cadherin ligation, masking of dileucine (LL) and tyrosine (Y) residues by p120-ctn, Rac/Cdc42 signaling, and the actin cytoskeleton (Carien M. Niessen et al., 2011).

cadherins masks the docking site for the adaptor protein AP-2 of the clathrin endocytosis pathway thus preventing subsequent endocytosis (Kowalczyk and Nanes, 2012). Alternatively, p120 could regulate cadherins trafficking by inhibiting RhoA that modifies actin dynamics. Indeed, inhibition of RhoA signaling blocked cadherins endocytosis and p120 mutant unable of RhoA binding stabilized cadherin at the plasma membrane (Chiasson et al., 2009).

The role of β-catenin and α-catenin in the regulation of cadherin endocytosis remains unclear. It has been shown that β-catenin was recruited by N-cadherin at the membrane ruffles where β-catenin mediated the internalization of N-cadherin (Sharma and Henderson, 2007). On the contrary, another studie showed a significative reduction of cadherin endocytosis in the absence of β-catenin binding (Tai et al., 2007).

To note, cadherins internalization may be acutely stimulated by cell signaling, partly through phosphorylation of cadherins or their binding partners. This involves various growth factors receptors signaling including FGF-FGFR, EGF-EGFR. For example, VEGF signaling actives Src that phosphorylates VE-cadherin favoring β-arrestin docking to drive the clathrin dependant internalization of VE-cadherin (Gavard and Gutkind, 2006). Conversely, increased expression of E-cadherin or N-cadherin inhibits internalization of FGFR (Bryant et al., 2005; Suyama et al., 2002). Interestingly, in these examples, the affect of the growth factor receptor signaling pathways and cadherins trafficking is bidirectional.

2.2 Cell migration

Cell-cell adhesion and cell migration are two important features for the organization of many embryonic tissues in development, of adult tissues undergoing repair after injury and of cancer evolution. The two features function in a two-way communication: cell-cell adhesion control and is required for coordinated multicellular movements or movement of one cell over other. In parallel, cells undergoing migration reorganize intercellular junctions between partners inside the groups or/and with cells of the new environement. Dysregulation of cell-cell junctions and cell migration are equally associated with numerous diseases such as congenital malformations, neurological disorders and cancer.

2.2.1 Methods to study cell migration

Several experimental systems exist to study cell migration. The most direct observation *in vitro* and *in vivo* are performed by time-lapse microscopy in combination with subsequent sample fixation and immunofluorescence imaging. Direct imaging of morphogenetic movements in transparent embryos is also a performant tool to study 2D and 3D cell migrations *in vivo*. For example,

live imaging of *zebrafish* or *Xenopus, Drosophila* embryos during neural tube closure or gastrulation stae are frequently used.

2.2.1.1 Transwell migration

In vitro, numerous models were developed to study the individual or collective migration of cells. The most well-known and used model is the Boyden chamber (Boyden, 1962). Improved, simplified and disposable versions of the original chambers were developed. This includes two rooms separated by a porous membrane through which cells can migrate from one chamber (upper) to the other (lower) containing different attractants or drugs. Migratory cells in the lower room are stained with cytology dyes or fluorescence probe helping the quantification. The transwell migration assay is used for many different cell types including epithelial, mesenchymal, brain and cancer cells lines as well as primary cells from the tridermic disk. This assay is low-cost, ready-to-use, simple, rapid and applicable for numerous types of cells at the same time. However, the disadvantages are that it is an endpoint assay; the optimal time of analysis has to be determined individually for each cell type tested.

2.2.1.2 Wound healing assay

The simplest approach to study collective migration is the 2D migration of a cell sheet to a free region in wound scratch assay or after releasing a constraint barrier. Cell movements can be calculated by measuring the decreased of uncovered region at different time points. Plate-coating with different adhesive proteins prior to cell seeding offers the possibility to study cell migration with different substrates. This technique is popularly used for its simple and rapid set-up, easy read-out and cheapness. Disavantages include the increase of migration speed just following the scratch or barrier realease and the fact that adhesive substrate is often pilled off in an uncontrolled manner. For 3D assays, clusters of cells are cultured in Extracelluar Cell Matrix (ECM), proteins allowing cells to obtain morphology close to physiologic living.

2.2.1.3 Micro-fabrication

Newest progress brougth out micro-fabrication techniques to produce either one-cell-size adhesive area for individual cell migration or variable size zone for collective migration with different protein surfaces (Vedula et al., 2014). These techniques provide powerful tools to study cell-cell and cell-substrate interactions. One of the most widely applied micro-fabrication techniques is microcontact printing. In this technique, an elastomeric, frequently PDMS, stamp with bas-relief features of cellular resolution is used to transfer a cell adhesion protein onto the substrate. The remaining non-covered area is treated with cyto-repulsive material. Thus, after cell seeding and non-adhered cell washing, cells adhere and migrate under confinement in one dimension. Micro-fabrication allows the reconstitutioin of tissue-like conditions for *in vitro* cell cultures. It is a highly efficient method to investigate the sensitivity and response of cells to specific microenvironemental cues. Indeed, given

that the surface of the PDMS can be charged with virtually any protein, micro-contact printing can be used to evaluate the impact of ECM and cell-cell adhesion proteins in cell migration. The geomety of the lines can also be modified, facilitating the study of the impact of physical constrains in cell migration.

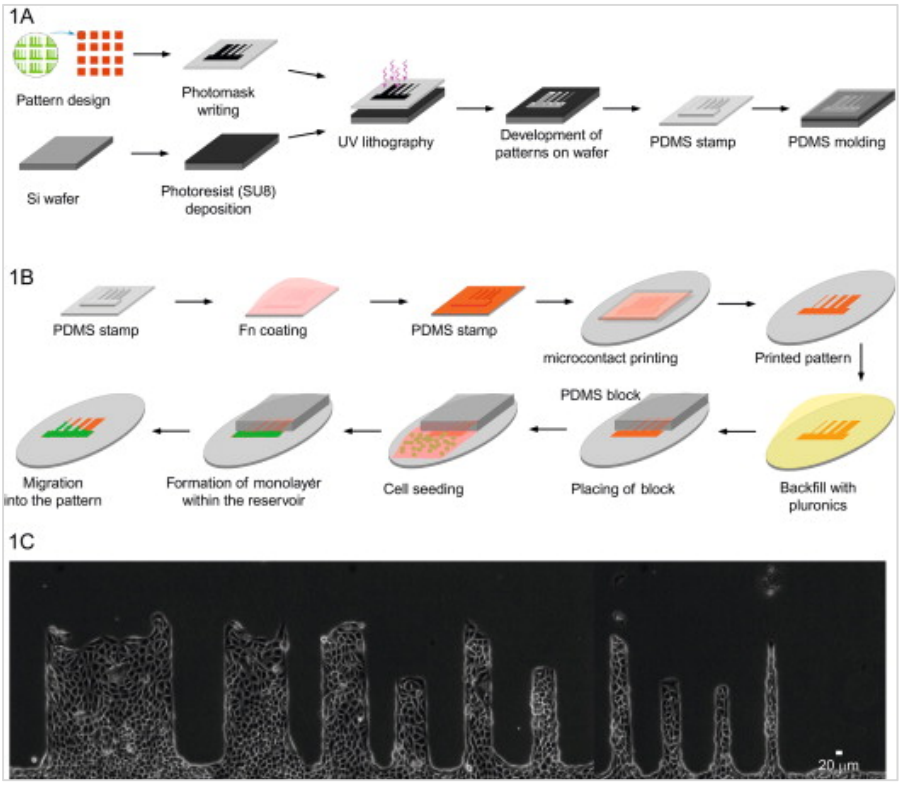


Figure 5: Schematic showing procedure for studying collective cell migration under varying geometrical confinements. (A) Soft lithography to obtain silicon masters with the pattern of interest. PDMS stamps are prepared from these wafers and used subsequently for microcontact printing. (B) Placing of a PDMS barrier on the microcontact-printed pattern such that cells are confined to the "reservoir" region of the pattern. The barrier is released when cells reach confluence in the reservoir. (C) Cell monolayer migrates from the reservoir into the fibronectin strips of different widths upon removal of the barrier (Vedula et al., 2014).

2.2.2 Cell migration mode

Cell-cell contacts regulate numerous biological processes including cell migration. The most relevant example is observed in cancer metastasis. Indeed, the capacity of cancer cells to migrate and invade subjacent tissue involves changes in the way cells attach to each other. Tumor cells escape from the original sites by destabilizing cell contacts, detaching from neighbouring cells, cross the extracellular matrix barriers, enter then exit the blood circulation to invade and occupy distant sites. Hence, the stability of cell-cell contacts control directly the capacity of cancer cells to disseminate and invade.

As a simple view, there are two main modes of cell migration, dependent on cell types and cellular environmentours. Some cells can migrate individually such as cells of immune/blood system or fibroblastic cells. Others remain associated to neighbours inside cell sheet and therefore move collectively (Friedl and Wolf, 2010).

2.2.2.1 Single cell migration

Migration of individual cells can be subdivided into ameoboid- and mesenchymal types of movement.

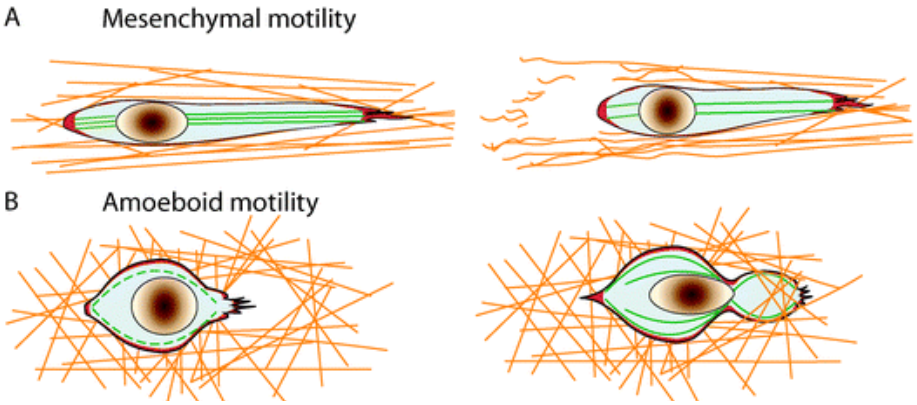


Figure 6 Single cell migration modes. Mesenchymal mode of motility with elongated spindle-shaped morphology, focal adhesions at the poles and enhanced stress fiber contractility. (B) Amoeboid mode of motility with blebby protrusions, rounded ellipsoid morphology and cortical contractility (Pathak and Kumar, 2011) .

2.2.2.1.1 Amoeboid migration

Amoeboid movement is characterized by cells moving as round, ellipsed shape without the involvement of strong cell adhesion to the substratum and formation of focal adhesions (Fas). Amoeboid cells migrate rapidly by formation of blebs at their front. A bleb is characterized by the disruption of the plasma membrane - actomyosin cortex link. Ameaboid migration follows by the reconstitution of the actomyosin network within the protruding bled (Yoshida and Soldati, 2006). In a confined environment, amoeboid cells migrate by pushing on their lateral environment thanks to a retrograde intracellular actomyosin flow and weak substrate interaction (Paluch et al., 2006). This mode of migration is employed by immune surveillance cells (dendritic cells) exiting the blood flow to patrol in tissue upon inflammation or by som cancer cells (Vargas et al., 2014).

2.2.2.1.2 Mesenchymal migration

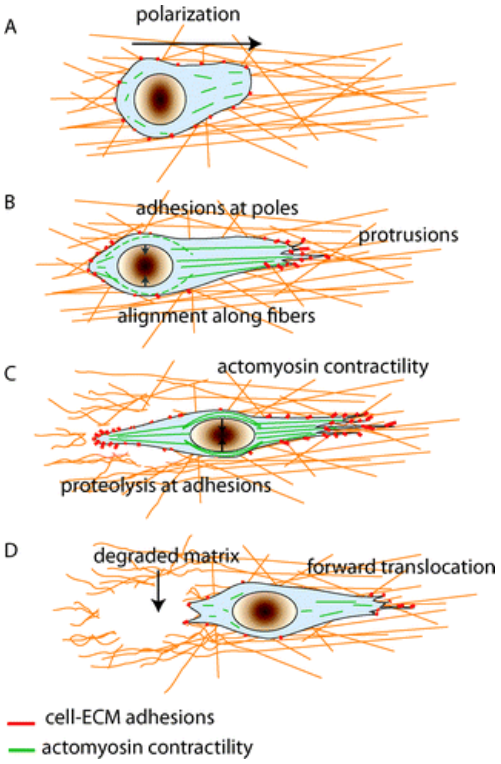


Figure 7: Cell migration in steps. (A) A cell interacts with the ECM and polarizes in one direction. (B) Extended protrusions at the leading edge probe the surrounding ECM fibers and form cell-ECM adhesions. (C) Further polarization and strengthening of adhesions is accompanied by a rise in actomyosin contractility and proteolytic degradation of ECM fibers at cell-ECM junctions. (D) Retraction of the trailing edge is followed by forward translocation of the cell and completion of the migration cycle. (Pathak and Kumar, 2011)

Mesenchymal movement involves strong focal attachment to the ECM via FAs, cytoskeletal contractility and elongated spindle-like cell body. Cells migrate through a cyclic process involving adhesion/de-adhesion and acto-myosin polymerisation/contractility (Blanchoin et al., 2014). The successive steps are: protrusion of the leading edge by actin polymerisation, attachment of protrusive membrane to new ECM areas, contraction of acto-myosin to promote the sliding and detachment of the cell rear. Attachment/detachment of the cell from the ECM is one of the most important checkpoints in this process. Fibroblasts, sarcoma cells and highly dedifferentiated tumor cells, which have undergone Epithelial to Mesenchymal Transition (EMT) display this mode of motility (Thiery, 2002).

2.2.2.2 Collective cell migration

In the case of collective migration, cells move in groups, with tight or loose association between each other and with protrusion that distinguish actively and directionally migrating cells in front from their followers. Front cells display large protrusion at the free edge while remaining adherent to neighbours cells at the back and exert pulling forces on them through AJs. At the back, trailing edge retraction is a collective process that simultaneously involves several cells (Friedl and Gilmour, 2009). Together, leading edge extension and force generation as well as rear retraction are shared function among cells that are coupled with each other. Such combination of cell migration and cell adhesion requires a fine-tuned crosstalk between cell-cell adhesion, cell-substratum adhesion and cell contractility (Montell,

2008; Rørth, 2007). Cell-cell contacts allow cytoskeleton dynamics at the leading edge while sinlencing other regions of the collective. As a consequence, intercellular coordination can improve or deprave the efficiency of collective migration. For example, increasing cell-cell adhesion converts dispersed cells towards collective and *vice versa*. On the contrary, reducing cell-cell adhesion by blocking cadherins causes the disruption of cell collectives towards individually migrating cell (Friedl et al., 2004).

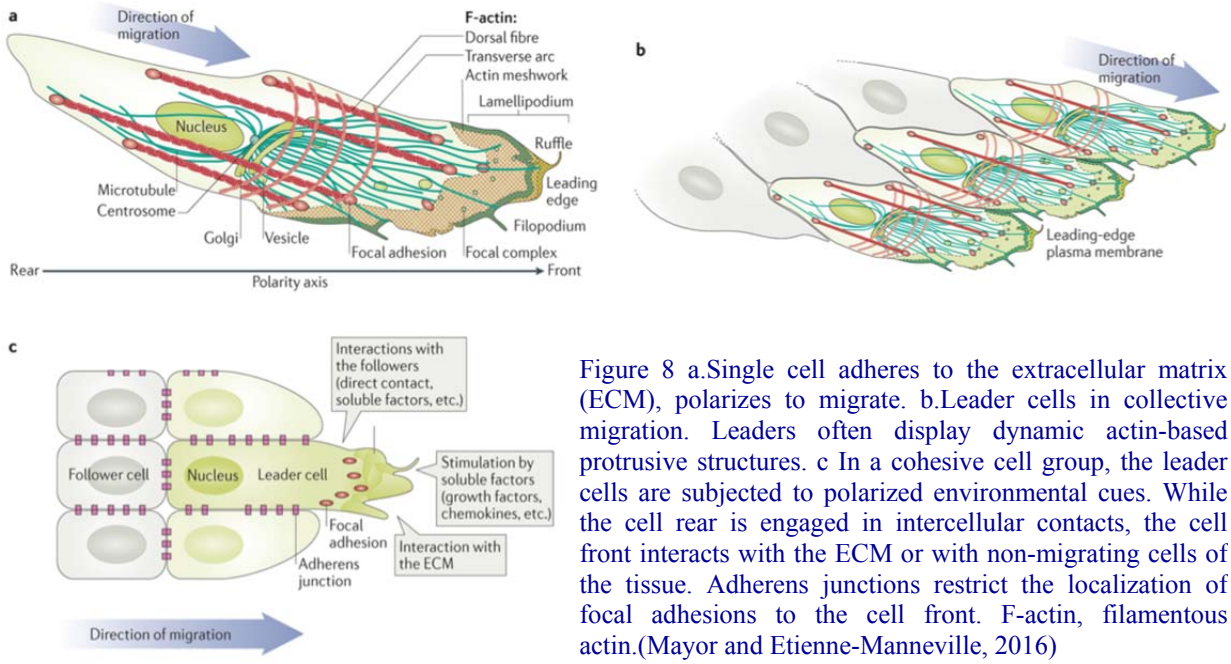


Figure 8 a.Single cell adheres to the extracellular matrix (ECM), polarizes to migrate. b.Leader cells in collective migration. Leaders often display dynamic actin-based protrusive structures. c In a cohesive cell group, the leader cells are subjected to polarized environmental cues. While the cell rear is engaged in intercellular contacts, the cell front interacts with the ECM or with non-migrating cells of the tissue. Adherens junctions restrict the localization of focal adhesions to the cell front. F-actin, filamentous actin.(Mayor and Etienne-Manneville, 2016)

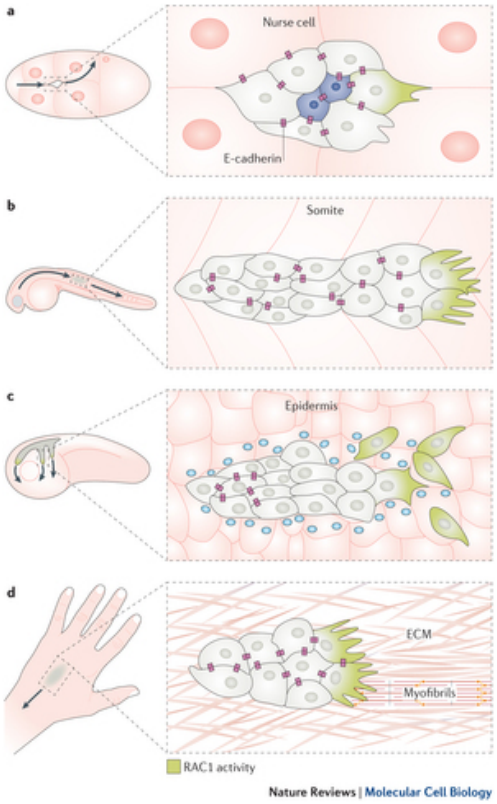


Figure 9: Example of collective migration in vivo. a. The border cell in *Drosophila melanogaster*. During oogenesis, anterior polar cells (purple) recruit neighbouring cells to form the border cell cluster and start migrating. b.The lateral line in zebrafish. The primordium migrates as a compact epithelial cluster with large polarized protrusions at the front. c. The neural crest in *Xenopus laevis*. The cephalic neural crest migrate as a cohesive cluster of cells that influence each other's behaviour.d. Cancer invasion. Cancer cells migrate collectively, although leader cells can acquire a more mesenchymal phenotype.ECM, extracellular matrix. (Mayor and Etienne-Manneville, 2016)

Collective migration is active in many developmental processes, from gastrulation to complete organogenesis and tissue homostasis maintenance. Gastrulation or wound healing are examples for cells that move extensively in a collective manner. Collective cell migration has been extensively studied *in vivo* in both vertebrate and intervertebrate models. For example, epithelial collective migration includes *Drosophila* border cells migration, branching and sprouting morphogenesis of *Drosophila* trachea and mouse retina. Mesenchymal cell collective migration includes neural crest and mesendoderm migration in *Xenopus* and *zebrafish* (Friedl and Gilmour, 2009).

2.2.2.2.1 Force generation during collective migration

During collective cell migration, cluster of cells move due to the traction forces generated by the individual cells pulling on the surrounding ECM. The leading row of cells at the front of the cluster play a mayor role in directing the motion of the rest of the sheet by having a distinct traction (Trepat et al., 2009). The anterior traction-forces generated by leader cell toward the substrate are balanced by the tensile forces at the cell-cell junctions with follower cells at the rear, and transmit forces across a longer distance and multiple cell bodies within moving cell sheets (Trepat and Fredberg, 2011). Follower cells can also engage in cell-substrate traction forces, possibly as a consequence of 'cryptic lamellipodia' that protrude underneath the neighboring cells (Farooqui, 2005) (Figure 6). Thus, both leader and, to a lesser extent, follower cells generate traction force toward the substrate, which is balanced with the forces extending across cell-cell bodies. Imbalance of these two force results in the net force driving cell migration.

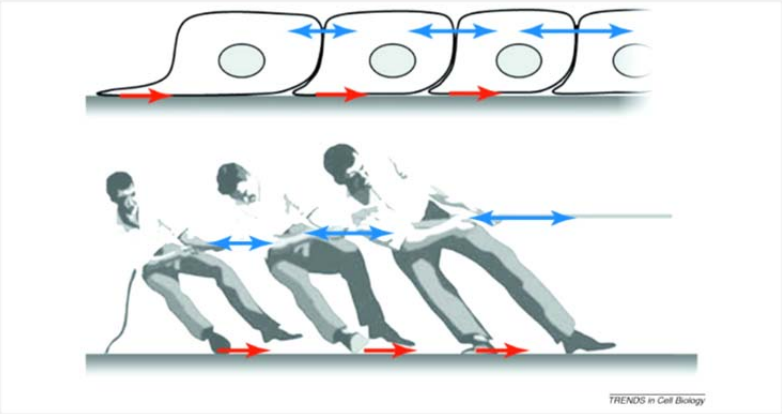


Figure 10: Cells use a tug–of–war mechanism to integrate local tractions (red) into long-range gradients of intra- and inter-cellular tension (blue). Tension in the monolayer reflects the spatial accumulation, or pile-up, of traction forces. Equivalently, the local traction force is the spatial derivative of the intercellular stress (Trepat and Fredberg, 2011).

Moving cell groups process directional information by intra and intercellular signaling. An integrated mechanocoupling program within the leader cell reinforces outward polarization, cyclic actomyosin coupling, force transmission, and negative feedback signaling to follower cells to guide the cell group (Ladoux et al., 2016). Along cell-cell junctions, signaling is exerted by the adhesion molecules themselves, including cadherins. In addition, forces transmitted at cell-cell junctions may induce conformational changes in mechanoresponsive molecules including vinculin and thereby trigger singaling events (del Rio et al., 2009). Together, the cell-matrix adhesion mediates the generation of traction forces that allow the protrusion of the leading edge, then followed by the translocation of the rest of the cell. Concomitantly, cell-cell adhesion allows the integrated mechanocoupling and guidance through the cell group to promote coordinatedly collective migration.

2.2.2.2.2 Adherens junction dynamics during collective cell migration

Cadherin adhesion can have distinct and apparently opposite effects on cell migration. Dependent on cell types and cellular environement, they can support cell migration or participate in contact

inhibition of cell migration. Stable cell-cell adhesion is essential for maintaining tissue integrity but cells need also to relocate, implying the existence of mechanisms for coordinating cell adhesion and cell migration. AJs mediate force transmission between cell-cell contacts in cell clusters to counteract the forces exerted between cells and serve as signaling platforms controlling cell polarity and directed migration. Variations in size and strength of AJs and FAs affect the distribution of forces throughout the cells, putting in game the spatial regulation of actomyosin contractility to control the coordinated cells movements, for example in convergent extension of epithelial tissue in *Drosophila* (Harris and Tepass, 2010).

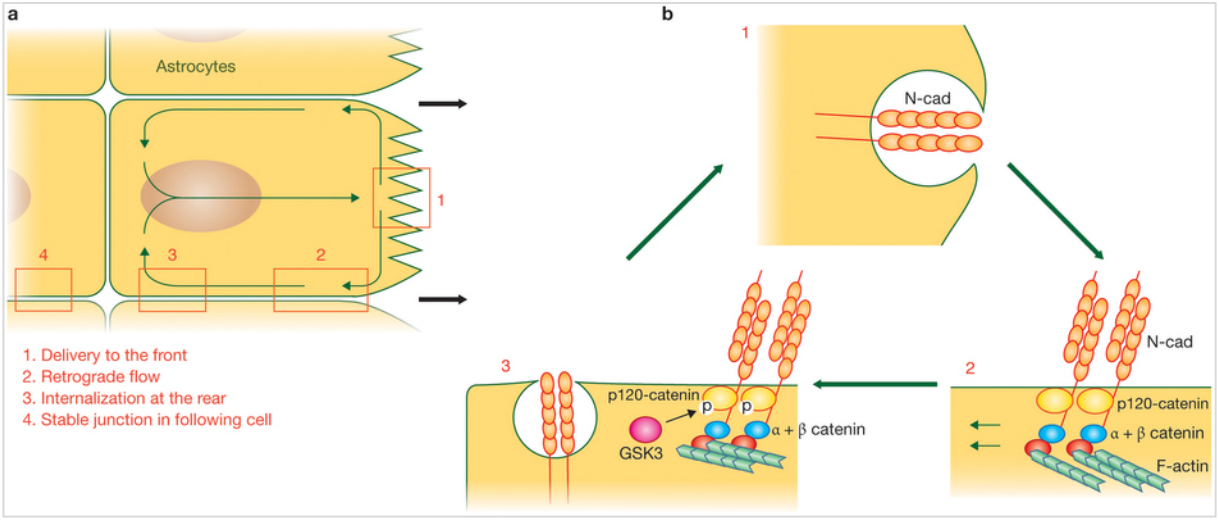


Figure 11 (a) An illustration of the collective migration of astrocytes and the key steps in AJ regulation. Cells of the first row of a migrating cell sheet are depicted with a jagged side representing the cell front. Thick black arrows represent forward cell movement. Dark green arrows in the cell represent cadherin recycling through steps 1, 2 and 3 (see b) compared to stable cadherin-based adhesions in the cells of the second row (step 4). (b) AJ cycling between the front and the back of the cell involves three events: (1) microtubule-dependent delivery of AJ components including N-cadherins (N-cad) to the front of the cell; (2) actin-driven retrograde flow of AJs along the lateral edges of the cell, which is driven by F-actin attachment to AJ complexes that include catenins (for example, p120-, α- and β-catenin) and N-cadherin; and (3) GSK3 phosphorylation of p120-catenin at the cell rear and removal of AJs from the cell surface by endocytosis, which is followed by trafficking of endocytosed N-cadherin to the front to repeat step 1. (Hirata et al., 2014)

In astrocytes monolayer, AJs formed between adjacent leading cells undergo a continuous retrograde movement in a wide range of cell, that is required for a coordinated collective migration (Peglion et al., 2014). This retrograde flow is associated with a retrograde flow of transverse actin fibers and is controlled by a front-to-rear gradient of p120 phosphorylation. As a consequence, p120 may regulate a front-to-rear trafficking of N-cadherin creating a free pool of cadherin complexes at the leading edge while weakening the interactions between leading cells and followers (Peglion et al., 2014). Cadherin disponibility contributes to the formation of AJs between protrusions of adjacent migrating cells and probably helps maintaining a linear front of migration. This cadherin retrograde flow requires a

polarized actin flow that itself depends on the geometry of cadherin-mediated contacts, creating a feedback mechanism, which could maintain the polarized organization of the actin cytoskeleton.

2.2.2.3 Cancer cell migration

Cancer metastasis engages different modes of migration in order for cells to reach and invade distant tissues (Friedl and Wolf, 2010). Local invasion is the result of protruding sheets, which still maintain the contact with the primary cancer site. This invasive mode is observed in epithelial cancers such as oral squamous cell carcinoma and mammary carcinoma, colon carcinoma and melanoma (Häger et al., 2013). On the contrary, cluster of cancer cells can completely be detached from their site of origin to invade distant tissues as seen in lung cancer, breast cancer and melanoma (Häger et al., 2013). Cells can detach from each other to escape the origin layer and migrate individually. In this case, cells adopt either ameboid-morphology-like as observed in lymphoma and small cell lung cancer, leukemia; or mesenchymal-type in fibrosarcoma and glioblastoma tumors. Alternatively, cells can maintain or reinforce adhesion with their neighbours and migrate collectively by pulling on the substrate by a tug-of-war mechanism (Ravasio et al., 2015). Therefore, cell-cell adhesion could be detrimental or repressive to cancer cell migration.

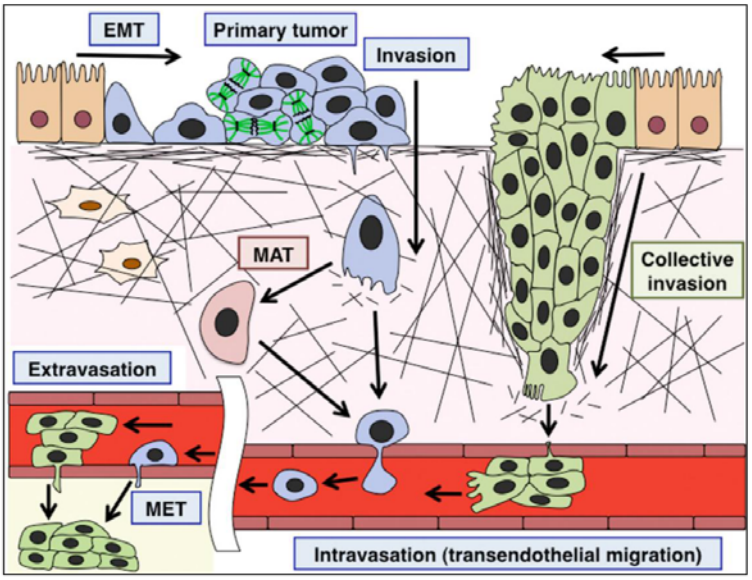


Figure 12 EMT-mediated invasion and collective invasion in cancer metastasis. Normal epithelial cells (orange cells) undergo EMT and form a primary tumor (blue cells). Some primary tumor cells invade and migrate into blood circulation as a multicellular strand (green cells). EMT: epithelial-mesenchymal transition, MAT: mesenchymal-amoeboid transition, MET: mesenchymal-epithelial transition. (Kawauchi, 2012)

2.3 N-cadherin and cell migration during tissue organization

2.3.1 N-cadherin

2.3.1.1 Generalities

N-cadherin was first identified in 1982 as a 130 kDa protein in the chick neuronal retina. While E-cadherin is primaly expressed in epithelial cells, N-cadherin is found in a variety of cell types including neural, heart, muscle, osteoblasts and fibroblasts. Structurely, N-cadherin shares common module with other members of the classical cadherins family containing 5 ECs. EC1 is the main domain participating in the homophilic interaction between N-cadherin of the neighboring cells. The EC4 domain of N-cadherin has been identified to interact with the ectodomain of FGFR, promoting breast cancer invasion (Hazan et al., 2000). Interestingly, by transfection of chimeras of E-and N-cadherin in squamous epithelial cells, EC4 was indentified to be essential for epithelial to mesenchymal transition and an increase in motility by N-cadherin expressing cells (Kim et al., 2000).

Like other members of the classical cadherin family, the cytoplasmic part of N-cadherin is complexed with catenins and actin regulator proteins, which are capable of regulating N-cadherin function. β-catenin binds to N-cadherin, which is responsible for association with α-catenin and hence for linking of the whole complex to the actin network under stress. The anchoring of complex N-cadherin-catenins to actin network reinforces N-cadherin mediated cell-cell contacts. Binding of N-cadherin and p120 is required for its stabilization at the cell membrane of C2C12 cells during myogenesis (Taulet et al., 2009) and of astrocytes during collective migration (Peglion et al., 2014). Moreover, cells expressing cytoplasmic domain truncated N-cadherin attached to N-cadherin ectodomain-coated substrate although the adhesion strength was reduced compared to cells expressing wild type N-cadherin (Shan et al., 2004), confirming that the cytoplasmic domain is required for strong cell-cell contacts. Catenins binding to N-cadherin could also negatively regulate cell-cell contacts strength through modification of N-cadherin prevalence at the cell membrane; this will be developed later in the same chapitre.

Different cadherins display different adhesive forces. N-cadherin mediated junctions are more labile and dynamic than E-cadherin based one. Using dual pipette assay, Chu et al. showed that disruptive force generated by N-cadherin mediated-junctions was 3-4 fold less than the one generated by E-cadherin (Thiery et al., 2012). This difference in adhesive property may explain cadherin dependent homophilic cell-sorting behaviors. For example, a switch of E- to N-cadherin has been observed during the separation of the neural tube from the embryonic ectoderm (Stepniak et al., 2009). Although N-cadherin typically forms homotypic homophilic interactions, it can bind to other cadherin such as R-cadherin in transfected L cells and in neurons (Shan et al., 2000).

2.3.1.2 Roles of N-cad mediated cell-cell contacts in tissues

2.3.1.2.1 Nervous tissue

N-cadherin is the most common classic cadherin in the vertebrate central nervous system (CNS) [126]. Classically, N-cadherin has been associated with the maintenance of tissue integrity through its mediation of cell–cell adhesion between stationary cells. Beside, it mainly contributes to cell-cell adhesion in neural protenitor cells (NPCs) and neurons (Hirano and Takeichi, 2012) to maintain the integrity of cerebral tissue architecture. Indeed, loss of function of N-cadherin by antibody in NPCs of the neural tube, retina and the brain cortex destroys the tissue architecture of the zebrafish forebrain and mouse cortex (Kadowaki et al., 2007; Masai et al., 2003). At the beginning of neurogenic phase, expression of N-cadherin is repressed inducing the detachment of NPCs from the apical region of neuroepithelium. This detachment is crucial for further neurogenesis and neuronal migration from the ventricular zone (Rousso et al., 2012).

At the onset of synaptogenesis, N-cadherin is found at presumptive synaptic sites where it can participate in their maturation (Togashi et al., 2002). Synapse maturation occurs in parrallele to the clustering of N-cadherin at the site of contact, both in vivo (Rubio et al., 2005) and in vitro (Bozdagi et al., 2000). Moreovre, overexpression of N-cadherin increased synapse stability (Mendez et al., 2010).

As peripheric nervous system development progresses, N-cadherin is down-regulated but persists in some glial cells types (mysenteric glia, DRG, non-myelinating Schwann cells). Interestingly, it is expressed at glia-glia and glia-neuron contacts, therefore stabilizes the structure of ganglia and the mysenteric, sciatic nerves. Using N-cadherin, the non-myelinating Schwann cells can attach to wrap the axon then wind around itselfs during axonal myelination, at the same time appose over myelinating Schwann cells to form the whole nerve structure (Corell et al., 2010).

2.3.1.2.2 Cardio-vascular tissue

When other tissues express more than one subtype of cadherins, heart muscle depends solely on N-cadherin. N-cadherin is strongly expressed in precardiac mesoderm and its expression continues throughout cardiomyocytes development up to adult differentiated myocardium (Radice, 2013). In mice, N-cadherin deficient myocytes are unable to maintain cell-cell contacts resulting in dissociated rounded myocytes (Radice et al., 1997). Furthermore, N-cadherin is required for heterotypic epicardial–myocardial cell–cell interactions. Disruption of these cellular interactions leads to myocardial cell hypoplasia (Luo et al., 2006). In adult myocardium, N-cadherin was found abundantly at intercalated discs (ICD) composed of three distinct junctions types: AJs, desmosomes and gap junctions. Loss of N-cadherin in the adult heart leds to disassembly of the ICD (Borrmann et al., 2006).

In the vascular system, N-cadherin is intimately associated with the formation and maintenance of blood vessels. In endothelial cells (ECs), N-cadherin could be nonjunctional thus freely difussing in the membrane, and responsible for interactions of ECs with mural cells such as pericytes or smooth muscle cells (Navarro et al., 1998), or junctional between ECs (Luo and Radice, 2005a). Inhibition of N-cadherin function by antibodies disrupted ECs-pericytes adhesive complexes and caused vessel wall permeability (Gerhardt et al., 2000). Interestingly, loss of N-cadherin in mice ECs caused a significant decrease in VE-cadherin and p120 levels. Also, N-cadherin is abundantly expressed in the vascular muscular smooth cells assuring the adhesion between the latter and ECs as mentioned above.

2.3.1.2.3 Skeletal tissue

N-cadherin mediated adhesion is important for the integrity of dermomyotome (Cinnamon et al., 2006). Pertubation studies demonstrated that N-cadherin plays a role in myoblast interaction and fusion (Mege et al., 1992). Transfection of N-cadherin in a myogenic cell line promotes skeletal myogenesis (Redfield et al., 1997).

2.3.1.2.4 Bone

N-cadherin is expressed at all stages of bone formation, although at various levels. Notablely, the expression of N-cadherin is increased at early stage of osteoblasts differenciation, and associated with enhanced cell-cell adhesion. Reversly, its expression is decreased in more mature osteoblasts-osteocyte which exhibit reduced cell-cell interactions (Ferrari et al., 2000). Therefore, a decrease in N-cadherin expression in osteoblasts leading to loss of cell–cell contacts may contribute to the change from the osteoblast to the osteocyte phenotype. Concordantly, conditional knockout of N-cadherin in mice reduces cell-cell adhesion and bone mass (Di Benedetto et al., 2010).

2.3.1.3 Regulation of N-cadherin prevalence at the cell surface

The prevalence of N-cadherin at the cell surface regulates the engagement of N-cadherin at cell-cell contacts, thus controlling junctional propreties. Trafficking of cadherins presents significant differences between members of the family. For example, Hakai- an ubiquitin ligase, binds E-cadherin in a tyrosine kinase dependant fashion targeting E-cadherin for internalization but does not bind N-cadherin (Fujita et al., 2002). β-arrestin whose recruitment upon Ser-phosphorylation of VE-cadherin leads to its endocytosis is not found in neither E-cadherin nor N-cadherin (Gavard and Gutkind, 2006).

p120 is important in the trafficking and maturation of the cadherin-catenin complex. Upon p120 silencing by siRNA, cadherin is rapidely degraded, probably via ubiquitination (Davis et al., 2003). Dissociation of the N-cadherin/p120 complex could have either negative or positive effects on membranous N-cadherin stabilization, dependent on cellular context and cell types. For example, Src-dependent phosphorylated-p120 uncouples from N-cadherin and is translocated to the cytosol. This

triggers the internalization of N-cadherin from the cell-cell adhesion site to an early endosomal compartment (Inumaru et al., 2009). Transfection of mutant N-cadherin lacking the p120 binding site in C1C12 cells destabilizes N-cadherin from cell junctions by impairing N-cadherin association with the lipid raft (Guillaume et al., 2013). In contrast, the same mutant induced aggregation of colon carcinoma cells (Aono et al., 1999). The loss of N-cadherin/p120 interaction promotes cadherin clustering at the cell surface and therefore adhesion-mediated stabilization of the synaptic contact (Rubio et al., 2005). Indeed, N-cadherin/p120 complex is found in small clusters at the surface of nascent synapses whereas mature synapses display large N-cadherin clusters, which are largely depleted of p120.

While p120 appears to be the key cadherin binding partner in regulating N-cadherin endocytosis, emerging evidences suggest that β-catenin also participates in the modulation of cadherins cell surface avaibility. β-catenin was reported to be internalized by macropinocytosis in cultured fibroblasts, and that internalized β-catenin colocalizes with N-cadherin (Sharma and Henderson, 2007). In ovarian cancer cells, disruption of AJs is provoked upon the internalization of N-cadherin/β-catenin complex by altering the phosphorylation status of β-catenin (Huang et al., 2012). Tai and al. observed that β-catenin was accumulated at the synaptic interface upon inhibition of N-cadherin turnover suggesting the negative role of β-catenin binding in N-cadherin endocytosis at neuronal synapse (Tai et al., 2007). Thus, β-catenin also participates in the modulation of cadherin at cell surface availability although the mechanistic pathways remain so far unclear. Given the conflicting evidences, more work is needed to understand how β-catenin and/or p120 might regulate cadherin endocytosis.

2.3.1.4 N-cadherin-dependent motile phenotype

The function of N-cadherin depends to a great extent on the cellular context. On one hand, N-cadherin mediates strong adhesion between cardimyocytes to maintain the structural integrity of the heart. On the other hand, N-cadherin promotes the formation of rather small AJs in other tissues (Stepniak et al., 2009). Beside, N-cadherin is known for conferring a motile phenotype (Hazan et al., 2000), contrary to E-cadherin, which is more often designated as a tumor suppressor (M. Fedor-Chaiken et al., 2003). During development, cells undergoing invagination during gastrulation upregulate their N-cadherin expression, while completely invaginated cells express abundantly E-cadherin (Hatta and Takeichi, 1986). Neural crest cells undergo EMT process characterized by a E-cadherin-to-N-cadherin switch to emigrate from the dorsal side of the neural tube (Scarpa et al., 2015). Similarly, cancer cells display frequently a E-cadherin-to-N-cadherin switch associated to their invasive and metastatic behaviors (Friedl and Gilmour, 2009). To investigate the difference in capacity to induce movement of N-cadherin and E-cadherin, which display however high sequence homology, Fedor-Chainken and colleagues studied the migration of mammary carcinoma cells transfected with different N-cadherin/E-cadherin chimera. They revealed a region common to both E-cadherin and N-

cadherin (AA699-710) that is required for the suppression of the migration of mammary carcinoma cells. However, this domain is masked in N-cadherin expressed by these cells (M. Fedor-Chaiken et al., 2003). Therefore, N-cadherin has no influence on the movement of MBD-MB-435 human breast melanoma cells, but it inhibits the migration of LM8 mouse osteosarcoma cells (Kashima et al., 2003). So, the cadherins-induced motility effect can be cell type specific.

2.3.2 N-cadherin and cell migration

2.3.2.1 Role of N-cadherin in cell migration during brain development

2.3.2.1.1 Examples of role of N-cadherin in cerebral single cell migration

N-cadherin tightly regulates neuronal migration from the first location of progenitors to the final site destined for mature neurons. Abnormal expression of N-cadherin in neural stem cells (NSC) causes abnormalities in neurogenesis and abnormal neuroblast migration (Guerra et al., 2015). N-cadherin-based migration is essential to tissue organization in the CNS, especifically in cortical layer formation (Cooper, 2013).

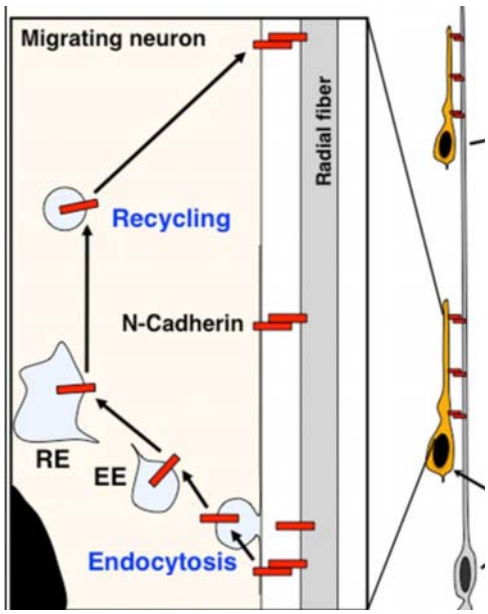


Figure 13 A scaffold cell-dependent migration in neural development. The migrating neurons in the developing cerebral cortex show radial glial fiber-dependent migration, which requires the endocytic trafficking of N-cadherin. RE : reticulum endoplasmic ; EE :early endosome (Kawauchi, 2012)

Scaffold cell-dependent, in which N-cadherin is tightly regulated, provides one mode of neuronal migration. In this process, N-cadherin binds the newborn neuron to glial fibers allowing them to glide, likely by the transfer of force, generated by the intracellular actin network (Franco et al., 2011). Interestingly, it has also been shown that N-cadherin is internalized, and recycled by the migratory neurons, which is substantial their migration (Kawauchi, 2012). Indeed, N-cadherin is necessary for the radial migration of neuronal precursors on radial glial fibers from ventricular wall to their final location in the cortical layers (Shikanai et al., 2011). N-cadherin allows mild cohesion between neurons and radial fibers favouring the migration of locomoting neurons. During this process, N-cadherin undergoes active endocytosis to maintain proper surface levels of N-cadherin. Inhibition of N-cadherin endocytosis increases surface N-cadherin, which enhances neuron-glial adhesion *in vitro* but inhibits radial migration *in vivo* (Shikanai et al., 2011). Moreover, knock down (KO) of N-cadherin inhibits radial migration (Jossin

and Cooper, 2011a). It appears thus that too much or too little surface N-cadherin is negative for glial migration. Similarly, movement of precerebellar neurons in the hindbrain is reduced when N-cadherin function is impaired (Taniguchi et al., 2006b). N-cadherin is also pivotal for the tangential migration of the future cortical interneurons, born in the medial ganglionic eminence (Luccardini et al., 2013).

At later developmental stages, N-cadherin regulates selective adhesion between neural cells and induces neurite outgrowth likely by interacting with actin treadmilling. N-cadherin substrate has been shown to stimulate neurite outgrowth *in vivo* (Plestant et al., 2014b), and to increase the migration of NPC to the demyelinated zone, *in vitro* (Klingener et al., 2014).

2.3.2.1.2 Examples of role of N-cadherin in cerebral cell collective migration

Besides controlling single migration of different derived neuronal cells, N-cadherin also regulates the collective migration of neural cell clusters happening at onset of brain development. In the chick, inhibition of N-cadherin by injection of antibody alters the directional migration of neural crest cells, which accumulates outside the neural tube (Duband et al., 1987). The latter is distorted, overgrown and folded.

Cephalic Neural Crest (CNCs) cells start their migration away from the neural tube as a cohesive cell population by dissociating from neighboring neuroepithelial cells (NE cells). This process, called delamination, relies on a qualitative and quantitative change of cadherin repertoire. CNCs are highly motile, they migrate extensively throughout the embryo after separation from NE cellss. Neighbor NE cells on the contrary have a transient motility, which is progressively restrictive during neural tube development (Duband et al., 2009). A dramatic increase in N-cadherin mediated cell-cell adhesion accounts for the reduction of migratory potential of NE cells. Indeed, the spatio-temporo pattern of N-cadherin expression in the NE cells matched extaclty with the progressive restriction of cell migration potential. Moreover, inhibition of N-cadherin function by antibody restored the migration capacity of explant of non-motile NE cells (Duband et al., 2009). N-cadherin equally mediates CNCs interaction during migration (Theveneau et al., 2010). Premigratory CNCs begin their migration by increasing expression of N-cadherin at both mRNA and protein levels (Théveneau et al., 2007; Xu et al., 2001). Inhibition of N-cadherin expression in Xenopus using antisense morpholinos, or overexpression shows dramatic effects on CNCs migration. These cells became highly motile, dispersed quicker than controls and produced numerous protrusions on top of each other with wide membranous overlapping (Theveneau et al., 2010) whereas too much of N-cadherin blocked completely CNCs migration. Concordantly, disruption of cell-cell adhesions mediated by N-cadherin leads to cell dispersion and loss of cell cooperation, causing loss of directional migration. On the contrary, cells that were strongly adherent with one another could undergo directional migration but failed to invade narrow spaces (Kuriyama et al., 2014a). Furthermore, it was shown that N-cadherin recycling is required for neural

crest migration in vivo, as it allows the collective to acquire enough tissue plasticity to pass through narrow spaces (Kuriyama et al., 2014b). This indicates that the level of N-cadherin must be correctly regulated in order for CNCs to sense each other and undergo proper coordinated migration.

2.3.2.2 N-cadherin and cell migration in other systems

2.3.2.2.1 N-cadherin and epithelial migration

N-cadherin is an enhancer of epithelial cell migration. Indeed, transfection of epithelial cells with N-cadherin induces a motile phenotype (Hazan et al., 1997). Experiments in cell lines show that forced expression of even low levels of N-cadherin in epithelial cells expressing E-cadherin stimulates cell migration (De Wever, 2004; Nieman et al., 1999). In epithelial cell culture on 3D collagen matrix, N-cadherin mediates and maintains cell-cell contacts between cells in the sheets and promotes cell collective migration (Shih and Yamada, 2012). These studies suggest that N-cadherin plays an active role in cell motility that E-cadherin cannot suppress.

2.3.2.2.2 N-cadherin and endothelial cell migration

Endothelial cells express two classic cadherins, VE-cadherin and N-cadherin. Contrary to VE-cadherin, the role of N-cadherin on vascular development including endothelial migration is poorly understood. During angiogenesis, N-cadherin is primordial to form the contact zones between pericytes and endothelial cells (Gerhardt et al., 2000). N-cadherin knock down results in enhanced EC migration compared with control whereas VE-cadherin loss of function had no effect of ECs motility (Luo and Radice, 2005a). The authors conclude that N-cadherin regulates angiogenesis by controlling endothelial cell proliferation and migration, in part, throuth VE-cadherin regulation at the cell surface. Concordantly, N-cadherin increases migration of endothelial cells both *in vivo* and in culture (Giampietro et al., 2012). Moreover, the expression and junctional localization of N-cadherin is negatively modulated by VE-cadherin, at least partly by a competition for binding with p120 and with β-catenin. Alltogether, these studies show a narrow inter-regulation between different subtype of cadherins to control cell-cell adhesion and cell migration.

2.3.2.2.3 N-cadherin and smooth muscular cell migration

During myocardial morphogenesis, N-cadherin expression is significantly increased in cells migrating toward the endocardium during the formation of the cardiac trabeculae and intercalated disks (Ong et al., 1998). Jones and collegues studies support a positive role of N-cadherin on vascular smooth muscle cells (VSMCs) migration *in vitro* (Jones et al., 2002). Blocking homophilic binding of N-cadherin by antibody suppressed spreading and migration of VSMCs during wound repair in vitro (Chrétien et al., 2010). Movement into the wound site in control cultures was highly oriented with leading edge cells aligning perpendicularly to the wound contrarily to N-cadherin antibody-treated-

cultures, which displays a disoriented migration without protrusion (Chrétien et al., 2010). This suggests that N-cadherin could enhance directed migration during repair. Interestingly, VSMCs in damaged artery upregulated N-cadherin expression while dowreagulating other cadherins (Moiseeva, 2001). Thus, N-cadherin may promote wound repair by forming labile cellular adhesion that facilitate dynamic cell-cell interactions and cell migration. This idea is concordant with the reversed effects of N-cadherin on VSMCs migration in (Chrétien et al., 2010; Lyon et al., 2010).N-cadherin neutralization does not have effects on VSMCs located away from the wound edge where VSMCs exhibit well-established contacts. Besides, they showed that DN N-cadherin expression causes effects all over the wound as this mutant disrupts existing N-cadherin mediated intercellular junctions (Lyon et al., 2010). Taken together, N-cadherin mediates a temporal as well as spatial dynamic change in cell-cell junctions to regulate the migratory capacities of VMSCs.

2.3.2.2.4 N-cadherin and bone cell migration

N-cadherin also plays a role during bone development since its complete blocking leads to impaired osteogenesis. Indeed, the potential migration of osteoblasts was enhanced by overexpression of N-cadherin (Xu et al., 2013). The severely altered expression and localization of N-cadherin would impair osteoblast cell-cell adhesion and result in increased migration (Gunduz et al., 2012). Again, the optimal expression level of N-cadherin could be a check-point of motile or non-motile phenotype in osteoblast cell lines, as we have seen for other cell types. Effectively, primary calvaria cells express a low level of N-cadherin with cytosolic localization is not sufficient to induce migration but primed to enhance migration. When the level of N-cadherin is significantly increased, osteoblast cells migrate. When osteoblast cells become more mature and express higher level of N-cadherin, they are less migratory (Gunduz et al., 2012).

2.3.2.3 E-cadherin-to-N-cadherin switch in Epithelial-to-mesenchymal transition and cell migration

An important step of cell migration control during development and disease is EMT. E-cadherin is expressed by most normal epithelial tissues and many epithelium-derived cancer cells have lost E-cadherin expression (Wheelock et al., 2001). Mesenchymal cells, which are more motile and less polarized than epithelial cells, typically express N-cadherin.

During EMT, epithelial cells leave their surrounding tissues and become motile, often resulting in invasive growth. The term of cadherin switch usually refers to a switch from expression of E-cadherin to N-cadherin, but also includes the unchanged expression of E-cadherin accompanied by turn-on of Ncad expression.

Cadherin switch may allow a select population of cells to separate from their neigbors for example during processes such as gastrulation, epiblast cell ingression through the primitive streak and neural

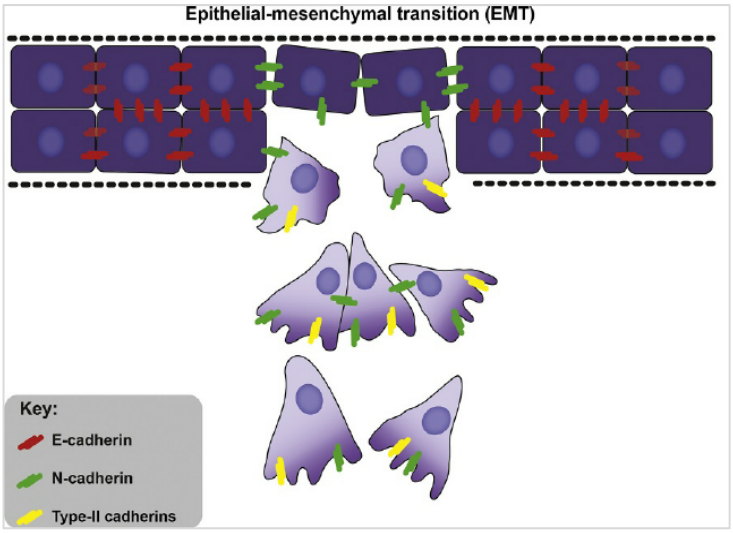


Figure 14: Cadherins switch epithelial-to-mesenchymal transition (EMT). Presumptive cells express E-cadherin (red parallel lines) and N-cadherin (green parallel lines) as cell – cell adhesion molecules. Prior to migration, cells undergo EMT. This process involves transcriptional repression of E-cadherin, to allow cells to separate from E-cadherin expressing environement. The expression levels of N-cadherin are slightly but not completely reduced, allowing cells to form transient contacts that mediate signaling during collective migration.

crest cells migration from the neural tube. It has been acquired that cells expressing different cadherins segregate from one another *in vitro* assay. In the other way, cadherins switch could happen to favor the adhesion of cells or cluster of cells to the new environment. Although there are many studies that relate the E-to-N-cadherin switch to motile phenotypes, the underlined mechanisms are poorly understood.

2.3.2.4 Upregulation of N-cadherin promotes invasiveness of cancer cells

N-cadherin is acknowledged as a marker of malignant state during cancer process. In epithelial cancer type development, cells acquire motility and invasiveness through EMT process. The most visible example is the switch from VE-cadherin to N-cadherin of endothelium cells to favor the transmigration of certain cancerous cells to invade; or the switch of N-cadherin in stromal cells to promote engraftment of tumor cells. Besides, numerous clinical studies have shown that N-cadherin and other "inappropriate" cadherins are expressed by cells from various tumors *in situ*, and thus there is a correlation between cadherin switch and tumor progression in human (Cavallaro et al., 2002). The switch of cadherin subtype expression is observed in breast cancer (Rieger-Christ et al., 2004), prostate cancer (Kolijn et al., 2015), lung cancer... Transfection of exogenous N-cadherin into breast cancer cells increase cells migration, cell invasion and metastasis (Hazan et al., 1997). Blocking of stromal N-cadherin reverses adhesion between cancer cells and the stroma, providing insight into the strong engraftment of cancer cells to invade (McAndrews et al., 2015). Inhibition of phosphorylation at Tyr860 of N-cadherin prevents the transmigration of transfected melanoma cells across the vessel wall (Qi et al., 2005). It was suggested that activation of Src leads to the phosphorylation of N-

cadherin and subsequent dissociation of β-catenin. This allows the transmigration of melanoma cells through the vessel wall.

To conclude, cadherins control cell migration during tissues organisation and development through adhesion as well as its signaling functions. Importantly, their expression level should be strictly regulated. Cadherin endocytosis and degradation play crucial roles in this dynamic control. By adjusting the rate of cadherin internalization, cells are able to quickly modify the strength of their adhesions and migration. The misregulation of cadherins endocytosis induces abnormal expression of cadherin and can thus cause abnormal migration in numerous pathologies. Moreover, there is likely an optimal level for migration also controlled through recycling at the plasma membrane.

2.4 FGFRs and cell migrations

Cancer is characterized by uncontrolled growth enhanced by important increase of growth factor and their receptors expression such as the couple: fibroblast growth factor (FGF) and its receptor (FGFR). As argued above, N-cadherin expression is equally inceased in cancer cells leading to dissemination and invasion. Moreover, FGFR and N-cadherin have similar feature of subcellular localization in similar physiologic as well as pathologic conditions, in cancer for example. Moreover, they share a lot of signaling partners. Thus, it is important to inquire the contribution of FGFR in N-cadherin mediated cell-cell contacts and cell migration.

2.4.1 FGFRs-FGFs family

FGFRs belong to Tyrosine Kinase Receptors (RTKs) family, which include Growth Factor Receptor family including Epidermal Growth Factor Recptor (EGFR), FGFR and Neuronal Growth Factor Receptor (NGFR). All RTKs have the same structural feature contain : an extracellular part controlling ligand interaction, a transmembrane domain and a cytoplasmic part responsible for kinase function (Schlessinger, 2000). The binding of a growth factor to the ligand-binding domain results in RTK activation and initiation of intracellular signalling cascade to induce cellular effects.

2.4.1.1 Structure and functions

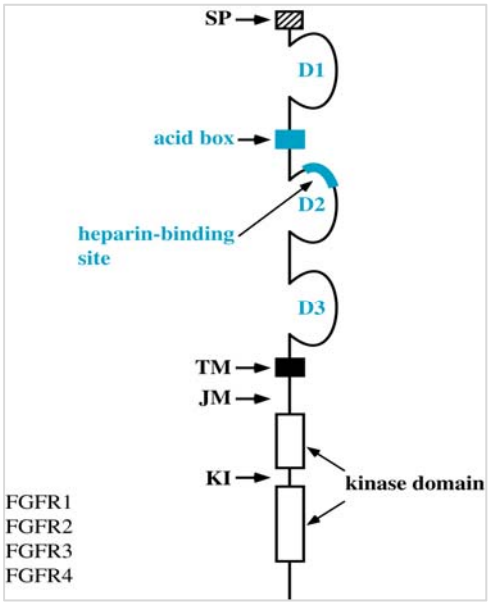


Figure 15: Schematic diagram of fibroblast growth factor receptors. D1, D2, and D3 represent immunoglobulin-like domains 1, 2, and 3. SP represents the signal peptide; TM represents the transmembrane helix; JM represents the juxtamembrane region. The heparin-binding site in D2 is marked by a thick black line.

FGFR constitutes one of the largest RTK families in vertebrates. FGFR family consists of 4 receptors named from FGFR1 to FGFR4. Expression of FGFR1, 2 and 3 have distinct expression patterns with overlaps in some tissues (Zhang et al., 2006). FGFR1 and FGFR2 exhibit broad expression in the embryo and later, in contrast with other FGFRs. FGFR1 is predominantly expressed in the brain and in mesodermal tissues in the embryo (Johnson and Williams, 1993). The ectodomain of FGFRs contains three Ig-like loops. The first Ig-like domain is postulated to play a role in receptor auto-inhibition. Between IgG I and IgGII is located a Cell Adhesion Molecule (CAM) homology domain (CHD) and a heparin-binding domain in the beginning of the IgG II. The second and third Ig-like domains of the receptors are sufficient for FGF binding and contribute to the preferences in the ligand binding by each FGFR subtype and isoform. Alternative splicing of the third Ig-like domain occurs in FGFR1-3,

generating the IIIa, IIIb and IIIc isoform of the receptor. Splicing IIIa form encodes a secreted extracellular FGF-binding protein with no known signalling capacity (Werner et al., 1992). The IIIb and c are regulated in a tissue-specific manner, such as the b isoform is restricted to epithelial lineages and the c isoform is preferentially expressed in mesenchymal lineages. Kinase domains of the four FGFRs as well as the auto-phosphorylation sites are relatively well-conserved (75–92% homology) with the closest homology between FGFR1 and FGFR2 and the biggest difference between FGFR1 and FGFR4 (Powers et al., 2000). However, there are some differences in tyrosine phosphorylation profile among FGFRs that may underline a different overall kinase activity and/or downstream signalling pathways.

FGFs are morphogens that control the patterning of the mammalian embryos. FGFs bind and activate alternatively spliced forms of FGFRs. They constitute a family of at least nine structurally realated heparin-binding peptides. Binding of FGF to FGFRs requires Heparan Sulfate (HS). During development, FGF gradients control the establishment of the dorsoventral body axis and the extension of the anteroposterior body axis. Studies show the presence of a rostral FGF-secreting signalling center composed of FGF 3, 8, 17 and 18 during brain development in mice embryos from E9.5 until E12.5 (Bachler and Neubüser, 2001). FGF can bind to extracellular matrix that serves as a reservoir of FGF thus promoting long range FGF signaling (Duchesne et al., 2012). In the presence of HS, FGFs stably binds FGFRs leading to the formation of 2:2:2 FGF-FGFR-HS dimers, which enable the signalling cascades inducing cellular responses.

2.4.1.2 Mechanisms of FGFR activation

The fixation of FGF induces the homo-dimerization of FGFRs allowing the cross phosphorylation of each receptor subunit and their activation. Subsequently, activated receptors bind adaptor proteins and phosphorylate cytoplasmic substrates, eliciting various downstream signalling pathways that control cell proliferation, migration and survival. The classical view postulates that RTKs including FGFRs are monomers in the absence of ligand, but dimerized upon ligand binding. However, the existence of unliganded FGFR dimers was recently revealed (Sarabipour and Hristova, 2016). The specificities in the cellular answer upon FGFs binding to FGFRs are unclear. Considering the same receptor, fixation of different FGFs trigger different downstream responses. Responses mediated by different receptors in response to the binding of the same ligand are also distinct. These specificities in cell responses could be related to the tendencies of FGFRs to dimerize in the absence of ligand (Sarabipour and Hristova, 2016). FGFR1 is predominantly monomeric, whereas unliganded FGFR 2 and 3 form homodimers that become phosphorylated. However, FGFs binding triggers structural changes in the FGFR dimers which increase FGFR phosphorylation, but are different for FGF1 and FGF2, establishing the existence of multiple active states (Sarabipour and Hristova, 2016).

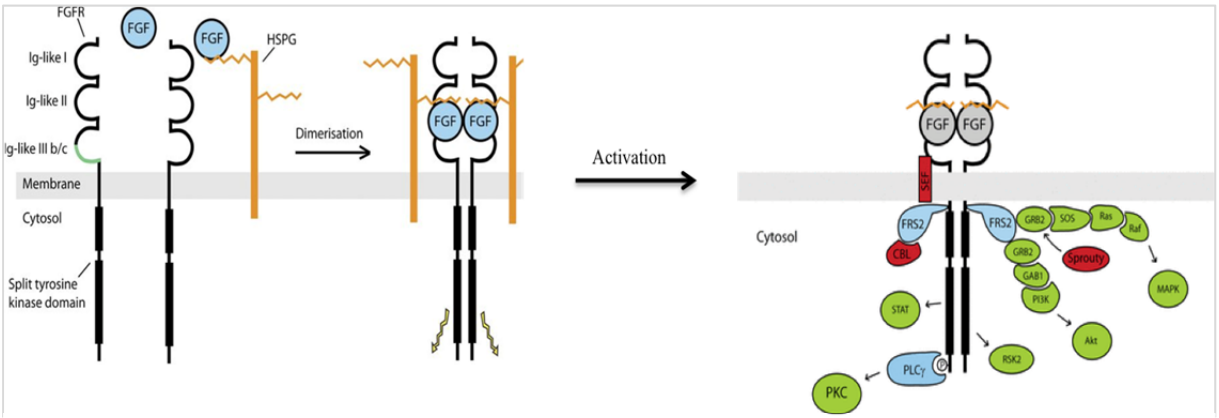


Figure 16 : 1) Complex formation between FGF, FGFR and HSPG initiates signalling .The basic structure of an FGFR is shown on the left-hand side. FGFRs are single-pass transmembrane RTKs with an extracellular part composed of three Ig-like domains (I–III), and an intracellular part containing a split tyrosine kinase domain. The complex formed by two FGFs, two heparan sulfate chains and two FGFRs causes dimerization and transphosphorylation by the kinases on several tyrosine residues in the intracellular part of the FGFRs, causing activation of downstream signalling cascades. (2) Intracellular signalling pathways downstream of FGFRs .After ligand-induced FGFR activation, several downstream signalling cascades are initiated. Two central players, FRS2 and PLCγ (in blue) bind directly to the receptors. FRS2 is constitutively associated with the receptor, and upon activation of the receptor kinase, it is phosphorylated on several tyrosine residues which, in turn, recruit two important signalling pathways, the Ras/MAPK and PI3K/Akt pathways. PLCγ binds to a phosphotyrosine in the C-terminal tail of FGFRs. PLCγ recruitment culminates in the activation of PKC. Several negative regulators are also associated with FGF signalling (in red). Soluble heparin (in orange), a highly sulfated polysaccharide, can take the place of HSPG in the FGF complex and is often used experimentally to increase FGF signalling. See the text for more details. (Wesche et al., 2011)

Phosphorylation of FGFRs activates signaling cascades involved in their internalization. It has been reported that the binding of FGF1 to FGFR1 and FGFR3 leads to the ubiquitination of the receptors targeting them to endocytosis and degradation (Haugsten et al., 2005). Thus, FGFs-FGFRs association states contribute also to FGFR trafficking, and consequently to FGFR crowding at the plasma membrane. Phosphorylated tyrosine residues of FGFRs serve as docking site for various adaptor proteins containing Src homology II domain (SH2). One prominent example is PLCγ that triggers the release of intracellular calcium to activate calcium-dependent proteins and this could also induce MAPK signalling. Some of the adaptor proteins are directly phosphorylated by FGFR such as FGFR substrate 2 (FRS2). FRS2 is rather specific to FGFRs. FRS2 binds to the juxtamembrane region of the FGFR, and upon activation of the receptor it becomes phosphorylated on several tyrosine residues, creating docking sites for additional adaptor proteins. By binding to phosphorylated FRS2, the adaptor GRB2 (growth-factor-receptor-bound protein 2) recruits the Ras/MAPK pathway (mitogen-activated protein kinase). Src is equally recruited to the plasma membrane by docking to phosphorylated FRS2 (Zhan et al., 1994).

2.4.1.3 Regulation of surface distribution

Although FGFR are relevant in both development and cancer progression, very little is known about FGFR trafficking (Haugsten et al., 2005). In the absence of the ligand, the abundance of unstimulated receptors at the cell surface is controlled via production and continuous recycling. Upon stimulation, the prevalence of FGFR at the plasma membrane is predominantly controlled by ligand-dependant receptor endocytosis; endocytosis and subsequent degradation attenuates signalling of FGFRs (Haugsten et al., 2005).

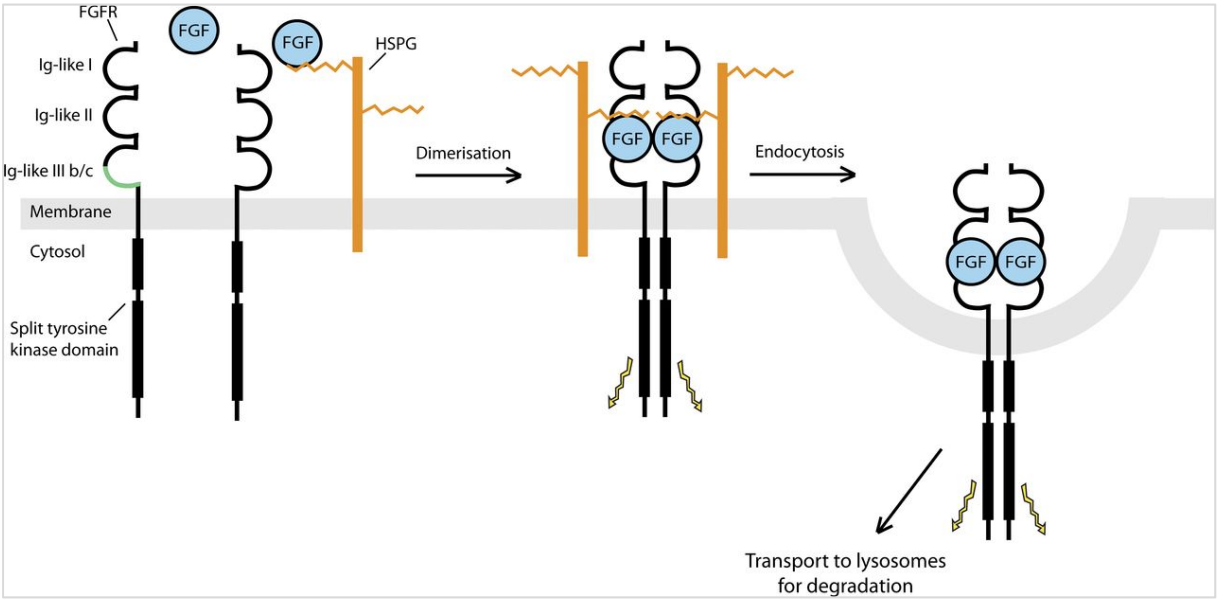


Figure 17: Complex formation between FGF, FGFR and HSPG initiates signalling .The basic structure of an FGFR is shown on the left-hand side. FGFRs are single-pass transmembrane RTKs with an extracellular part composed of three Ig-like domains (I–III), and an intracellular part containing a split tyrosine kinase domain. The complex formed by two FGFs, two heparan sulfate chains and two FGFRs causes dimerization and transphosphorylation by the kinases on several tyrosine residues in the intracellular part of the FGFRs, causing activation of downstream signalling cascades. After activation, the complex is internalized by endocytosis and transported to lysosomes for degradation. (Wesche et al., 2011)

2.4.2 FGFR and cell migration during development and tissue organization

2.4.2.1 FGFR expression and role in migration during early development

FGF and their receptors are known to regulate cell migration during early development and organogenesis. Gastrulation defects were reported in FGFR1 mutant mice and in *Zebrafish* or *Xenopus* embryos injected with mRNA coding for a DN form of FGFR1 (DN-FGFR1) (Amaya et al., 1993; Griffin et al., 1995). Blocking of FGFR1 disrupts the migration of epiblastic cells toward the anterior pole with accumulation of these cells at the posterior streak (Deng et al., 1994). In *Drosophila*, FGFR is essential for directional cell migration and establissment of several mesodermal lineages

(Gisselbrecht et al., 1996). During gastrulation, the primary effect of the FGFR is to promote the spreading of mesoderm cells after their invagination (Beiman et al., 1996). In *Drosophila*, a mutation in FGFR2 results in the failure of mesodermal cells to migrate away from the midline during gastrulation (Gisselbrecht et al., 1996). These are also evidences of FGFR1 role in the regulation of cell adhesion and/or cell migration. Rossant's lab showed that FGFR regulates the morphogenesis and migration of mesodermal cells by differentially regulating intercellular adhesion of progenitor population in the primitive streak (Ciruna and Rossant, 2001). In the early 90, a *Drosophila* FGFR receptor homolog was proved to be essential for migration of tracheal cells (Kl et al., 1992). Live imaging in *Drosophila* embryos demonstrated that FGFR signalling leads to dynamic cytoskeletal reorganizations during migration (Dossenbach et al., 2001). Specific deletion of FGFR1 in late CNS development showed its necessary role in mid-brain and hindbrain development. FGFR1 appears to modify cell-cell contacts for maintaining a cohesive organization between the mid- and hind-brain (Trokovic et al., 2003).

2.4.2.2 Regulation of FGFR in neural crest cells migration

During neural crest cells (NCCs) migration, FGFR1, 2 and 3 have been detected at mesencephalic axial levels. There are accumulating evidences for important roles of FGFRs in the migration of NCCs. Blocking FGFR singaling caused NCCs to accumulate dorsally at the posterior half of each somite where repulsive molecules normally drive NCCs away (Jia et al., 2005). Expression of a DN-FGFR1 in NCCs cells induced an early emigration from the neural tube (Martínez-Morales et al., 2011). Cardiac NCCs expressing FGFR1 display increased migratory properties in the presence of FGF8, involving MAPK pathway (Sato et al., 2011). NCCs motility is controlled by a chemotactic FGF2-FGFR1 signaling. Indeed, anti-FGF2-neutralizing antibodies preventing its fixation to FGFR1 stops the migration of these cells (Kubota and Ito, 2000). Hypomorphic FGFR1 allele perturbing FGFR1 functions leads to defects in NCCs migration into the 2nd branchial arch, responsible for cranio-facial morphogenesis (Trokovic et al., 2005).

2.4.2.3 FGFR in epithelial migration

The presence of FGFR is required for the migration of epithelium tracheal cells in *Drosophila* and for their ability to recognize external guiding cues (Klambt et al., 1992). Indeed, mosaic studies of tracheal progenitor cells suggested that a single FGFR expressing cell is sufficient for sheet extension and tracheal branch formation (Ghabrial and Krasnow, 2006). The most relevant role of FGFR in keratinocyte epithelium migration is shown during the cicatrisation of the skin in mice, consisting in migration of epithelial sheet toward the lost epithelial area to recover the integrity of the skin. FGFR2 expressed by keratinocytes is upregulated and activated by FGFs in wounded skin (Steiling and Werner, 2003). A mouse expressing a DN-FGFR2 in keratinocytes displays severe delay in cicatrisation (Werner et al., 1994). Along the same line, mice lacking FGFR1 and FGFR2 in

keratinocytes show severely impaired re-epithelialization. This defect is associated with an impaired migration of keratinocytes.

2.4.2.4 FGFR in angiogenesis

FGFRs play also important roles in angiogenesis. FGFR1 is mainly expressed in endothelial cells. However, FGFR2 expression is frequently detected. When FGFR1 controls proliferation, migration and formation of endothelial tubes, FGFR2 contributes solely to the motility of endothelial cells through activation of the MAPK pathway (Nakamura et al., 2001). FGF2 activation of FGFR1 is essential for EC migration and survival during vasculogenesis (Nourse et al., 2007). Futhermore, FGFR1 silencing or drug-mediated kinase activity significantly inhibited the migration of Human Vessel Endothelial Cells (HUVEC), blocking the organization of capillary networks (Hu et al., 2013). Furthermore, the Src-dependent phosphorylation of Tyr 653/654 on FGFR1 is required for endothelial cell migration induced by fibronectin (Zou et al., 2012). Targeting FGFR1 reduces endothelial sheet migration spead by regulating at least in part VE-cadherin mediated cell–cell adhesion. This VE-cadherin induced mechanical coordination of migrating sheet allows maintenance of cohesion when boundary cells move into open space (Vitorino and Meyer, 2008). It was further shown that FGFR affects both the directionality and speed of migrating cells by activating differently and separately two pathways: PI3K and PLCy (Kim et al., 2014). Furthermore, transfection of DN-FGFR1 in HUVEC cells lead to the loss of endothelial cell-cell contacts. This disruption is due to decoupling of p120 from VE-cadherin and subsequent dissociation of AJs and tight junctions in both arteries and veins (Murakami and Simons, 2009).

2.4.2.5 FGFR and migration of muscular cells

FGFR regulates migration of muscular cells development. Infusion of chicken somite with FGF stimulates the migration of muscle precursors to the limb buds where they differentiate into muscle fibers. In contrast, transfection with a truncated dominant negative mutant of FGFR1 prevents myoblast migration into the limb bud (Itoh et al., 1996). Following vascular injury or in association with a variety of diseases, smooth muscular cells (SMCs) acquire a proliferative phenotype characterized by enhanced cell proliferation and migration. Expression of FGF/FGFR is detected in SMCs in rat injured artery leading to neointima formation (Lindner and Reidy, 1993). Moreover, FGFR is required for longitudinal visceral muscle fiber (LVFM) cells migration since FGFR expression as well as its downstream MAPK pathway are turned on when these cells migrate. Loss of FGFR results in the absence of LVFM migration and complete failure of longitudinal visceral fibers to forms whereas circular fibers are relatively undisturbed (Mandal et al., 2004). Silencing FGFR in human VSMCs reduced migration of these cells (Rauch et al., 2005). Other study showed the role of FGFR in VSMCs migration without activating MAPK pathway with increased Rap2 which regulates actin dynamic (Poling et al., 2011).

2.4.2.6 FGFR activation increases cancer aggressiveness

FGF/FGFR signalling has been associated to cancer development as well. FGFRs promote proliferation and migration of numerous cancer cell types (Coleman et al., 2014; Künstlinger et al., 2015; Parish et al., 2015). Genes encoding for FGFRs undergo a large panel of mutations and rearrangements in cancers (Parish et al., 2015). Mutational activation of FGFR2 was reported in endometrial cancer cells, and drug treatment against FGFR2 kinase activity decreases the migration and invasion of these cells (Kim et al., 2015). Interestingly, drug-mediated inhibition of FGFRs delocalizes FGFR1 and FGF2 from the plasma membrane to the cytoplasm and the nucleus. Moreover, this drug-induced membranous delocalization reduces invasion of pancreatic cancer cells (Coleman et al., 2014), as well as invasion of breast cancer cells (Chioni and Grose, 2012). Along the same line, nuclear accumulation of FGFR1 correlates with the ability of head and neck squamous carcinoma cells to escape from the primary tumour site, to invade stroma and migrate to distant sites (Nguyen et al., 2013). Interestingly, blockade of FGFR1 activity reduces cell growth and invasion (Nguyen et al., 2013). An increase in cell contact-dependent migration and invasion of colorectal cancer cells, triggered by Src-dependent FGF2/FGFR1 signalling pathway, has been reported (Knuchel et al., 2015). Finally, targeting FGFR activity by specific inhibitors blocks downstream signalling pathways, impairs proliferation and induces apoptosis of breast tumour cells (Dey et al., 2010). Cell migration and invasion has also been reported to be enhanced by FGF10/FGFR2 signalling in pancreatic cancers (Nomura et al., 2008). To sumerize, enhanced activity of FGFRs is generally associated to cancer cells aggressiveness.

2.5 Synergies between FGFR and N-cadherin in developmental processes

Thus, the two kinds of receptor (N-cadherin and FGFRs) are involved in the control of cell migration during normal development, and their deregulation is correlated with cancer progression. However, the crosstalk between N-cadherin and FGFR, as well as the combined effects of their deregulations, are barely understood, as developed below.

2.5.1 N-cadherin/FGFR cross-talk

Importantly, a synergistic action of N-cadherin and FGFRs was demonstrated in the regulation of the pluripotency of epiblast stem cell (Takehara et al., 2015), of ovarian cell survival (Trolice et al., 1997) and of osteogenic cell differentiation (Debiais et al., 2001). N-cadherin stabilizes FGFRs to maintain pluripotency of mouse epiblast stem cells (mEpiSC) upon FGF2 stimulation. Indeed, the overexpression of N-cadherin prevents the downregulation of FGFRs at the plasma membrane after FGF2 addition in mEpiSC cells (Takehara et al., 2015). Moreover, downstream effectors of FGFR signalling, such as ERK and Akt, are significantly activated in cells overexpressing N-cadherin. At the opposite, N-cadherin silencing accelerates FGFR degradation, suggesting that N-cadherin inhibits FGFR protein degradation (Takehara et al., 2015).

FGFR and N-cadherin are both involved in the survival of granulosa cells (GCs) and ovarian surface epithelial cells (ROSEs) in the rat gonad generative cycle (Peluso et al., 1996; Trolice et al., 1997). GC and ROSE cell death is regulated by growth factors including FGF, but depends also on cell surface N-cadherin (Trolice et al., 1997). Neutralization of N-cadherin by antibodies reduces the phosphorylation level of FGFR by 50% in GCs and ROSEs, even in the absence of FGF. Authors speculate that both homophilic N-cadherin binding and FGF/FGFR binding enhance FGFR Tyr phosphorylation, and that subsequent activation of FGFR triggers downstream signalling to prevent apoptosis of aggregated cells (Peluso et al., 1996; Trolice et al., 1997). It was further suggested that N-cadherin regulates FGFR phosphorylation by interfering with the capacity of FGF to bind.

The expression of a constitutively active form of FGFR increases cell-cell adhesion in human osteogenic cells (Debiais et al., 2001). Moreover, an increase of N-cadherin expression at cell-cell contacts occurs upon FGF2 treatment in a Src-dependent manner. Thus, activated FGFR reinforces N-cadherin osteoblastic cell-cell junctions. Interestingly, E-cadherin level in these cells was not affected by FGF2 treatment, showing the specific effect of FGF/FGFR complex activity on N-cadherin functions. From above studies, we appreciate the mutual crosstalk of FGFR and N-cadherin, involving the stabilization at the plasma membrane of these two proteins and leading to the reinforcement cell-cell contacts and FGFR signalling pathways.

2.5.2 N-cadherin/FGFR complex promotes axonal outgrowth

The Doherty's lab pioneered the study of the functional interaction between FGFR and N-cadherin during neurite outgrowth *in vitro* (Williams et al., 1994). Recombinant N-cadherin induces neurite outgrowth through the activation of signalling cascade (lipase/CAM kinase, MAP Kinases and PI3 kinase pathways) known to act downstream of FGFRs (Doherty et al., 2000; Williams et al., 1994). Moreover, the expression of DN FGFR inhibits axonal growth, as well as FGFR phosphorylation stimulated by N-cadherin (Brittis et al., 1996). Similarly, it has been reported a crosstalk between FGFR and immobilized N-cadherin or cadherin-11 to promote neurite outgrowth (Boscher and Mège, 2008). Indeed, biomimetic surfaces grafted with recombinant N-cadherin or cadherin-11 stimulates neurite outgrowth in the absence of FGF, and this response is severely reduced by FGFR inhibitor treatment. These observations suggested that FGFR activation is primordial for neuritogenesis induced by N-cadherin-mediated adhesion. Beside, FGFRs and N-cadherin are co-clustered at the surface of neuronal cells (Michelle A. Utton et al., 2001). These results suggest that FGFRs and N-cadherin interact at the cell membrane, triggering FGFR activation signaling.

2.5.3 Synergies between FGFRs and N-cadherin in cancer invasion and metastasis

2.5.3.1 N-cadherin/FGFR crosstalk exacerbates cancer cell dissemination

In lung cancer cells, FGFR expression is positively correlated with ZEB expression, a transcription factor promoting N-cadherin expression and inducing migratory, invasive cell properties (Gemmill et al., 2011; Ware et al., 2013). Direct evidence of a FGFR and N-cadherin complex formation came from biochemical analysis in breast cancer cells (Suyama et al., 2002). Moreover, N-cadherin prevents FGFR from undergoing ligand-induced internalization, which results in its stabilisation at the plasma membrane and ultimately in sustained FGFR signalling (Suyama et al., 2002). N-cadherin/FGFR signalling enhances ERK phosphorylation, leading to invasive phenotype development of tumor cells (Hulit et al., 2007). N-cadherin co-precipitates with FGFR in human pancreatic β-cells (Cavallaro et al., 2001). In the case of mice xenografted with human pancreatic cancer cells, inhibition of FGFR leads to decreased expression of N-cadherin and to the reduction of cancer cell motility. As a consequence, FGFR inhibition in pancreatic cancer cells reduces invasive properties and improves sensitivity to chimiotherapy (Taeger et al., 2011).

Several molecular aberrations have been implicated in the pathogenesis of pituitary tumours, among which the production of an alternative cytoplasmic isoform of FGFR4 which lacks most of the extracellular domain (Ezzat et al., 2006). Truncated FGFR4 expressing tumours show reduced membrane levels of N-cadherin. The pivotal role of N-cadherin as a mediator of cell growth was demonstrated by experiment using small interfering RNA. N-cadherin silencing promotes invasive

growth in xenografted mice (Ezzat et al., 2006). Interestingly, selective inhibition of FGFRs with PD173074 restores membrane distribution of N-cadherin, and significantly reduces the tumour growth and invasion, emphasizing the critical partnership of N-cadherin and FGFRs in promoting invasion in pituitary cancer development.

2.5.3.2 N-cadherin/FGFR and neoangiogenesis

N-cadherin and FGFRs also induced tumour angiogenesis (Presta et al., 2005). Knock down or drug inhibition of FGFRs perturb angiogenesis during tumour development (Murakami et al., 2008). Endothelial cells express both N- and VE-cadherins, and deletion of either of these cadherins leads to severe vascular defects in mice (Carmeliet et al., 1999; Giampietro et al., 2012). However, endothelial cells overexpressing N-cadherin (N cells) express higher levels of FGF2 than endothelial cells overexpressing VE-cadherin (VE cells) (Giampietro et al., 2012). In N cells, the phosphorylation of FGFR substrate 2a (FRS2a) and the MAPK pathway activation are increased in comparison with VE cells. Consistently, inhibition of FGF2/FGFR1 signalling strongly reduces migration of N cells.

As reported above, N-cadherin can interact with FGFRs in tumour cells, thus limiting its internalization and subsequently increasing its signalling activity (Hulit et al., 2007; Qian et al., 2013; Suyama et al., 2002). Surprisingly, VE-cadherin, but not N-cadherin, co-immunoprecipitates with FGFR1 in endothelial cell extracts, and authors suggested that VE-cadherin may antagonize FGFR activation. As a matter of fact, the higher level of FGFR signalling in N cells may parallel a higher migratory phenotype required for the sprouting and the elongation of newly vessels. In contrast, VE-cadherin, once engaged at cell-to-cell junctions, would promote vascular stability by limiting cell motility and growth, in accordance with observations made in transgenic mice. An inhibitory action of VE-cadherin on FGFR signalling may contribute to this process (Giampietro et al., 2012).

Another study reported that the attenuation of N-cadherin function in endothelial cells by antagonistic peptides results in the impairment of cadherin-mediated endothelial interaction and causes apoptosis (Erez et al., 2004). This effect appears to be mediated by FGFR signalling, since N-cadherin inhibitory peptides reduces FRS2 phosphorylation, and exogenous addition of FGF2 completely rescues the phenotype (Erez et al., 2004). Taken together, it is reasonable to believe that N-cadherin and FGFRs establish a reciprocal crosstalk to drive tumour angiogenesis.

2.5.3.3 Cadherins, FGFR and transmigration

The upregulation of N-cadherin to the detriment of E-cadherin observed in some invasive cancer cells could be correlated with the fact that the endothelium abundantly expresses N-cadherin. Thus, the E- to N-cadherin switch may facilitate the adhesion of cancer cells on endothelial cells required for transendothelium migration, as reported for melanoma cells (Qi et al., 2005). Interestingly, although

FGFR1 was reported to co-immunoprecipitate with N-cadherin in some breast cancer cells (Hazan et al., 1997) and to contribute with N-cadherin to mammary cancer cell metastasis (Qian et al., 2013). In contrast, FGFR1co-immunoprecipitates with VE-cadherin but not with N-cadherin in melanoma cancer cells undergoing transendothelial migration (Qi et al., 2005). Thus, a more complex communication between FGFR and subtype-cadherins would exist during cancer cell migration and transmigration.

2.5.4 Cross-talk between classic cadherins and RTKs in cancer

There are additional evidences concerning the specific crosstalk between RTKs and cadherins in the control of cell migration, metastasis and neoangiogenesis (Chiasson-MacKenzie et al., 2015; Mary Fedor-Chaiken et al., 2003; Lugo-Martínez et al., 2009; Mateus et al., 2007; Sasaki et al., 2013), or between VE-cadherin and VEGFR (Lampugnani et al., 2006).

2.5.4.1 E-cadherin and EGFR crosstalk

Like FGFR, EGFR is upregulated in numerous cancer cell types (Salomon et al., 1995). Many data argue for a cross-talk between the E-cadherin and EGFR pathways in the regulation of cancer cells growth (Chiasson-MacKenzie et al., 2015; Mary Fedor-Chaiken et al., 2003; Lugo-Martínez et al., 2009; Mateus et al., 2007; Sasaki et al., 2013). It has also been reported that E-cadherin binds to EGFR via its extracellular domain leading to a reduced EGFR activation (Al Moustafa et al., 2002). Moreover, activated receptor induces the endocytosis of E-cadherin, down-regulating E-cadherin levels at the cell surface and in AJs. E-cadherin mutations in gastric cancers, preventing its binding to EGFR, lead to an increased activation of the EGFR, and results in increased cell migration (Mateus et al., 2007). Other studies suggested that E-cadherin may stabilize EGFR at cell-cell contacts through modulation of the contractility of the cortical actomyosin network by involving Merlin/NF2 (Chiasson-MacKenzie et al., 2015). Importantly, EGFR does not associate with and is not activated by N-cadherin in epithelial cells (Mary Fedor-Chaiken et al., 2003), further supporting the hypothesis that there are specific functional interactions between cadherin subtypes and RTK members. It was reported this year an E-cadherin-based force transduction pathway triggering global changes in cell mechanics affecting also cell-ECM interactions (Muhamed et al., 2016). Interestingly, this pathway involves EGFR and a PI3K pathway. Altogether, these reports indicate that E-cadherin-mediated cell adhesion, EGFR related pathways and cell mechanics are linked. As cell mechanics has strong incidence on cell-cell and cell-ECM adhesions, this feedback loop may be of primary importance of the regulation of cell migratory behaviour.

2.5.4.2 VE-cadherin and VEGFR

In the same line, VE-cadherin was reported to bind to VEGFR2 and VEGFR3 at the plasma membrane of endothelial cells (Coon et al., 2015; Esser et al., 1998). The recruitment of VEGFR2 by VE-

cadherin activates the receptor, leading to the weakening of cell-cell contacts. As a consequence, the vessels barrier is more permeable and cell sprouting and migration are favoured (Esser et al., 1998). Dejana group studied the mechanism by which VE-cadherin regulates VEGFR2 signalling. When VE-cadherin is absent or not engaged at cell-cell contacts, VEGFR2 is endocytosed more rapidly and remains in endosomal compartments for a longer time (Lampugnani et al., 2006). At the opposite, pharmacological inhibition of VEGFR2 stabilizes endothelial barrier junctions maintained mainly by VE-cadherin accumulated at AJs. Src protein is involved in the phosphorylation of VEGFR induced by VE-cadherine (Pirotte et al., 2011) and Src inhibition produces the same result. These inhibitions lead to the reduction of extravasion and lung cancer metastasis (Weis et al., 2004).

2.5.5 Molecular bases of RTK/cadherin crosstalks

Possible regulation of N-cadherin availability at the cell surface

One way to tightly control N-cadherin activity and thus cell-cell adhesion is to regulate its abundance at the cell surface. N-cadherin recycling properties may allow the direct regulation of N-cadherin amount at the plasma membrane. The catenin p120 is acknowledged to stabilize N-cadherin at the cell-cell contacts by regulating its biogenesis (Davis et al., 2003; Reynolds and Roczniak-Ferguson, 2004) and/or preventing its internalization (Chen et al., 2003). Interestingly, p120 lacking cancer cells show more malignant and invasive proprieties (Reynolds and Carnahan, 2004). Besides, phosphorylation of p120 has pro-tumorigenic activity in renal and breast cancer (Kourtidis et al., 2015). Interestingly, phosphorylation of p120 by Src has been reported to prevent its interaction with N-cadherin cytoplasmic tail (Kourtidis et al., 2015).

3 THESIS OBJECTIVES

To summerize, existing literature reports a functional relationship between FGFR and N-cadherin, leading to neurite outgrowth, increased invasion of prostate cancer cells and malignancy of breast cancer cells. This suggests that N-cadherin and FGFR synergize to generate signals, which alter migratory and invasive behaviours. Moreover, a crosstalk between E-cadherin/ EGFR or between VE-cadherin/VEGFR, has been shown. These finding highlight the pivotal role of cadherin family and growth factor receptors in physiology and pathology, and that various cell type specific cadherin/tyrosine kinase growth factor receptor cross-talk to regulate these processes.

Even separated impacts of cadherins on growth factor receptors on these processes have been widely explored; little is known about effects of their combined activites on cell-cell adhesion and cell migration. Therefore, my thesis focuses on evaluating the synergistic activities of FGFR1 and N-cadherin in the regulation of cell-cell contact stability and on two modes of cell migration: individual and collective ones. The project aim to address the following issues: (1) The reciprocal consequences of FGFR1 and N-cadherin on the strength of N-cadherin mediated cell-cell junctions. (2) The impacts of FGFR1 on the mechanotransduction of N-cadherin mediated cell-cell contacts. (3) Effect of FGFR1 and N-cadherin crosstalk on single cell migration. (4) Effect of FGFR1 and N-cadherin crosstalk on collective cell migration. (5) Cellular pathways and signalling events regulating FGFR1-dependent N-cadherin adhesion and migration.

I developpe here below the notable results obtained during my thesis, which are presented in two manuscripts.

The first manuscript studied the synergistic functions between FGFR1 and N-cadherin in the regulation of cell-cell contacts and individual cell migration. We used the model of scaffold-migration in which individual cell, such as neuron or cancer cells, migrates over the surface of their neighbors, as developed above. Cell migration is regulated by the strength of cell-cell adhesion forming between these two cells. Too strong adhesion hinders the detachement from the support and too weak adhesion is not sufficient for inducing new attachment, which is essential for cell body translocation. In this model of migration, N-cadherin is frequently required for adhesion between the migrating cell and the scaffold cell.

Likely, because N-cadherin forms more dynamic and labile cell adhesions compared to one generated by E-cadherin. The role of FGFR in regulating cell migration through modulation of cell adhesion has not been shown. However, a direct interaction between FGFR and N-cadherin has been reported. Furthermore, cadherin was shown to stabilize FGFR at the cell surface decreasing its ligand-induced endocytosis. Interestingly, this effect was not observed in cells co-expressing FGFR with E-cadherin, suggesting a specific response of FGFR to N-cadherin. In the first place, we studied the impact of FGFR1 expression on the migration of single N-cadherin expressing cell over N-cadherin coated surface. In the second place, we evaluated adhesive properties of N-cadherin mediated junctions in the absence or presence of FGFR expression. We next studied the effect of FGFR1 expression on N-cadherin endocytosis, as a mechanism to modulate cell-cell contacts strength by regulating N-cadherin prevalence at the cell surface. Finally, we looked for a molecular explanation of N-cadherin endocytosis, focusing on p120 and Src involvement.

The second manuscript unraveled the crosstalk of FGFR and N-cadherin on collective cell migration using cell expansion method. Efficacy of collective migration depends on the coordination and cooperation betweens cells in the migrating cell sheet. Thus, cells move together by affecting the behaviors of each other by communicating through cell-cell contacts. The first work showed the regulation of FGFR1 on N-cadherin mediated cell-cell contacts. The second work studied the impacts of this regulation at a collective level as well as its consequence for the migration of C2C12 cell monolayers, which express endogenous N-cadherin. During collective migration, cells at the front are in contacts with the free space inducing them to spreads and somes adopt leader behaviors. Leaders cells drag and direct the whole sheet to move forward. Moreover, it has been shown that these cells express growth factor receptor and are sensitive to grow factor. Therefore, we inquired the differences of N-cadherin mediated cell-cell junctions behaviors at the front or at the back of the migrating cell sheet. We also studied the role of FGFR1 in directing the movement of C2C12 monolayer. In parallel, the impacts of FGFR1 on the cytoskeleton organization as well as N-cadherin bound proteins are analyzed.

For both works, we generated DsRed-N-cadherin, GFP-FGFR and DsRed-N-cadherin/GFP-FGFR stably expressing HEK cell lines or GFP-FGFR stably expressing C2C12 cells to easily follow the two proteins. Thanks to microfabrication technique, we were able to study two modes of cell migration on different protein-coated surfaces and spatial environements. This

technique is very reproducible and allows the control of initial condition as well as of the environment in which cells migrate.

During my thesis, i also participated in the project of P.O.Strale who studied “The formation of ordered nanoclusters controls cadherin anchoring to actin and cell-cell contacts fluidity”, which was published in the Journal of Cell Biology in July 20 2015. Moreover, with my director of thesis, we published a review about “N-cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations” in European Journal of Cell Biology in January 2016.

4 RESULTATS

Manuscrit 1

FGFR-N-cadherin cross talk and effect on cell-cell contact stability and migration

Thao Nguyen[1], Laurence Duchesne[2], Nicole Bogetto[1], Chandra Murade[1], Benoit Ladoux[1,2], René-Marc Mège[1]

[1]Institut Jacques Monod, CNRS, Université Paris Diderot, Paris, France; [2] Institut de génétique et développement de Rennes, Université de Rennes, [3]MechanoBiology Institute, Singapore.

Abstract

The cell adhesion protein N-cadherin (N-cad) initiates signal transduction by interacting with several signaling proteins such as Fibroblast Growth Factor Receptor (FGFR). However, the underlying mechanism and what are the consequences for the cell remain open questions. We show here that FGFR1 overexpression reduces the migration of N-cadherin expressing cells on recombinant Ncad-Fc coated surfaces. Both proteins are co-recruited at cadherin-mediated cell contacts supporting a crosstalk between N-cadherin and FGFR in regulating cell adhesion and migration. FRAP analysis of FGFR and N-cadherin at cell-cell contacts shows that the mobile fraction of both proteins is reduced when the two molecules are co-expressed, in particular FGFR dynamics closely follows that of N-cadherin with an increased immobile fraction at cell-cell contacts. Furthermore, N-cadherin stabilization at cell-cell contacts requires the FGFR activity. Cell surface biotinylation analysis showed that FGFR stabilizes N-cadherin at the cell membrane by decreasing its internalization. We confirmed a more important recruitment of N-cadherin at the cell-cell contact by FGFR, and this through a decrease of N-cadherin internalization using flow cytometry experiments. As a consequence, FGFR reinforces N-cadherin-mediated-cell-contact strength as shown by magnetic tweezer experiments. FGFR1 expression increases the recruitment at the cell membrane of the catenin p120, a well-known regulator of cadherin trafficking and decreases its phosphorylation. FGFR also triggers Src activation in N-cadherin-bound immunocomplexes. Both p120 and Src are

involved in the negative regulation of N-cadherin-mediated migration by FGFR1. Based on these findings we propose a positive feed-back loop between N-cadherin and FGFR at adhesion sites limiting N-cadherin-based single cell migration.

Introduction

Cell migration is a complex and central process of tissue organisation during embryogenesis, morphogenesis and wound healing. Dysregulation of cell migration is associated with numerous diseases such as congenital malformations, neurological disorders and cancers. We can distinguish two main modes of migration, depending on the substrate on which cells migrate. Most of cells during morphogenesis and cancer development migrate on, or through, an ExtraCellular Matrix (ECM) thanks to their integrin receptors (Huttenlocher and Horwitz, 2011). Alternatively, others as border cells in the *Drosophila* egg chamber (Zaromytidou, 2014), vertebrate neuronal precursors (Jossin and Cooper, 2011b; Luccardini et al., 2013) or cancer cells in some situations migrate directly on the surface of other cells using cadherins as adhesion receptors. Moreover, cells while migrating may remain associated to their neighbours inside sheets or cohorts cell and therefore move collectively (Arboleda-Estudillo et al., 2010; Cai and Montell, 2014; Dumortier et al., 2012). In this case, cell migration is narrowly regulated by cadherin-mediated cell-cell adhesion. As a result, changes in expression profile of cadherins and associated proteins have major impacts on cell migration during development (Fung et al., 2008; Hong and Brewster, 2006) and metastasis (Hazan et al., 2000; Kashima et al., 2003).

Cadherins, which are the intercellular homophilic ligands of *Adherens Junctions* (AJ), are involved in the cohesion and homeostasis maintenance of all solid tissues. This is particularly true for the Epithelial cadherin (E-cadherin) which is recognized as a tumor suppressor (Van Roy and Berx, 2008). Cadherins provide anchorage between neighboring cells thanks to their interaction with the contractile actomyosin network through the adaptor proteins catenins α, β and p120 (Buckley et al., 2014). The Neuronal form, N-cadherin, mediates however weaker cell-cell adhesion and has been associated to cell migration in a large range of tissues, in both physiological and pathological processes (Derycke and Bracke, 2004). During neural development in mice, N-cadherin ensures mild cohesion between neurons and radial glial cells allowing neurons to adhere and migrate on radial glial cells (Franco et al., 2011; Jossin and Cooper, 2011b). During this process, N-cadherin undergoes

active endocytosis maintaining proper cell surface levels and allowing the effective locomotion of neurons (Jossin and Cooper, 2011a). N-cadherin is also required for proper long distance migration and maintained polarization of tangentially migrating interneuron precursors (Luccardini et al., 2013). *In vitro*, N-cadherin has been long recognized as a stimulating substrate for neurite outgrowth (Bard et al., 2008; Boscher and Mège, 2008; Matsunaga et al., 1988; Williams et al., 1994). Two pathways have been involved in N-cadherin-induced neurite outgrowth: the mechanical coupling of adhesion sites to the actomyosin tread milling generating the traction forces necessary to propel the growth cones (Bard et al., 2008; Giannone et al., 2009) and the activation of an FGFR-dependent biochemical signalling cascades (Boscher and Mège, 2008; M A Utton et al., 2001; Williams et al., 2001, 1994).

FGFRs (Fibroblast Growth Factor Receptors) belong to the family of single pass transmembrane Receptors Tyrosine Kinases (RTK). FGFRs, activated by the binding of their cognate ligands FGFs trigger numerous intracellular signalling cascades orchestrating key cellular events during development and pathogenesis, including cell adhesion and migration (Lemmon and Schlessinger, 2011). In mice, the abnormal expression of FGFR1 disrupts the migration of epidermal cells from the primitive streak (Deng et al., 1994; Yamaguchi et al., 1994)(Ciruna et al., 1997), by regulating intercellular adhesion of progenitors (Ciruna and Rossant, 2001; Ciruna et al., 1997; Partanen et al., 1998; Yamaguchi et al., 1994). In *Drosophila*, the migration of tracheal cells requires FGFR signaling regulating dynamic cytoskeletal reorganizations(Chu et al., 2013; Lebreton and Casanova, 2016, 2014; Peterson and Krasnow, 2015). Dysfunctions of N-cadherin or/and FGFR induce pathological migrations that are most visible in the case of metastasis. Many types of cancer cells acquire motility and invasiveness by upregulating N-cadherin to the detriment of E-cadherin (Li et al., 2001) (Rieger-Christ et al., 2004) (Kolijn et al., 2015) (Nakashima et al., 2003; Taeger et al., 2011). Transfection of exogenous N-cadherin into breast cancer cells increases cell migration, invasion and metastasis (Hazan et al., 1997). FGF/FGFR signalling has been associated as well to migration of numerous cancer cells (Coleman et al., 2014; Künstlinger et al., 2015; Parish et al., 2015). FGF10/FGFR2 signalling enhances pancreatic cancers cell migration and invasion (Nomura et al., 2008). Interestingly, drug-mediated inhibition of FGFRs delocalizes FGFR1 and FGF2 from the membrane to the cytoplasm and the nucleus of pancreatic cancer cells (Coleman et al., 2014) and this delocalization reduces invasion.

While the impacts of FGFR and N-cadherin activities on cancer cells migration have been widely explored separately, little is known about their combined effects on cell adhesion and migration. Existing literature reports on a synergistic action between N-cadherin and FGFRs in the regulation of the pluripotency of epiblast stem cell (mEpiSC) (Takehara et al., 2015), ovarian cell survival (Trolice et al., 1997) and osteogenic cell differentiation (Debiais et al., 2001). The overexpression of N-cadherin in mEpiSC cells prevents the downregulation of FGFR at the plasma membrane after FGF2 addition (Takehara et al., 2015). The expression of a constitutively active form of FGFR increases the expression of N-cadherin reinforcing cell-cell adhesion in human osteogenic cells (Debiais et al., 2001). A functional relationship between FGFR and N-cadherin, leading to neurite outgrowth (Boscher and Mège, 2008; Williams et al., 1994) has been reported. FGFR and N-cadherin are co-clustered at the surface of neuronal cells (M A Utton et al., 2001). Moreover, the expression of a dominant negative FGFR inhibits axonal growth as well as FGFR phosphorylation stimulated by N-cadherin (Brittis et al., 1996). FGFR and N-cadherin are found in the same immunocomplex in breast cancer cells (Suyama et al., 2002). N-cadherin prevents FGFR from undergoing ligand-induced-internalization, resulting in FGFR stabilisation at the plasma membrane, and ultimately in sustained FGFR signalling (Suyama et al., 2002). In mice xenografted with human pancreatic cancer cells, inhibition of FGFR leads to the decreased expression of N-cadherin and to the reduction of cancer cell motility (Taeger et al., 2011). Altogether, these data suggest that N-cadherin and FGFR synergize to generate signals which alter migratory or/and invasive behaviours (Hazan et al., 2000; Lamszus et al., 2005).

To further study the reciprocal effects of FGFR1 and N-cadherin, we expressed both receptors in HEK cells and analyzed the consequences on the stability of the two receptors as well as on cell-cell adhesion and N-cadherin-dependent cell migration. Using a single cell migration test Ncad-Fc coated lines, we show here that FGFR1 expression reduces N-cadherin-dependent cell migration. FRAP experiments revealed a co-stabilization of the two proteins at the cell-cell contacts. We further show that this results in an increased association of cadherin complexes to the actomyosin network resulting in the strengthening of N-cadherin-mediated cell-cell contacts. We show next by biochemical analysis the direct interaction between FGFR1 and N-cadherin as well as the activation of the FGFR1 induced by N-cadherin-mediated adhesion. We then questioned the effect of FGFR1 on the regulation of N-cadherin membrane trafficking as a possible mechanism to regulate N-cadherin turnover at cell-cell contacts and the involvement of the catenin p120 and the kinase Src in this

regulation. Altogether these data support the hypothesis that FGFR1 activity strengthens N-cadherin mediated cell-cell adhesion, and as a result decreases N-cadherin-dependent single cell migration, because cells have more difficulty to destabilize the interaction with the substrate, required for efficient migration.

Results

FGFR1 expression inhibits N-cadherin-expressing cell migration on N-cadherin coated substrates

To mimic the migration of cells over other cells, we developed a model in which DsRed tagged N-cadherin expressing HEK cells (Ncad cells) were seeded on Ncad-Fc-coated stripes as described in Materials and Methods. To study the role of FGFR1 in the regulation of N-cadherin-dependent single cell migration, we studied the impact of GFP tagged Fibroblast Growth Factor Receptor 1 (FGFR1) expression on the migration of these Ncad cells (Ncad / FGFR cells). Ncad expressing and Ncad / FGFR1 coexpressing cells were seeded on Ncad-Fc-coated lines prepared by a microstamping method and then followed by videomicroscopy over 20 hours. The trajectories of single cells were then extracted by manual tracking and analysed thanks to ImageJ Track. Ncad cells were very mobile covering a total displacement of 900 µm over 20 hours (Fig. 1 A and B). They migrate at a speed of 29,9 ± 4.7 µm/h (Fig.1 C). In contrast, Ncad / FGFR cells migrate more slowly and some of them were totally immobile. The displacement of these cells was strongly reduced, and cells exhibited numerous stops in between migration phases (Fig. 1 A and B). Concordantly, the migratory speed of Ncad / FGFR cells (5.6 ± 2.8 µm/h) was drastically decreased compared to Ncad cells (Fig. 1 C). To determine whether these differences were directly related to FGFR1 expression and activity we further analysed the migration of Ncad / FGFR cells treated with FGFR kinase inhibitor (Fig.1 A-C). Treatment of Ncad / FGFR cells with the inhibitor restored migratory capacities similar to Ncad cells with a migration speed of 22.9 ± 1.3 µm/h, indicating that the inhibition of migration observed in cells expressing FGFR1 requires the activity of the receptor.

We then analysed the persistence of cell migration in each condition, defined as the ratio between *l*, the net displacement of a cell between the first and the last position of the

analysis, and *L*, the total distance covered by the cell during this period. This parameter reflects the ability of cell to maintain its direction of motion; it is inversely related to the inversion of migratory direction of the cells on the line. Ncad cells are rather persistent in their migration (Fig. 1C, middle). FGFR1 co-expression significantly decreased this persistence, an effect that was prevented by treatment with the FGFR inhibitor. Interestingly, the instantaneous velocity of Ncad cells increased continuously during the first seven hours of migration then reached a plateau and remained constant over time (Fig. S1). In contrast, instantaneous velocity of Ncad / FGFR cells continuously decreased over time, a trend that was reversed by treatment with the FGFR inhibitor. In agreement with the observed pauses of Ncad / FGFR cells and their reduced instantaneous velocity, this indicates that FGFR1 expression strongly impaired the migration of Ncad expressing cells on Ncad-Fc substrates in a process depending of its kinase activity.

FGFR1 decreases N-cadherin-mediated adhesion

We observed that Ncad / FGFR cells were much more elongated and spread on the lines than Ncad cells, with high presence of Ncad foci along the interface between cell and the substrate. Inhibiting the FGFR kinase alleviated these effects (Fig. S2A). Thus the reduced migration of these cells could be associated to an increased adhesion to the Ncad-Fc coated lines, by analogy with the reported inverse relation between the strength of integrin mediated adhesion of cells on fibronectin and their migration capabilities (Ouyang et al., 2013). The mean migratory speed of the cells was thus plotted as a function of cell spreading area for the three conditions (Fig. 1C). We found an inverse correlation between these two parameters: the more the cells spread on the substrate, the slower they migrate. Ncad cells clustered in a region of small cell area / high migratory speed, whereas Ncad / FGFR cells clustered in a region of the graph with high cell area/low migratory speed. Ncad / FGFR cells treated with FGFR inhibitor behave as Ncad cells. These results strongly suggest that FGFR decreases Ncad single cell migration on Ncad-Fc substrate, by modulating N-cadherin-mediated adhesion and spreading.

To confirm this effect on N-cadherin-mediated adhesion, we analysed the accumulation of DsRed-Ncad (N-cadherin fused to DsRed) at contact sites in clusters of HEK cells grown in regular fibronectin-dependent tissue culture conditions. Cell-cell contacts of

Ncad / FGFR cells appeared more robust and straight compared to those of Ncad cells and Ncad / FGFR cells + inhibitor (Fig. S2B). Analysis of DsRed-Ncad intensities at the cell-cell contacts confirmed a significant increase of the DsRed signal in Ncad / FGFR cells that was dependent on the kinase activity of the receptor. Interestingly, higher resolution imaging of cells migrating on Ncad-coated lines showed that Ncad cells detached and reattached frequently from/to the Ncad-Fc substrate. This was accompanied by the formation of blebs during the phases of low spreading and more efficient motion (Sup. Movie 1). At the opposite, Ncad / FGFR cells remained attached and rarely detached to/from the substrate (Sup. Movie 2). Inhibition of FGFR restored dynamic attachment/detachment of the cells to the Ncad-Fc substrate allowing Ncad / FGFR cell movement (Sup. Movie 3). These observations suggest that FGFR1 reinforces the adhesion of Ncad cells to the Ncad-Fc substrate to prevent their migration.

N-cadherin and FGFR1 are co-stabilized at cell-cell contacts

We hypothesized that FGFR1 increased N-cadherin-mediated cell-cell adhesion by affecting the dynamics of N-cadherin molecules at these sites. To test this hypothesis and at the same time follow the dynamics of FGFR1, we performed dual wavelength FRAP experiments at the cell-cell contacts of HEK cells expressing DsRed-Ncad or GFP-FGFR1 alone, or both molecules at the same time (Fig. 2A, example for DsRed-Ncad cells). When N-cadherin was expressed alone, we detected a mobile fraction at the cell-cell contacts of 61.3± 2.7 %, in agreement with previous reports (Lambert et al., 2007). The expression of FGFR1 significantly decreased the mobile fraction of N-cadherin (38.2 ± 3.4 %). The treatment with the FGFR kinase inhibitor restored levels (62.3 ± 2.3 %) found in DsRed-Ncad expressing cells (Fig. 2B).

In a reverse manner, N-cadherin co-expression decreased the mobile fraction of FGFR1 at cell-cell junctions, which was of 58.9 ± 2.3 % in the absence of N-cadherin and dropped to 44.0 ± 3.7 % when N-cadherin was expressed. To test whether this action on FGFR1 mobility at cell-cell junction was specific to N-cadherin, we co-transfected the GFP tagged receptor with E-cadherin fused to mCherry (mCherry-Ecad) in HEK cells. Importantly, E-cadherin expression did not affect the mobility of FGFR1 molecule at the cell-cell contacts (Fig. 2C). To further confirm the specific dialogue between FGFR1 and N-cadherin in the

regulation of their mobility at the cell-cell contacts, we also performed FRAP on E-cadherin in Ecad / FGFR coexpressing cells (Fig. 2D). The presence of FGFR1 did not modify the mobile fraction of E-cadherin detected at the cell-cell contacts. Thus, FGFR1 and N-cadherin specifically co-stabilize each other at the cell-cell contacts. This is in direct agreement with the increased recruitment of N-cadherin observed at cell-cell contacts in co-expressing cells and the increased spreading of these cells on the Ncad-coated lines.

FGFR strengthens N-cadherin-mediated cell-cell contacts

To further study the impact of N-cadherin stabilization at the cell-cell contacts induced by FGFR1, we disrupted N-cadherin mediated cell-cell contacts, by chelating Ca^{2+} ions by the addition of EGTA in Ncad, Ncad / FGFR cells cultured on fibronectin coated surfaces, and followed by live imaging the evolution of cell-cell contacts (Fig. 3). Ncad cells separated quickly form each other resulting in a dispersed distribution of round-shape cells after 2 minutes (Fig. 3A, upper panels). On the contrary, Ncad / FGFR cells remained attached together showing a dense cell layer at the same time scale. Inhibition of FGFR kinase activity in these cells increased cell dispersion (Fig. 3A, upper panels). At higher magnification, Ncad mediated cell-cell junctions appeared completely disrupted in Ncad monolayers after 2 min of treatment, in contrast to those of Ncad / FGFR cells which still remained robustly cohesive (Fig. 3A, lower panels). Ncad / FGFR cells treated with the FGFR inhibitor presented both intact and disrupted cell-cell contacts (Fig. 3A, lower panels). Thus, FGFR1 activity strengthens N-cadherin-mediated cell-cell contacts inside the monolayer as revealed by the resistance to EGTA-induced contact disruption. This result was confirmed by kymograph analysis (Fig. 3B) as well as quantitative analysis of cell-cell contact life-time following Ca^{2+} depletion (Fig. 3C). This FGFR1-induced resistance of N-cadherin-mediated cell-cell contacts upon Ca^{2+} chelation was abolished by inhibiting FGFR kinase function. Altogether, these results indicate that the kinase activity of the receptor is required for the enhanced resistance of N-cadherin mediated cell-cell contacts observed upon FGFR1 expression.

FGFR stiffens the anchoring of N-cadherin to actin network and increases the rupture force of N-cadherin mediated cell-cell contacts

The mechanocoupling between cadherin junctional complexes and the underlying actomyosin network is a major actor of cadherin downstream signalling leading to the reinforcement of cell-cell contacts (Mège et al., 2006). The analysis of retrograde flow of F-actin in the lamellipodia of cells spread on an adhesion molecule coated substrate has been shown to directly inform on the coupling of adhesion receptors to the actin treadmilling (Plestant et al., 2014a; Strale et al., 2015); a decreased tread milling speed correlating with an increased friction between the cytoskeleton flowing underneath the plasma membrane and the membrane-anchored adhesion site (Mitchison and Kirschner, 1988). To investigate the role of FGFR1 in the association of N-cadherin receptors to F-actin, we thus first quantified the actin retrograde flow in the lamellipodia of C2C12 myogenic cells spread on Ncad-Fc coated surfaces as described in (Plestant et al., 2014a). C212 cells which express endogenous N-cadherin were transfected with LifeAct-GFP, seeded on Ncad coated surface, treated or not with the FGFR inhibitor, then live imaged using spinning disk to visualize and quantify the actin retrograde flow (Fig. 4; Sup. Movie 4 and 5). The speed of the F-actin rearward flow was increased by 40% in cells treated with the FGFR inhibitor compared with cells treated by the vehicle alone, indicating that the coupling of N-cadherin adhesion complexes to F-actin is decreased after treatment with the inhibitor. At the opposite, the low speed of actin retrograde flow observed in untreated C2C12 cells reflects a stronger association of N-cadherin adhesion complexes to the actin network compared to C2C12 cells depleted in FGFR activity (Fig. 4B). This result suggests that in these myogenic cells, likely expressing endogenously an isoform of FGFR (Kontaridis et al., 2002), the FGFR activity constitutively stimulates the functional coupling of N-cadherin to the actin cytoskeleton.

The reinforced association between N-cadherin and the actin network as shown in C2C12 cells should increase F-actin recruitment at the N-cadherin-mediated adhesion. To test this hypothesis, we imaged doublets of Ncad or Ncad / FGFR HEK cells transfected with LifeAct-GFP seeded on fibronectin coated strips and analysed the F-actin as well as N-cadherin signal intensities along the cell, from junctional end to free end (Fig. 5). We observed by comparing Ncad / FGFR and to Ncad cells, that FGFR1 expression enhanced actin staining intensity at the junctional end (Fig. 5A). Moreover, the intensity curves of actin and N-cadherin displayed similar profiles in the case of Ncad / FGFR cells (Fig. 5B). These

observations support the role of FGFR1 in reinforcing N-cadherin and F-actin coupling at the cell-cell contacts.

To directly test whether this positive effect of FGFR1 on the mechanical coupling of N-cadherin to the underlying cytoskeleton had an effect on the strength of N-cadherin-mediated adhesions, we probed the response to force of Ncad-Fc-coated magnetic beads bound to Ncad or Ncad / FGFR cells, using magnetic tweezers. Beads were let to interact with the cell surface for 30 minutes. They were then probed for displacement under force or detachment by approaching the magnetic rod near each individual bead (Sup. Movie 6, 7; Fig. 6A). The semi-quantitative analysis of bead behaviour showed that beads adhesion was weaker on Ncad cells compared to Ncad / FGFR cells since more beads were displaced or detached from the cell surface as the magnetic coil was approached. Indeed, more than 80% of beads were moved or detached from the membrane for Ncad cells whereas less than 20% behave similarly for Ncad / FGFR cells. Moreover, the inhibition of the FGFR kinase activity restored bead detachment/displacement in proportions similar to those observed for Ncad cells induces beads detachment (Fig. 6B, middle).

For the population of beads that were detached under force, the distance between bead and the magnetic rod at which the bead was teared off the cell membrane (breaking distance) was recorded (Fig. 6B, left). The mean breaking distance was of 28,5 ± 0.9 µm for Ncad cells and 14.3 ± 0.6 µm for Ncad / FGFR cells, respectively. Inhibition of FGFR in Ncad / FGFR cells increases the breaking distance (21.4 ± 0.9 µm, Sup. Movie 8). As the magnetic force sensed by the bead is inversely and exponentially dependent of the distance to the magnetic rod (See Material and Method and Fig. 6A), one can extract after calibration the actual force at which the adhesions formed between Ncad-Fc beads and the plasma membrane of transfected cells was disrupted (Fig. 6B, right). Consistently, FGFR1 increased the disruption force of Ncad mediated cell-cell contacts from 5.9 ± 0.1 nN to 7.3 ± 0.1 nN. Furthermore, inhibition of the receptor activity significant attenuated this effect (6.5 ± 0.1nN), which proves that FGFR activity increases mechanical resistance of N-cadherin cell adhesions.

Altogether, these data indicate that FGFR1 activity is required for the increase in stability of N-cadherin complexes at cell-cell contacts, their coupling to the underlying cytoskeleton and finally the strength of the N-cadherin-mediated contacts induced by FGFR1 overexpression.

Direct interaction between N-cadherin and FGFR1 leads to sustained activation of FGFR1

The effect of FGFR1 overexpression on N-cadherin contact strengthening we described so far requires the kinase activity of the receptor although no exogenous FGF ligand was added. Furthermore, FGFR1 and N-cadherin are co-stabilized at the cadherin-mediated cell contacts. Therefore, we hypothesized that the increased residence of FGFR at cell-cell contacts induced by N-cadherin-mediated adhesion may directly induce the FGF-independent activation of the receptor as previously reported in neuronal cells (Boscher and Mège, 2008) This may rely on direct interaction of these two proteins. To confirm this hypothesis, we performed Biasensor experiments in which the captor surface was covered with the FGFR1 ectodomain of over which a solution of Ncad-Fc fragment was perfused (see Material and Methods). The Ncad-Fc molecule bound to the FGFR1 ectodomain with a Kd = 31 ± 5 nM (Fig. 7A). This direct interaction was confirmed by co-immunoprecipitation in extracts of HEK cells co-expressing the two proteins (Fig. 7B). Indeed, anti-GFP immunoprecipitation of the tagged receptor co-precipitated DsRed tagged N-cadherin. Interestingly, the co-precipitation of N-cadherin was strongly reduced when FGFR kinase activity was inhibited.

To further test whether this interaction may induce FGFR activation, we followed the phosphorylation of the tagged receptor by performing anti-phosphotyrosine western blotting following FGFR1 immunoprecipitation (Figure 7C). FGFR1 phosphorylation was significantly increased in Ncad / FGFR cells compared to FGFR1 only expressing cells. To further provide evidence that FGFR1 activation could be induced by N-cadherin-mediated adhesion, we performed Ca^{2+} switch in C2C12 cells and followed the activation of a well-known downstream target of FGFR activity: ERK1/2 (Fig. S3). As a control we showed that ERK1/2 phosphorylation was significantly increased in C2C12 cells extracts after treatment of the cells with FGF2 and that this phosphorylation was prevented in C2C12 cells pretreated with FGFR inhibitor (Fig. S3A). To test whether FGFR activation was dependent on N-cadherin engagement, we performed P-ERK1/2 immunoblotting in extracts of cells incubated with and without Ca^{2+} and FGFR inhibitor. Addition of 2 mM Ca^{2+} for 10 minutes on Ca^{2+} depleted C2C12 cells to trigger N-cadherin engagement significantly increased ERK1/2 phosphorylation. This Ca^{2+} switch induced ERK1/2 phosphorylation was not observed in the

presence of the FGFR inhibitor (Fig. S3B). Altogether, these observations strongly suggest that N-cadherin engagement at cell-cell contacts sustains the activation of the FGFR1.

In conclusion, our results show a two-way communication between FGFR1 and N-cadherin resulting likely from their direct interaction. The recruitment and stabilization of FGFR1 by N-cadherin at cell-cell contacts allow its activation. The activation of FGFR1 increases N-cadherin recruitment and stabilization at cell-cell contacts that strengthen cell-cell adhesion and reduce N-cadherin-dependent cell migration.

FGFR1 increases N-cadherin prevalence at the cell membrane through downregulation of N-cadherin endocytosis.

One way to increase N-cadherin activity and thus cell-cell adhesion is to regulate its abundance at the cell surface. To determine whether FGFR1 expression increases N-cadherin prevalence at the cell membrane of Ncad and Ncad/FGFR HEK cells, we performed biotin labelling of cell surface proteins followed by immunoblotting with anti-N-cadherin, with or without previous streptavidin immunoprecipitation, to reveal total and cell surface contents in DsRed-tagged N-cadherin, respectively. Anti-N-cadherin immunoblots revealed that the fraction of biotin-labelled N-cadherin, thus the fraction of N-cadherin present at the plasma membrane, was significantly increased in Ncad / FGFR cells compared to Ncad cells, and that this increase was prevented by treatment with the FGFR inhibitor (Fig. 8A). Therefore, the expression of FGFR1 significantly increases the fraction of N-cadherin accumulated at the plasma membrane in a process depending on its kinase activity.

Then, we questioned the role of FGFR1 on the regulation of N-cadherin endocytosis as a possible mechanism to regulate N-cadherin turnover at cell-cell contacts also using biotin surface labelling. Plasma membrane proteins were labelled with biotin, then endocytosis was allow to resume before cell surface exposed biotin were cleaved, while endocytosed biotin-labelled proteins were protected from cleavage. Quantitative analysis by Western blotting of uncleaved biotin-labelled N-cadherin and total N-cadherin in Ncad and Ncad / FGFR cells revealed that FGFR1 expression significantly decreasing the fraction of internalized N-cadherin (Fig. 9A, upper). In order to confirm that FGFR1 expression plays on N-cadherin endocytosis, we treated Ncad and Ncad / FGFR cells with hydroxyl-dynasore that inhibits

completely endocytosis process. As expected, N-cadherin levels found in the endocytosed fraction as well as in membranous fraction of Ncad cells and Ncad / FGFR cells are similar (Fig. 9A, lower). This result is in accordance with higher levels of N-cadherin at the plasma membrane in Ncad / FGFR cells, suggests that FGFR1 expression reduces the endocytosis rate of N-cadherin, leading to the stabilisation of N-cadherin-mediated cell contacts.

Next, we confirmed the inhibitory role of FGFR1 on N-cadherin endocytosis by combined flow cytometry and single cell imaging. Thanks to segmentation of the plasma membrane and internal part of the cells from the bright field images, we analysed the distribution at the plasma membrane and in the cytoplasm of DsRed-Ncad in 5 x 10^5 Ncad and Ncad / FGFR cells (Fig. 9B). The internalization score given by the signal intensity per surface unit in the internal part was of 1.32 and 1.09 for the Ncad cells and Ncad / FGFR cell populations, respectively. These data support the hypothesis that FGFR1 expression indeed decreases N-cadherin endocytosis.

Then, we analysed with the same approach the recruitment of N-cadherin at cell-cell contacts, by analysing this time doublets of Ncad or Ncad / FGFR cells (3 x 10^5 doublets for each condition) (Fig. 8B). The average normalized intensity per pixel of N-cadherin accumulation at the cell-cell interface in the doublet was of 331 U.I versus 251 U.I for Ncad / FGFR and Ncad cells populations, respectively) (Fig. 8C, upper). The analysis of bright detail intensities (BDI) considering only the intense foci of N-cadherin at the interface between the two cells revealed that N-cadherin was more recruited in clusters in Ncad / FGFR cells than in Ncad cells (Fig. 8C, lower).

These results indicate that the FGFR1 regulates N-cadherin prevalence at the plasma membrane through regulation of N-cadherin endocytosis and enhances N-cadherin trapping at the cell-cell contacts leading to enhance cell-cell contacts strength.

The catenin p120 is involved in FGFR1-dependent stabilization of N-cadherin mediated cell-cell contacts and inhibition of cell migration

The protein p120 is known to stabilize N-cadherin at the cell-cell contacts by directly linking to its cytoplasmic domain. This binding prevents the internalization of Ncad from the plasma membrane (Reynolds and Carnahan, 2004). Thus, we asked whether FGFR1

expression could affect the interaction between p120 and N-cadherin. The preliminary analysis of the distribution GFP-p120 in cell doublets seeded on fibronectin coated lines revealed that FGFR1 expression increased p120 recruitment at the cell-cell contacts (Fig. S4). Enhanced p120 recruitment could explain the enhanced stabilization of N-cadherin at the cell-cell contacts induced by FGFR1. To verify this hypothesis, we realized FRAP experiments on dsRed-Ncad at cell-cell contacts of Ncad / FGFR cells and DsRed-Ncad3A in Ncad3A / FGFR cells expressing. The Ncad3A mutant N-cadherin was described to be impaired for its binding to p120 (Chazeau et al., 2015).The recovery of fluorescence after photobleaching of N-cadherin in Ncad3A / FGFR cells was significantly increased compared to the recovery of N-cadherin in Ncad / FGFR cells (Fig. 10A). Ncad3A in the presence of FGFR1 displayed a fluorescence recovery similar to wild type Ncad in cells expressing N-cadherin alone (compare Fig. 10A and Fig.2B). These results suggest that the binding of N-cadherin to p120 is involved in the stabilization of N-cadherin at cell-cell contacts induced by FGFR1 expression.

To see whether this affects N-cadherin-mediated cell migration, we compared the individual cell migration of Ncad / FGFR and Ncad3A / FGFR cells on Ncad-Fc coated lines (Fig. 10 B, C). Ncad3A / FGFR cells migrated faster than Ncad / FGFR cells with a high migration speed at 45.0 ± 1.6 μm/h compared to 7.9± 5.1 μm/h for the latter's. Ncad3A / FGFR, which migrated without notable stops recovered the migratory capacity of Ncad expressing only cells (45.0 ± 1.9 μm/h). We appreciated as well the reverse correlation between migration speed and cell area: Ncad3A / FGFR cells had small spreading area and fast migration speed whereas Ncad / FGFR cells were characterized by a higher spreading area and a lower migration speed (Fig. 10B, right).

Thus, preventing the binding of N-cadherin to p120 strongly increases N-cadherin mobility at the cell-cell contacts leading to the higher capacity of migration on Ncad-coated substrates.

FGFR1 alters p120 phosphorylation independently of Src activation

Detachment of p120 from N-cadherin has been reported to induce internalization of the latter and the cytosolic translocation of p120 (Davis et al., 2003). Thus, we looked at the

distribution of N-cadherin and p120 in membranous and cytosolic subfractions of Ncad or Ncad / FGFR cell extracts using cell subfractionation methods (Fig. S5). Western blotting analysis revealed that N-cadherin was mainly found in cell membrane fraction in the presence and in the absence of FGFR1. However, a small fraction of N-cadherin was found in the cytosolic fraction in Ncad cells, which was not found when FGFR1 was expressed. In contrast, most of p120 was found in the cytosol, with only a small fraction associated to membranes. Interestingly FGFR1 expression decreased the level of cytosolic p120 in Ncad / FGFR cells compared to Ncad cells lacking FGFR1 (Fig. S5). Thus, these experiments revealed the presence of a cytosolic subfraction of N-cadherin and p120 molecules in Ncad cells that is strongly reduced by FGFR1 expression. Phosphorylation of p120 has been reported to induce its detachment from cytoplasmic tail of cadherins (Ireton et al., 2002; Roura et al., 1999). We thus analysed the phosphorylation state of p120 engaged in complex with N-cadherin in the presence or the absence of FGFR and FGFR inhibitor (Fig. 11A). FGFR1 expression strongly decreased the phosphorylation of N-cadherin-associated p120 in a process dependent on FGFR kinase activity.

As p120 is a substrate of Src kinase, itself a downstream target of FGFR, we also analysed the effect of a Src kinase family inhibitor on the p120 phosphorylation level in the presence of FGFR1 (Fig. 11A). Unexpectedly, the Src inhibitor significantly increased the level of p120 phosphorylation levels observed in untreated Ncad / FGFR cells. These results clearly show that p120 is phosphorylated neither by FGFR nor Src family kinase although both activities are involved in the regulation of p120 phosphorylation. However, Src may play an indirect role in the phosphorylation of p120 or more generally in response to FGFR1 activation.

Src is activated by FGFR1 expression and is involved in the negative effect of the receptor on N-cadherin mediated migration

Thus, we investigate the recruitment of Src to N-cadherin immunocomplexes with or without FGFR1 expression. Src was coimmunoprecipitated at similar levels independently of FGFR1 expression and activity (Fig. 11B). FGFR1 expression however leaded to an increase in Src phosphorylation of Tyrosine 418-situated in the catalytic domain of Src, and the inhibition of FGFR activity totally prevented this increase in phosphorylation (Fig. 11 B),

leading to the conclusion that FGFR activates Src. In order to determine the role of Src in the modulation by FGFR1 of N-cadherin-mediated cell migration, we submitted Ncad/FGFR cells to the single cell migration assays on Ncad-Fc-coated lines in the absence and in the presence of Src family inhibitor PP2 (Fig. 11 C). Inhibition of Src family restores migratory capability of Ncad/FGFR cells similar to the one of Ncad cells, indicating that Src inhibition prevented the negative effect of FGFR1 expression N-cadherin-mediated migration. Together, we conclude from these results that FGFR1 expression triggers the activation of Src and a decrease in phosphorylation of p120 that may both regulate the migratory response of the cells.

Discussion

N-cadherin facilitates cell migration in numerous physiological and pathological processes whereas FGFRs plays the role of both an enhancer or a repressor of cell migration depending of the context. Crosstalk between N-cadherin/FGFR, E-cadherin/ EGFR or VE-cadherin / VEGFR have been reported, suggesting that cell type specific cadherin/tyrosine kinase growth factor receptor cross-talks have more general impact during developmental and pathological processes (Nguyen and Mège, 2016). Although N-cadherin and FGFRs are up-regulated in many type of cancer cells associated to increased migration and invasion, the crosstalk between these two receptors in cell migration remains so far unclear.

In this work, we describe a new type of crosstalk between N-cadherin and FGFR1 regulating the dynamics and strength of cadherin-dependent cell-cell adhesion as well as cell migration. To mimic the N-cadherin-dependent migration of single neural and cancer cells over neighbouring cells *in vivo*, we studied the individual migration on N-cadherin-coated lines of N-cadherin or N-cadherin / FGFR expressing HEK cells. It is known for other adhesion receptors that cell migration is enhanced by mild adhesion between cellular adhesion receptors and adhesion proteins on the substratum allowing cell detachment/attachment, whereas a strong adhesion would act to the detriment for migration. Accordingly, we found an inverse correlation between N-cadherin-mediated cell migration speed and N-cadherin-mediated cell adhesion suggesting that the more cells adhere and spread, the less they migrate.

FGFR1 expression clearly increases adhesion of N-cadherin expressing cells to the Ncad-Fc substrate, decreasing cell migration. Cells expressing only N-cadherin show cyclic alternations of their adhesion, allowing them to migrate whereas cells also expressing FGFR1 cells remained spread on the cadherin-coated substrate. This difficulty of N-cadherin / FGFR cells to detach from the substrate is the result of strong N-cadherin adhesion between the cell and the substrate. Probing cadherin adhesion resistance to force thanks to magnetic tweezers indicates that the strength of N-cadherin contacts is indeed increased by FGFR1. In particular the rupture force of Ncad/Ncad trans interactions was estimated at 6 nN in the absence and increased up to 8 nN in the presence of FGFR1. These values are in the same range of that was estimated in Dufour's lab which is at 7.7 ± 1.4 nN for doublet of N-cadherin expressing cells formed after putting two single cells in contact for 30 minutes (Y.-S. S. Chu et al., 2004).

Through magnetic tweezers experiments also suggest that FGFR stimulates the

anchoring of N-cadherin to the actin network, this was confirmed by the increased F-actin recruitment at actual N-cadherin mediated cell-cell contacts and the higher resistance N-cadherin mediated cell-cell contacts upon Ca^{2+} chelation in the presence of FGFR1. It was also supported by the analysis of the actin retrograde flow in the lamellipodia of cells spread on N-cadherin which was reduced upon FGFR1 expression, denoting with an increased friction between the cytoskeleton flowing underneath the plasma membrane and the membrane-anchored adhesion site (Mitchison and Kirschner, 1988). The development of a E-cadherin-based Fret-sensor allowed to objectivise a pulling force applied by the actomyosin network on epithelial cell-cell junctions (Borghi et al., 2012). Pulling forces generated by the actomyosin cytoskeleton is resisted by an increased cadherin mechanosensitive coupling to the cytoskeleton; thereby increase in N-cadherin-actin coupling may trigger contacts separation at higher force (Y.-S. S. Chu et al., 2004; Martin et al., 2009). This could be the explanation of the enhanced strength of N-cadherin-mediated cell-cell contacts in FGFR expressing cells. .

FGFR1 stabilizes N-cadherin complexes accumulated at the cell-cell contacts as shown by the decrease N-cadherin mobility at the cell-cell contacts measured by FRAP. We do not know if FGFR1 regulates directly the “cis” or “trans”-clustering of N-cadherin that may affect its stability at the plasma membrane regulates the anchoring of N-cadherin to actin filaments or other unidentified regulatory processes (Kowalczyk and Nanes, 2012). However, all the action of FGFR1 requires the kinase activity of the receptor, meaning that it needs to be activated. This activation is initiated by N-cadherin itself and does not require exogenous FGF. We think that the increased prevalence of FGFR1 at the cell-cell contact membranes enhances its activation independently of FGF ligand. Interestingly, we provide evidence of a specific two-way communication between N-cadherin and FGFR1. Indeed, the dynamics of these two proteins follow closely each other and their mobility is decreased to the same level at cell-cell contacts of co-expressing cells. N-cadherin decreases FGFR1 mobility and *vice versa* reversely; this effect is specific for the complex N-cadherin / FGFR1 since it is not observed with E-cadherin. Moreover, our work shows a direct interaction between N-cadherin and FGFR1 through their extracellular domains. This may be essential for FGFR1 activation by increasing the density of receptors at cell-cell contacts. Thus, N-cadherin may induce FGFR1 activation independently of FGF binding, likely by increasing FGFR1 prevalence at the plasma membrane in cell-cell contacts.

In the other direction of the cross-talk, one way to control N-cadherin activity and thus cell-cell adhesion is to regulate its abundance at the cell surface. We observed that sustained activation of FGFR1 increases N-cadherin levels at the plasma membrane, and its recruitment at cell-cell contacts. Consequently, FGFR1 may also strengthen N-cadherin mediated cell-cell adhesion and render them more resistant against disruption by this mean. N-cadherin recycling may allow the direct regulation of N-cadherin quantity at the plasma membrane. Cell extract subfractionation suggested that FGFR1 decreases N-cadherin endocytosis. The catenin p120 is acknowledged to stabilize N-cadherin at the cell-cell contacts by regulating its biogenesis (Davis et al., 2003; Reynolds and Roczniak-Ferguson, 2004) and/or preventing its internalization (Chen et al., 2003). Binding to p120 has a stabilizing function because it masks a conserved dileucine motif in N-cadherin juxtamembrane domain that is necessary for endocytosis (Miyashita and Ozawa, 2007). We show that FGFR1 increases p120 recruitment at N-cadherin-dependent cell-cell contacts and contrarily decreases its cytosolic distribution. This is correlated with decreased levels of N-cadherin in the cytosol. FRAP analysis showed that N-cadherin binding to p120 is required for the stabilization of junctional N-cadherin by FGFR1. Moreover, N-cadherin unable to bind p120 does not respond to FGFR1 in term of migration on Ncad-Fc coated lines. These findings suggest that p120 is involved in the decreased endocytosis of N-cadherin triggered by FGFR1. We do not know however at this point if this phosphorylation of p120 causes its detachment from N-cadherin and thus induces N-cadherin endocytosis. Even if p120 was acknowledged as Src substrate, in our model, Src is not involved in the regulation of p120 phosphorylation and association to N-cadherin. However, both of Src activation and decrease of p120 phosphorylation are involves in the stability of N-cadherin mediated cell-cell contact and cell migration, independently.

Taken together, these data suggest the existence of a signaling pathway controlled by FGFR1 and N-cadherin to regulate cadherin-dependent cell-cell adhesion and cell migration. FGFR1 and N-cadherin are co-recruited and co-stabilized, increasing their abundance at the cell-cell adhesions. This leads to sustained activation of FGFR1, which in turn strengthens N-cadherin, mediated cell-cell contacts. Adhesion between cells and N-cadherin expressing substrate is increased therefore decreasing cell migration (Fig. 12) . This decreased adhesion could be used by cancer cells to engraft to the vessel wall and to the host tissue, thus may enhance the invasion.

Figure 1

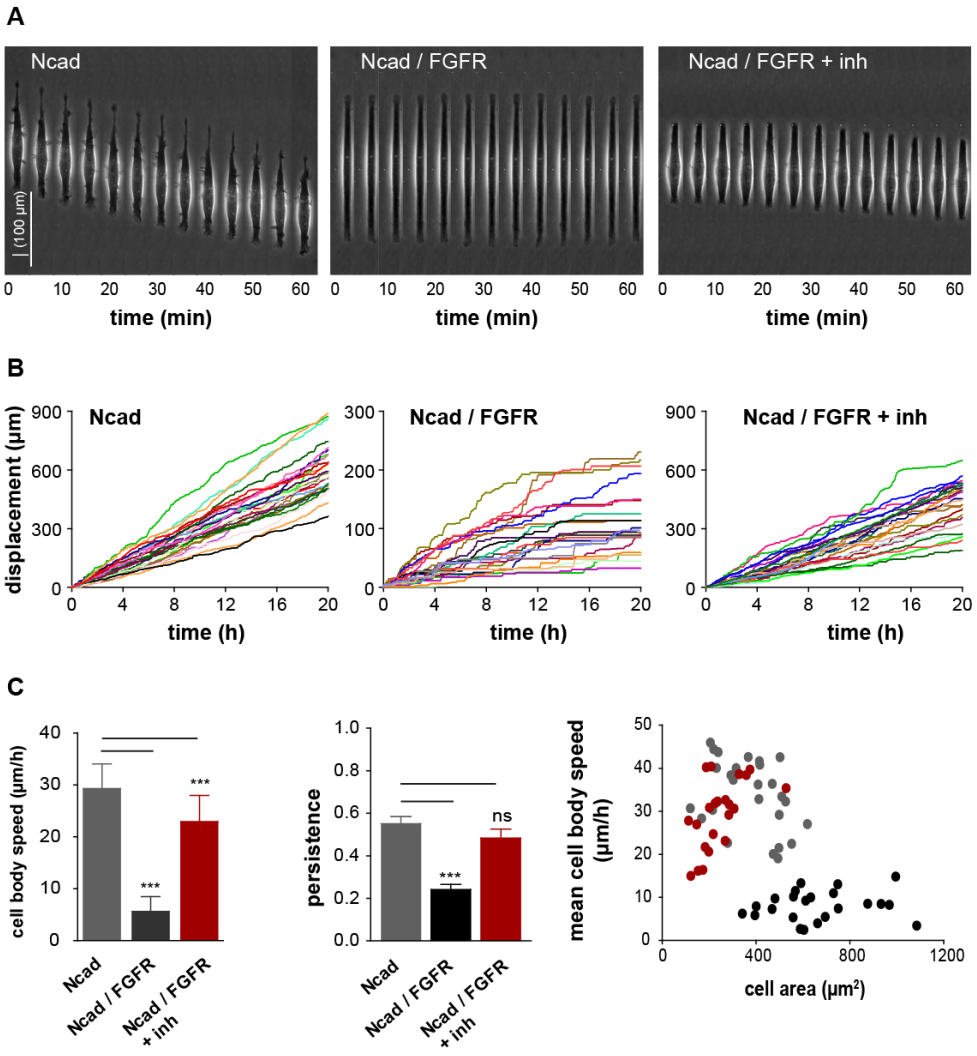


Figure 1: FGFR decreases the migration of individual N-cadherin expressing cells on N-cadherin coated surfaces. DsRed-Ncad (Ncad cells) or DsRed-Ncad / GFP-FGFR coexpressing HEK cells (Ncad / FGFR cells) were imaged in phase contrast every 6 minutes during 20 hours. (A) Example of 1 hour long cell displacements on Ncad-coated substrates for Ncad , Ncad / FGFR cells and Ncad / FGFR cells treated with 20 nM of FGFR inhibitor (Ncad / FGFR + inh). (B) Plots show the displacement in function of time for Ncad (left), Ncad / FGFR (middle) and Ncad / FGFR + inh cells (right) with n = 26, n= 22, n = 25 cells, respectively. Single cell tracking was performed over 20 hours. Ncad cells moved quickly and continuously. Ncad / FGFR cells had a decreased displacement and displayed numerous stops. Inhibition of FGFR in Ncad / FGFR cells restored their capacity of migration. (C) Histograms show the mean speed (left), persistence (middle) and mean speed in function of cell spreading area (cell area) (right) for Ncad, Ncad / FGFR and Ncad / FGFR + inh cells. Ncad / FGFR cells migrate more slowly and with a lower persistence thanNcad cells. Inhibition of FGFR in Ncad / FGFR cells restored partially the migration speed and persistence. The migration speed and the cell area are inversely correlated. (***, $p \leq 0.0001$, Anova multi-comparison test, Newman-Keuls post-test).

Figure 2

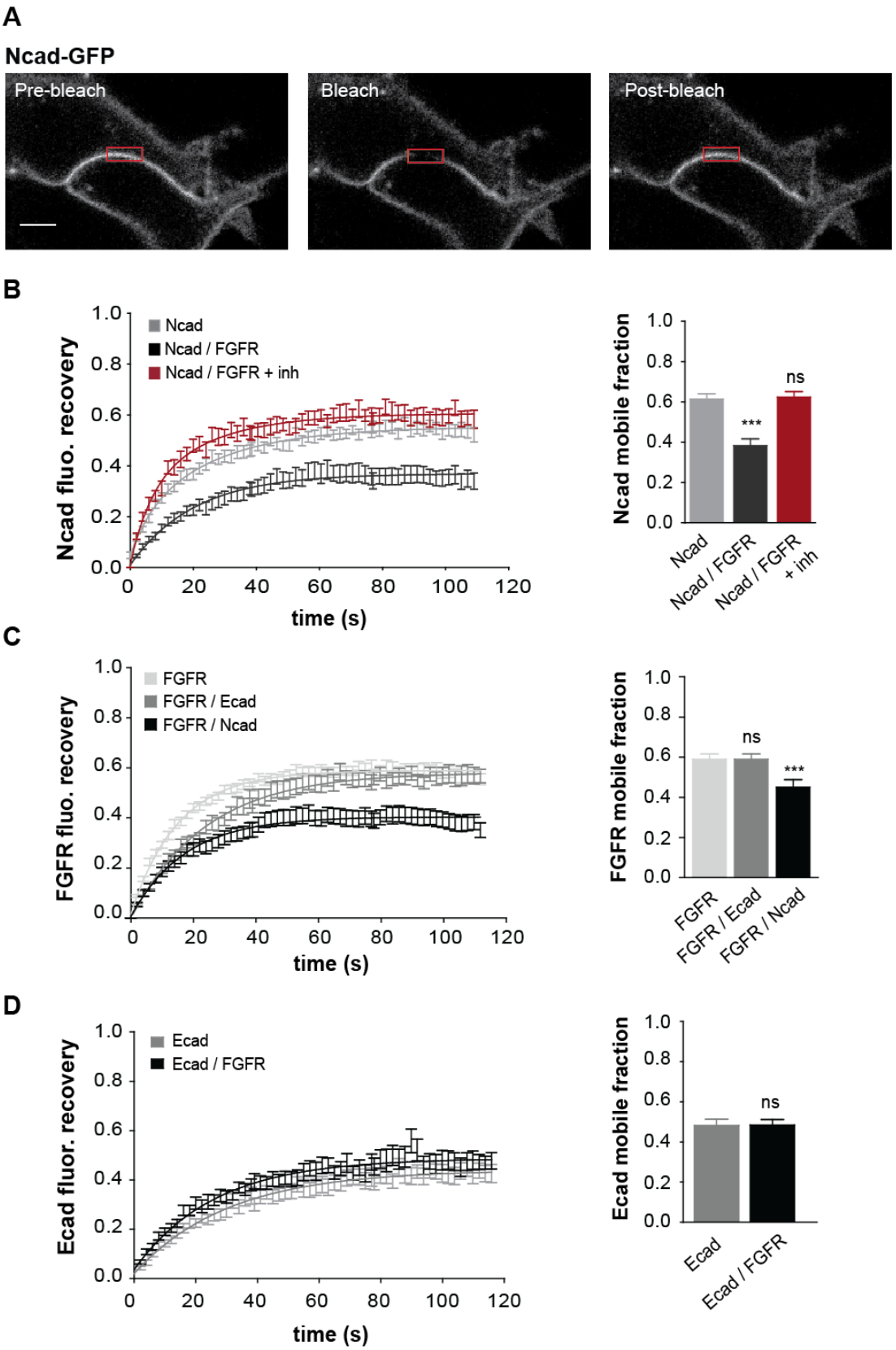

A
Ncad-GFP
Pre-bleach
Bleach
Post-bleach
B
Ncad fluo. recovery
Ncad
Ncad / FGFR
Ncad / FGFR + inh
time (s)
Ncad mobile fraction
***
ns
Ncad
Ncad / FGFR
Ncad / FGFR + inh
C
FGFR fluo. recovery
FGFR
FGFR / Ecad
FGFR / Ncad
time (s)
FGFR mobile fraction
ns
***
FGFR
FGFR / Ecad
FGFR / Ncad
D
Ecad fluor. recovery
Ecad
Ecad / FGFR
time (s)
Ecad mobile fraction
ns
Ecad
Ecad / FGFR

Figure 2: FGFR and N-cadherin co-stabilize at cell-cell contacts. (A) Representative images of FRAP experiments: DsRed-Ncad signal before (Pre-bleach), immediately after bleaching (Bleach) and 110 sec after the bleach (Post-bleach) performed at the cell-cell contacts of DsRed-Ncad HEK cells. Red squares represent the bleached region. Scale bar: 40 µm. (B) (Left) DsRed-Ncad normalized fluorescence recovery curves for Ncad (grey), Ncad / FGFR (black) and Ncad / FGFR + inh (red) cells ($n \geq 20$). (Right) Colum graph (mean ± SEM) shows the mobile fraction of Ncad molecule at cell-cell contacts. *** $p \leq 0.001$; ns: non-significant, Anova multiple comparison test, $n \geq 20$). (C) (Left) Normalized fluorescence recovery curves for GFP-FGFR molecules at cell-cell contacts in FGFR (bright grey), FGFR / Ecad (gray) and FGFR / Ncad (black) cells ($n \geq 15$). (Right) Colum graph (mean ± SEM) shows the mobile fraction of FGFR molecules at cell-cell contacts. *** $p \leq 0.0001$; ns: non-significant, Anova multi-comparison test, $n = 14$). (D) (Left) Normalized mCherry fluorescence recovery curves at cell-cell contacts in mCherry-Ecad (grey) and mCherry-Ecad / FGFR (black) cells, respectively ($n \geq 20$). (Right) Colum graph (mean ± SEM) shows the mobile fraction of mCherry-Ecad molecule at cell-cell contacts. ns: non-significant, student t-test, $n = 18$).

Figure 3

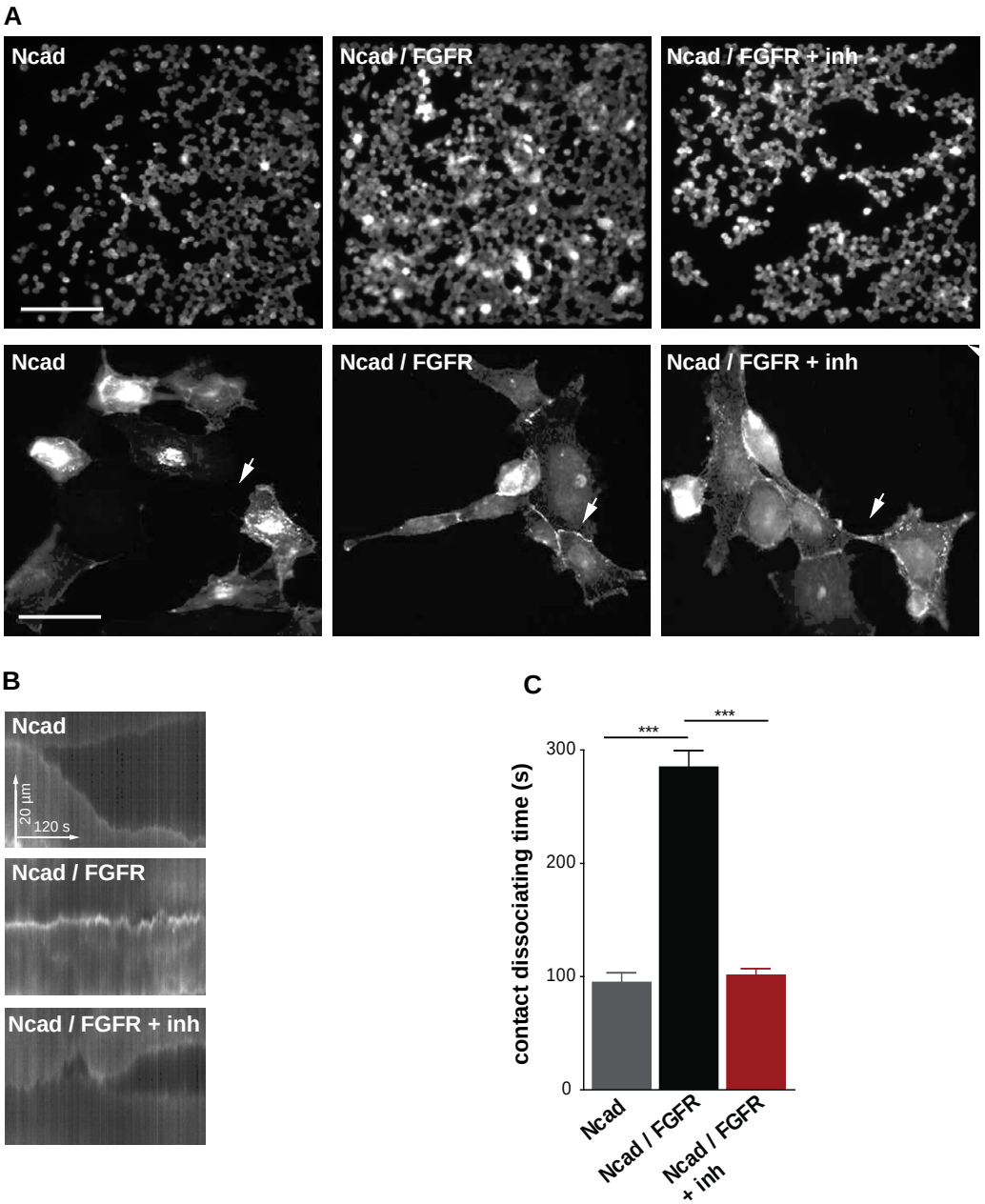


Figure 3: FGFR enhances the resistance of N-cadherin mediated cell-cell contacts. Ncad, Ncad / FGFR, Ncad / FGFR + inh cells were seeded on 200 µm^2 square surfaces coated with fibronectin. (N-cadherin was tagged with DsRed). At confluence, cells were treated with 20 mM EGTA then imaged every 30 seconds during 15 minutes. Scale bar = 40 µm. (A) Still images of cells in different conditions at 20X (upper lane) and 60X magnification (lower lane) after 5 minutes of EGTA treatment. Arrows indicate cell-cell contacts (B) Kymographs of DsRed-Ncad mediated contacts of Ncad, Ncad / FGFR and Ncad / FGFR + inh cells upon EGTA treatment. (C) Histogram shows estimated contacts life-time in different conditions. ** $p \leq 0.01$; *** $p \leq 0.001$, Anova multi comparison test, Newman-Keuls post-test, n = 60 contacts. FGFR increases the resistance of N-cadherin mediated cell-cell contacts against EGTA-induced disruption.

Figure 4

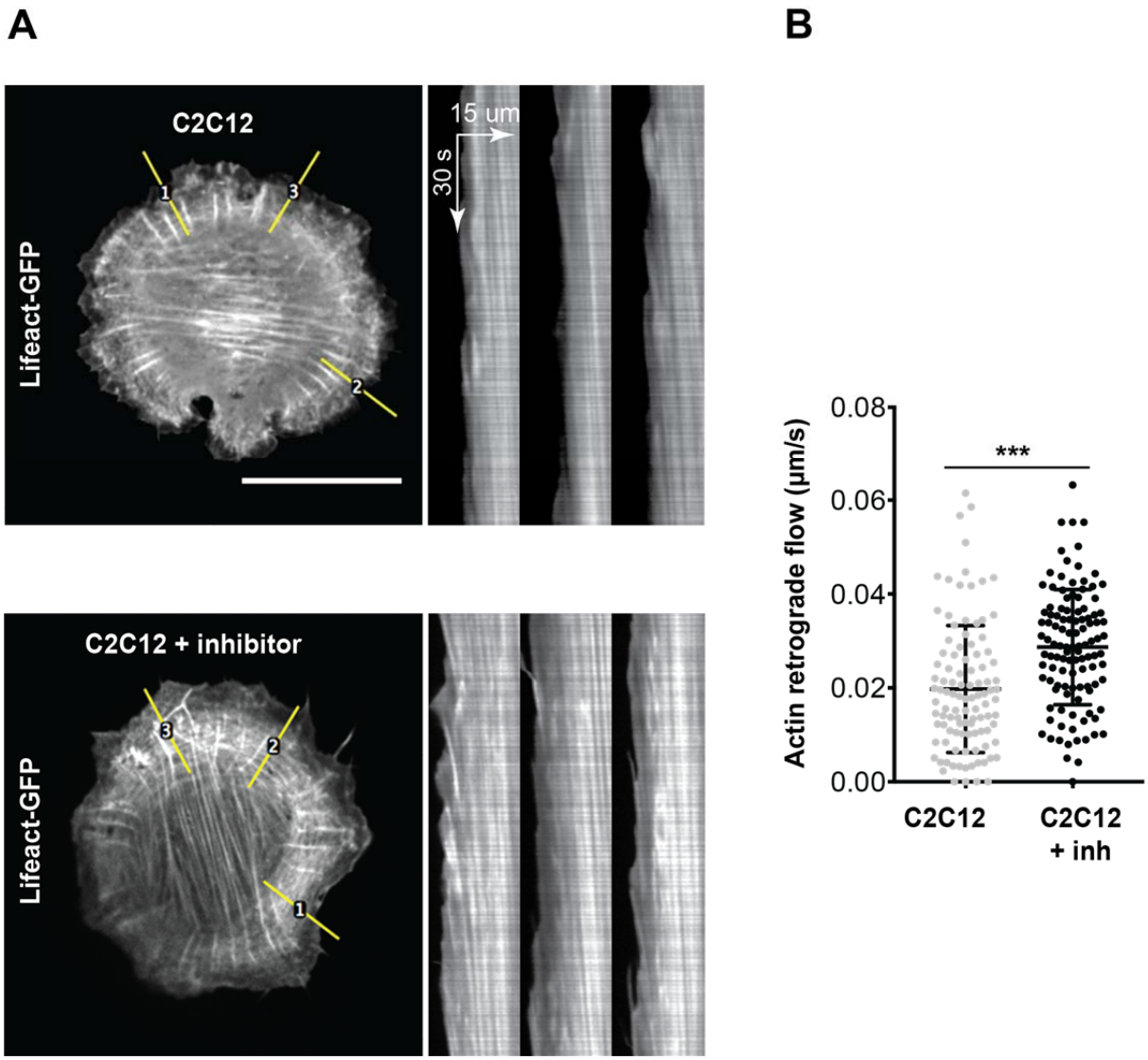


Figure 4: FGFR decreases actin retrograde flow in C2C12 cells spread on Ncad-Fc substrates. LifeAct-GFP expressing C2C12 cells were seeded on Ncad-Fc substrates for 2 hours, treated with DMSO or FGFR inhibitor for 1 hour, and then subjected to spinning disk live-cell imaging for 5 minutes at a frequency of one image per 30 seconds. (A) (Left) Still Images of LifeAct-GFP distribution. Bar = 20 µm. (Right) The actin retrograde flow was quantified by kymograph analysis (yellow lines 1–3, 2 pixel width, perpendicular to the cell membrane in Ncad dense region). (B) Histogram shows the means of actin retrograde flow speed for C2C12 + DMSO (n = 140 kymographs from 24 cells) and C2C12 + inh (n = 156 kymographs from 25 cells) cells. The actin retrograde flow was significantly faster in C2C12 + inh cells than in C2C12 cells (**** $p \leq 0.0002$, Student's t test).

Figure 5

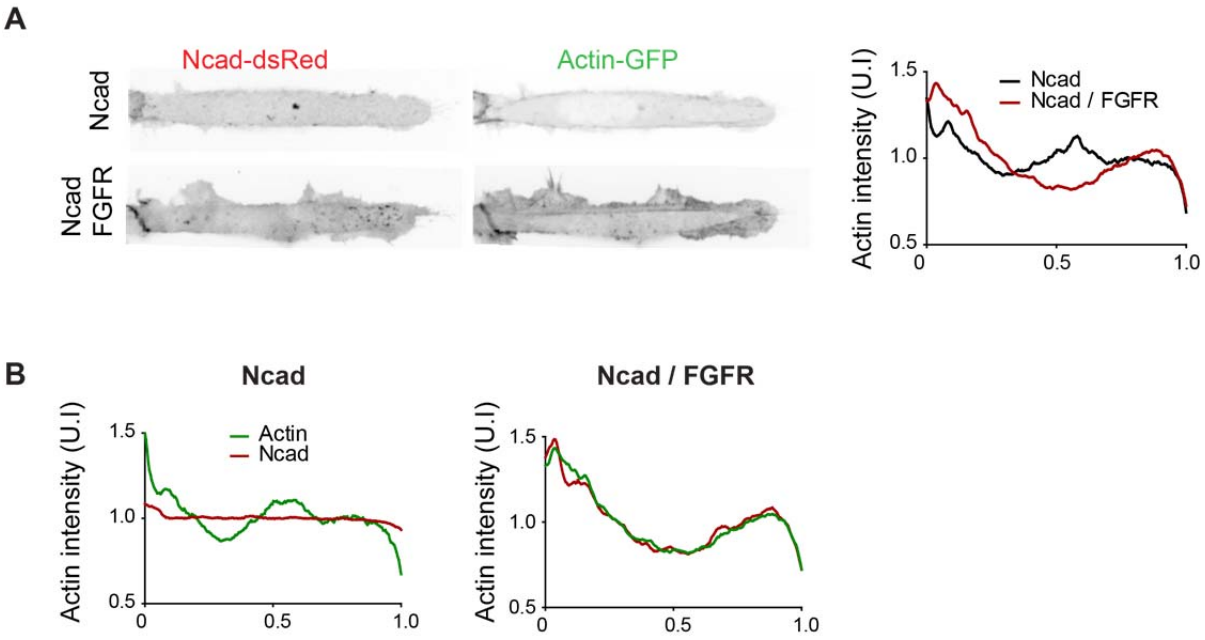


Figure 5: FGFR promotes N-cadherin and F-actin recruitment at the cell-cell contacts. DsRed-Ncad and DsRed-Ncad / Flag-FGFR stable HEK cell lines were transfected with GFP-actin and seeded on fibronectin coated stripes. Only doublets of cell were imaged. Projected GFP-actin and DsRed-Ncad intensity on the y axis were calculated by Matlab and plotted versus cell length (x axis) with 0 value defined as junctional end and 1 value as free end. (A) (Left) Still images of GFP-actin and DsRed-Ncad in one cell of the doublet. (Right) Graph shows actin intensity versus cell length in Ncad and Ncad / FGFR cells. FGFR increased accumulation of actin at the cell-cell contact. (B) Supperposition of Ncad and actin intensity along the cell length in Ncad (left) and Ncad / FGFR (right) conditions.

Figure 6

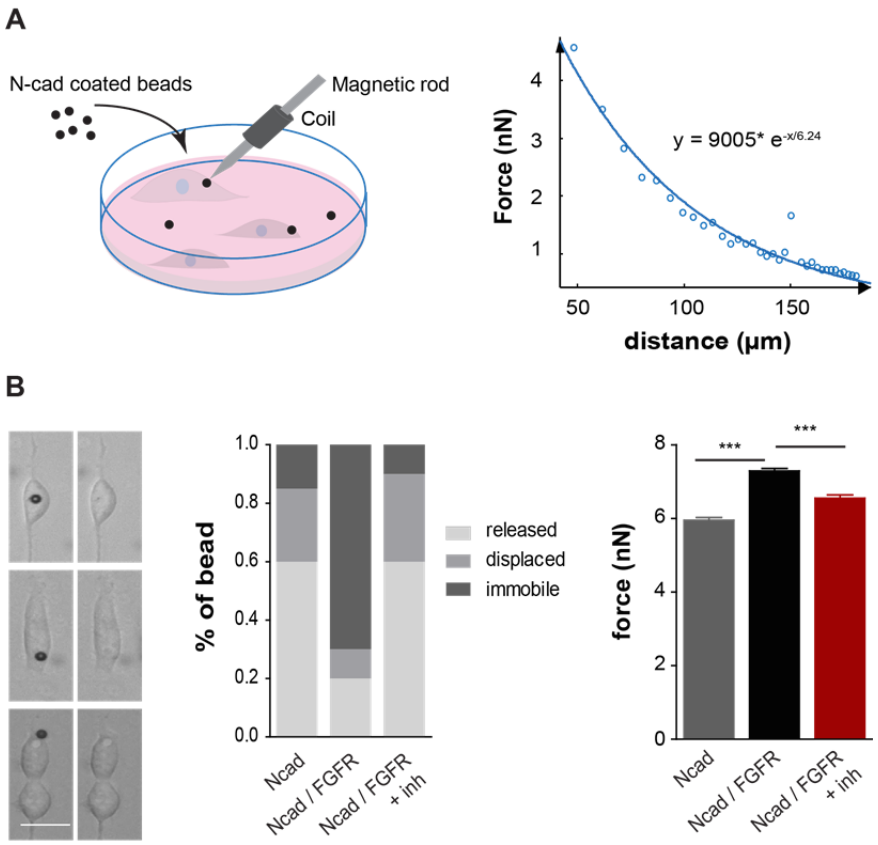


Figure 6: FGFR reinforces N-cadherin anchoring to actin network strengthening N-cadherin mediated cell-cell contacts. (A) Magnetic tweezers experimental set up and calibration. (Left) Set up. A pointed-iron tip was wrapped with a copper coil under tension to generate a magnetic field. The tension was controlled through a LabVIEW interface. The positioning of the tweezers was further controlled by a micromanipulator that allowed translational movement across all three axes with nanometer precision to position the magnetic field in the vicinity of beads. Ncad or Ncad / FGFR HEK cells were incubated with 4.5 µm magnetic Ncad-Fc coated beads for 30 minutes, then unbound beads were washed out. Cells and the moving tip were imaged in phase contrast every 10 milliseconds during 2 minutes. (Right) Graph of calibration of magnetic tweezers. 4.5 µm beads were mixed with PEG Mn700 (η = 25 Pa.s at 25°C) and processed to the same acquisition as mentioned above. Beads were tracked during acquisition time using ImageJ tracking. The force applied to a magnetic bead was calculated respecting Stoke equation $\upsilon = F/6\pi\eta r$, where η is the dynamic fluid viscosity and r is the radius of the bead. The disruption force of cell-bound beads is exponentially inverse correlated with the breaking distance. (B) Ncad mediated cell-cell contacts disruption force in Ncad, Ncad / FGFR and Ncad / FGFR + inh cells. (Left) Illustrations of beads before and after detachment from the cell membrane. (Middle) Distribution in three classes (release, displacement, and immobility) of responses of Ncad coated beads bound to the magnetic field for Ncad (n=60), Ncad / FGFR (n=65) and Ncad / FGFR + inh (n=50) cells. (Right) Calculated (based on Stoke equation) disruption forces for Ncad bead cell adhesions, , for Ncad, Ncad / FGFR and Ncad / FGFR + inh HEK cells. *** $p \leq 0.001$, (Anova multi comparison test, Newman-Keuls post-test).

Figure 7

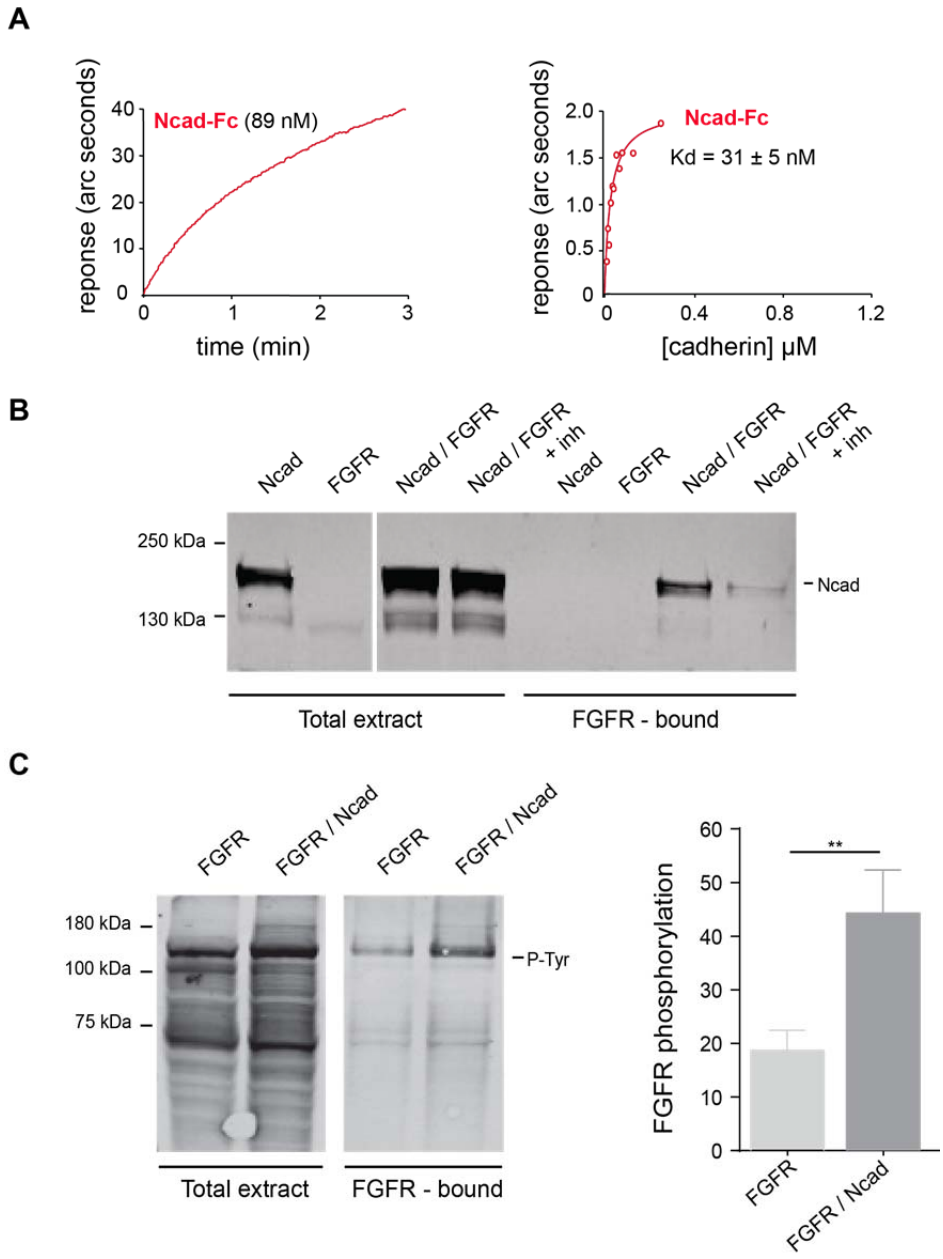


Figure 7: N-cadherin and FGFR associate directly leading to increased activation of FGFR. (A) Optical biosensor experiments were performed using recombinant extracellular domain of FGFR1 immobilized on gold surface. The subsequent addition of hNcad-Fc induces refractive index changes. Graphs show characteristics of the interaction between Ncad and FGFR. (B, C) Immunoprecipitation of tagged FGFR in cell extracts followed by immunobloting of showing the interaction between Ncad and FGFR. FGFR-GFP tagged proteins were immuno-precipitated from soluble cell lysates using GFP-Trap. Extracts from GFP or Ncad-dsRed transfected HEK cells served as controls. Input and FGFR-GFP bound proteins were subjected to Western Blot analysis to detect Ncad (B) or P-Tyr (4G10 antibody) (C). Histogram in C shows the percentage of phosphorylation of FGFR in the different extracts. *p≤0.1; ** p≤0.01; ns: non-significant, Student's *t* test.

Figure 8

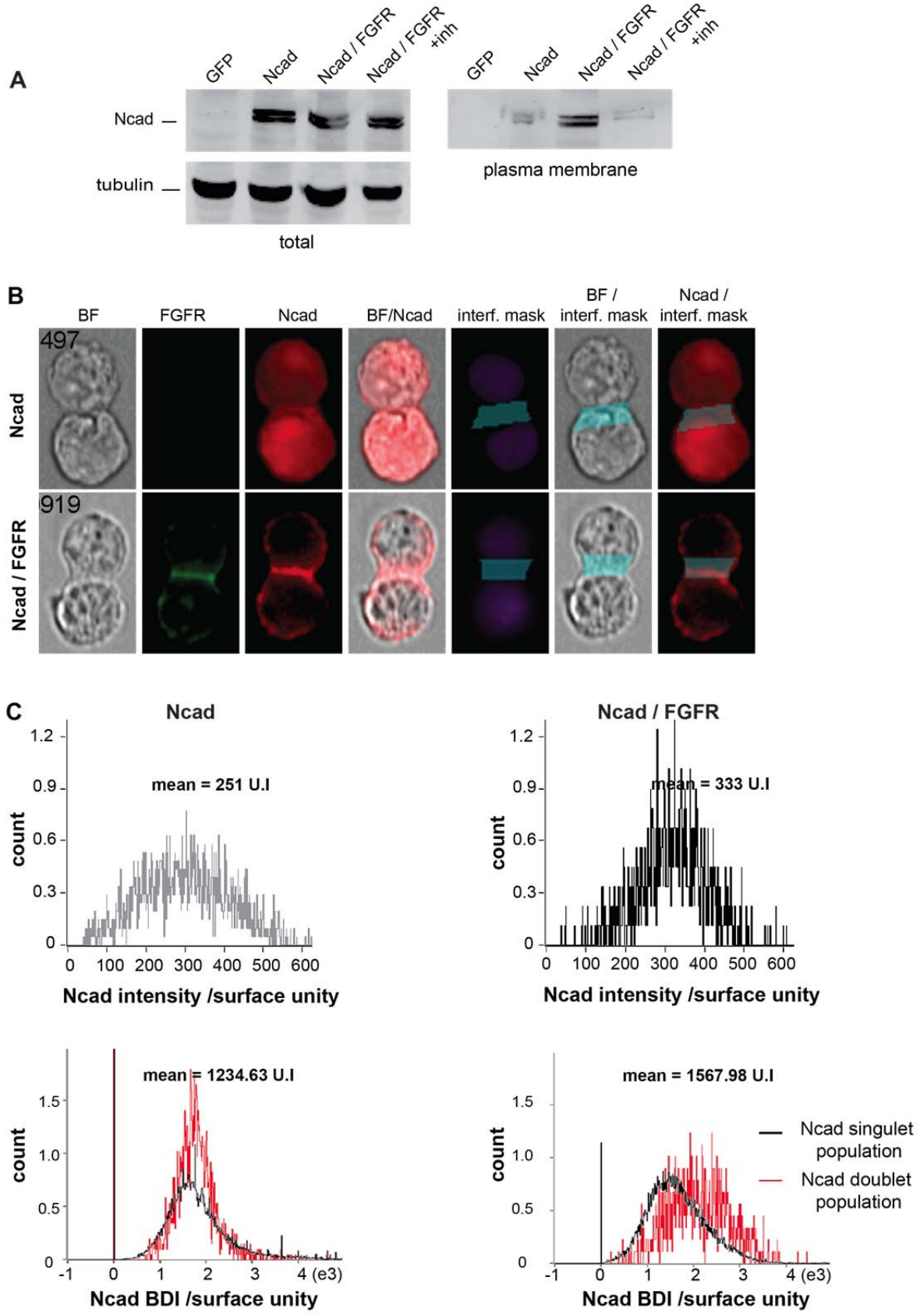

A
GFP
Ncad
Ncad / FGFR
Ncad / FGFR +inh
Ncad
tubulin
total
plasma membrane
B
BF
FGFR
Ncad
BF/Ncad
interf. mask
BF / interf. mask
Ncad / interf. mask
Ncad
Ncad / FGFR
497
919
C
Ncad
mean = 251 U.I
count
Ncad intensity /surface unity
Ncad / FGFR
mean = 333 U.I
mean = 1234.63 U.I
mean = 1567.98 U.I
Ncad singulet population
Ncad doublet population
Ncad BDI /surface unity

Figure 8: FGFR increases N-cadherin recruitment at the cell-cell contacts. (A) Plasma membrane separation using surface labeling technique. After surface biotynilation, and removal of unfixed biotin, cells were immediately lysed and subjected to biotin precipitation by streptavidin. GFP transfected HEK cells were used as control. Total extracts and plasma membrane fractions were immunoblotted with anti-N-cadherin antibodies. FGFR expression enhanced the recruitment of N-cadherin at the plasma membrane. (B+C) Analysis of Ncad recruitment at cell-cell interface by flow cytometry. DsRed-Ncad and DsRed-Ncad / GFP-FGFR HEK cells were mildly detached using non-enzymatic buffer, fixed, stained with Dapi then processed to cytometry analysis. Cells were imaged in Bright field, Dapi (405 nm wave length), Ncad-dsRed (560 nm wave length) and FGFR-GFP (480 nm wave length). Experiences were repeated 4 times, over populations of 150.000 cells for each condition in each experiment. (B) Determination of mask for calculating DsRed-Ncad recruitment at the cell-cell contacts. Firstly, the 4 pixels interface mask was determined as a region centered at the dimmest pixel between the 2 nuclei (bleu). The interface mask was applied to the bright field channel to determine the surface area of the cell-cell contact, then to the fluorescence channel to count the intensity of DsRed-Ncad at the cell-cell contacts. (C) (Upper) DsRed-Ncad recruitment was determined by the rapport between the Ncad fluorescence intensity and the surface area of cell-cell contacts. FGFR increased the Ncad recruitment at cell-cell contacts (215 U.I versus 333 U.I). (Lower) The puncta Ncad accumulation at the cell-cell contact was determined by a feature of IDEAS analysis assessing for the intensity of bright spots that have radii of 4 pixels after neglecting background staining, called bright detail intensity (BDI). The BDI of single cells population was used as control. FGFR increased the formation of Ncad foci at the intercellular junction compared to cell membrane (1567.98 versus 1234.63).

Figure 9

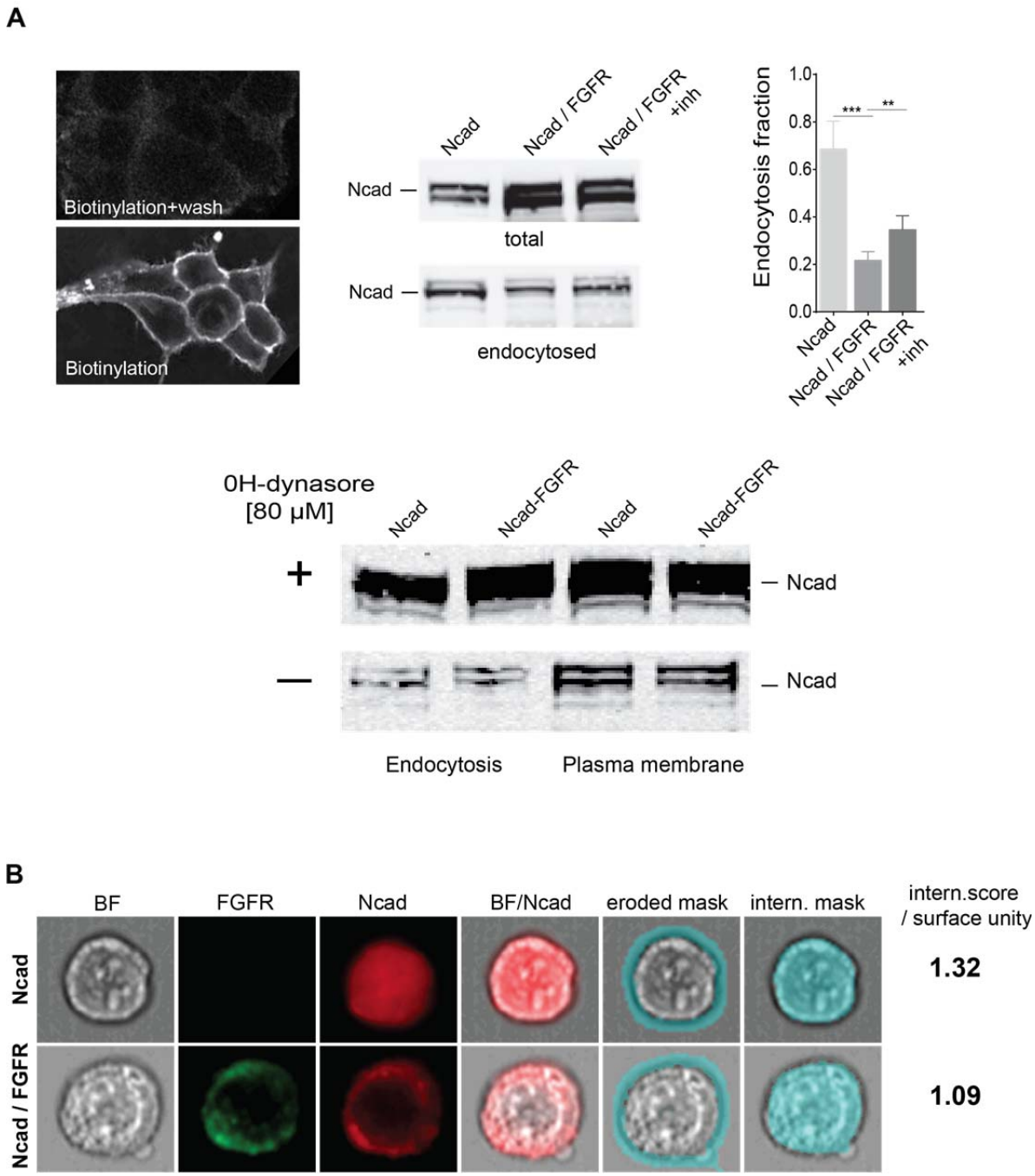


Figure 9: FGFR decreases N-cadherin endocytosis process. (A) Endocytic fraction separation using biotin surface labeling. (Left) Control of surface labelling by streptavidin-cy5 fixation and control of endocytosis protein detection. (Middle)The quantity of endocytosed Ncad in Ncad, Ncad / FGFR and Ncad / FGFR +inh HEK cells is detected by anti-N-cadherin immunoblotting. (Right) Graph shows the ratio of endocytosed versus total Ncad in each extract. FGFR expression decreases N-cadherin internalized fraction in cells. **p≤0.01; *** p≤0.001; ns: non-significant, Anova multiple comparison test, n = 3 experiments. (B) Ncad immunoblots for different

cellular and experimental conditions, treated or not with 80 µM hydroxyl-dynasore. **p≤0.01; *** p≤0.001; ns: non-significant, Anova multiple comparison test, n = 3 experiments. FGFR affects N-cadherin endocytosis. (C) Analysis of Ncad endocytosis by flow cytometry. Ncad, Ncad / FGFR HEK cells were mildly detached by EGTA treatment, fixed then processed to cytometry analysis. Cells were imaged in Bright field, Ncad-dsRed (560 nm wave length) and FGFR-GFP (480 nm wave length). Experiences were repeated 4 times, over populations of 150.000 cells for each condition in each experiment. Firstly, the morphology mask was applied to bright field images. Then, 4 pixels were evenly eroded from the border of the mask in order to exclude the cell membrane from the mask. The resulting mask was applied to the fluorescence channel. The internalization feature was then applied to the final mask in order to calculate the internalization score. FGFR reduces the internalization score of Ncad molecule about 17% (1.095 U.I versus 1.3208 U.I).

Figure 10

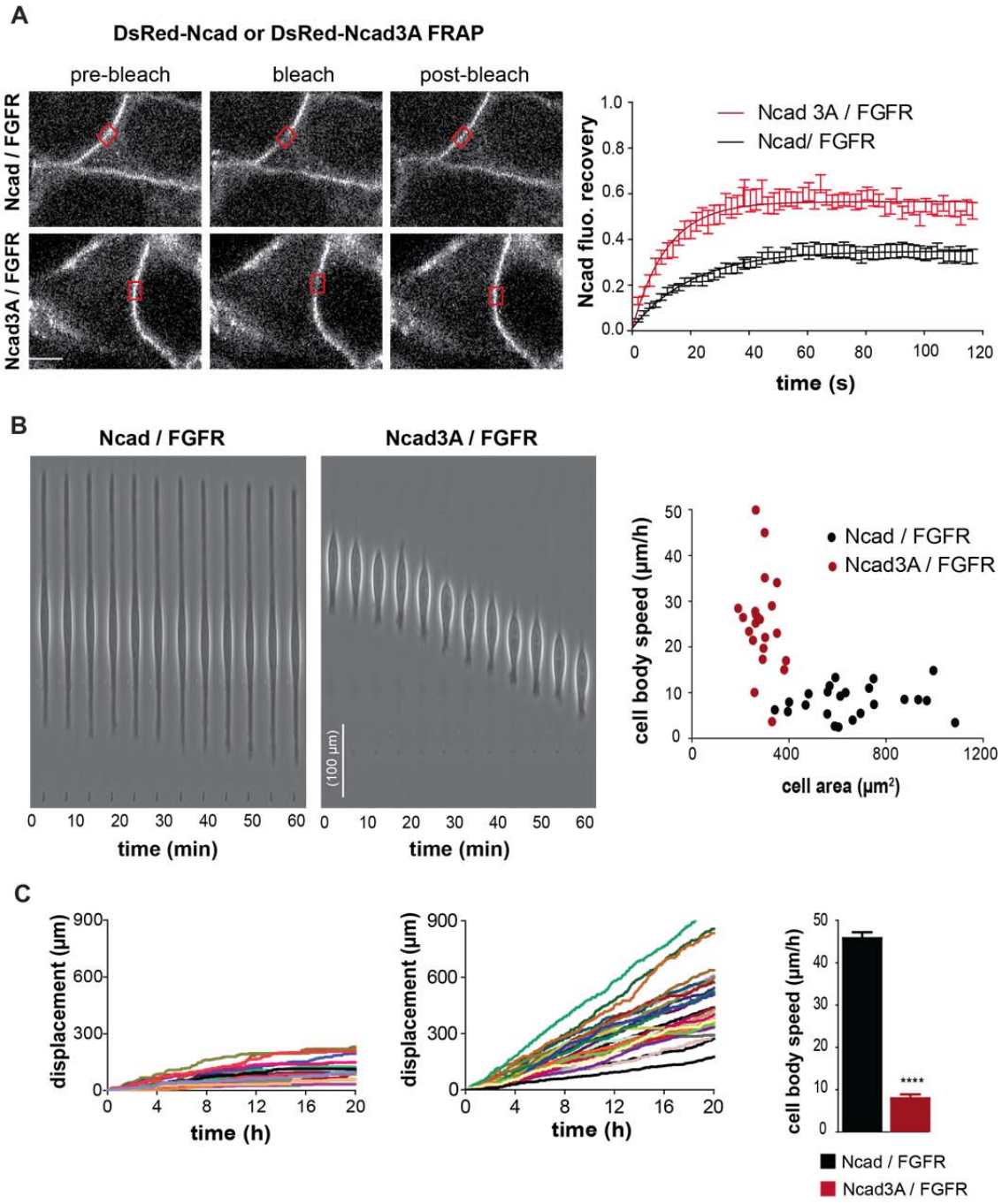


FIGURE 10: p120 is required for stabilization of N-cadherin at the cell-cell contact and for decreased migration induced by FGFR expression. HEK cells stably expressing GFP-FGFR1 were transfected with either DsRed-Ncad or mCherry-Ncad3A. (A) DsRed-Ncad or mCherry-Ncad3A to perform FRAP experiments. (Left) Representative images of DsRed-Ncad or mCherry-Ncad3A signal before (pre-bleach), immediately after bleaching (bleach) and 110 sec after the bleach (post-bleach) performed at the contacts of Ncad-FGFR and Ncad3A-FGFR cells. Red squares represent the bleached regions. Scale bar: 40 µm. (Right) Ncad and Ncad3A normalized fluorescence recovery curves for Ncad-FGFR (black) and Ncad3A-FGFR

(red) cells, respectively ($n \geq 20$). (B) (B) Ncad / FGFR, Ncad3A / FGFR cells were seeded on Ncad-Fc coated stripes. Cells were imaged in phase contrast every 6 minutes during 20 hours. (Left) Example of Ncad / FGFR (left) and Ncad3A / FGFR (middle) individual cell displacements over 1 hour. (Right) Histogram shows the cell areas in function of time. (C) Plots show the displacement in function of time for Ncad / FGFR (left), Ncad3A / FGFR (right) cells with respectively n =30, n= 40 cells. Histograms show the mean speed of Ncad / FGFR (black), Ncad3A / FGFR (red) cells (****, $p \leq 0.0001$, Anova multi-comparison test).

Figure 11

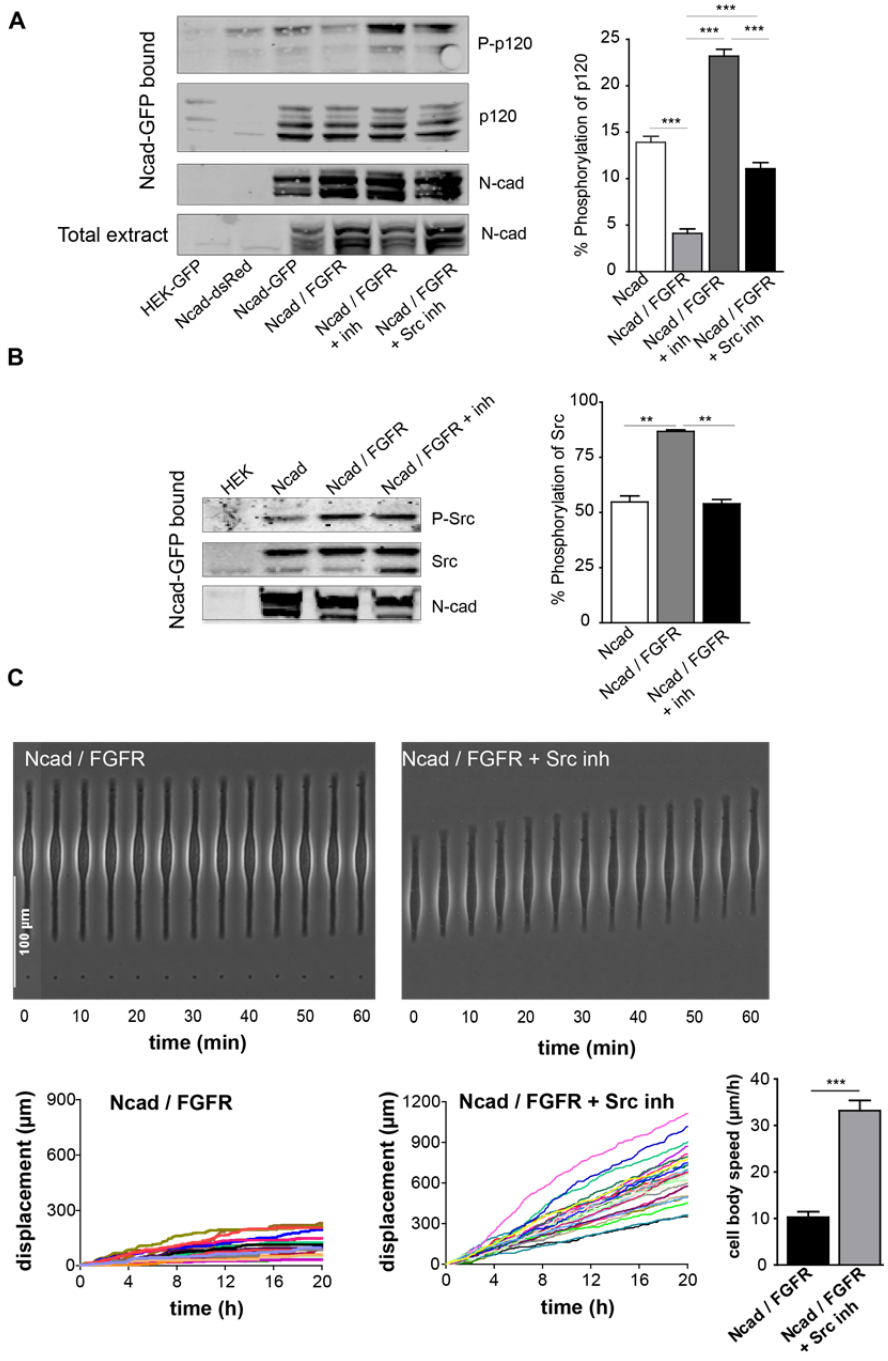


Figure 11: FGFR decreases phosphorylation of p120 and activates Src. (A) HEK cell extracts were immunoprecipitated with anti-N-cadherin then immunoprecipitates were immunoblotted with antibodies against N-cadherin, p120 and P-p120. Percentage of p120 phosphorylation was evaluated by the ratio of P-p120 band's intensity and p120 band's intensity. (B) Western blotting of Src and P-Src in the Ncad precipitated complex in different conditions. Histogram shows the percentage of phosphorylation of Src calculated by the ratio of Src and P-p120 intensity. (C) Migration on Ncad-Fc coated lines of Ncad / FGFR and Ncad / FGFR treated with Src inhibitor. Graph shows the displacement in function of times and histogram shows the migration speeds.

Figure 12

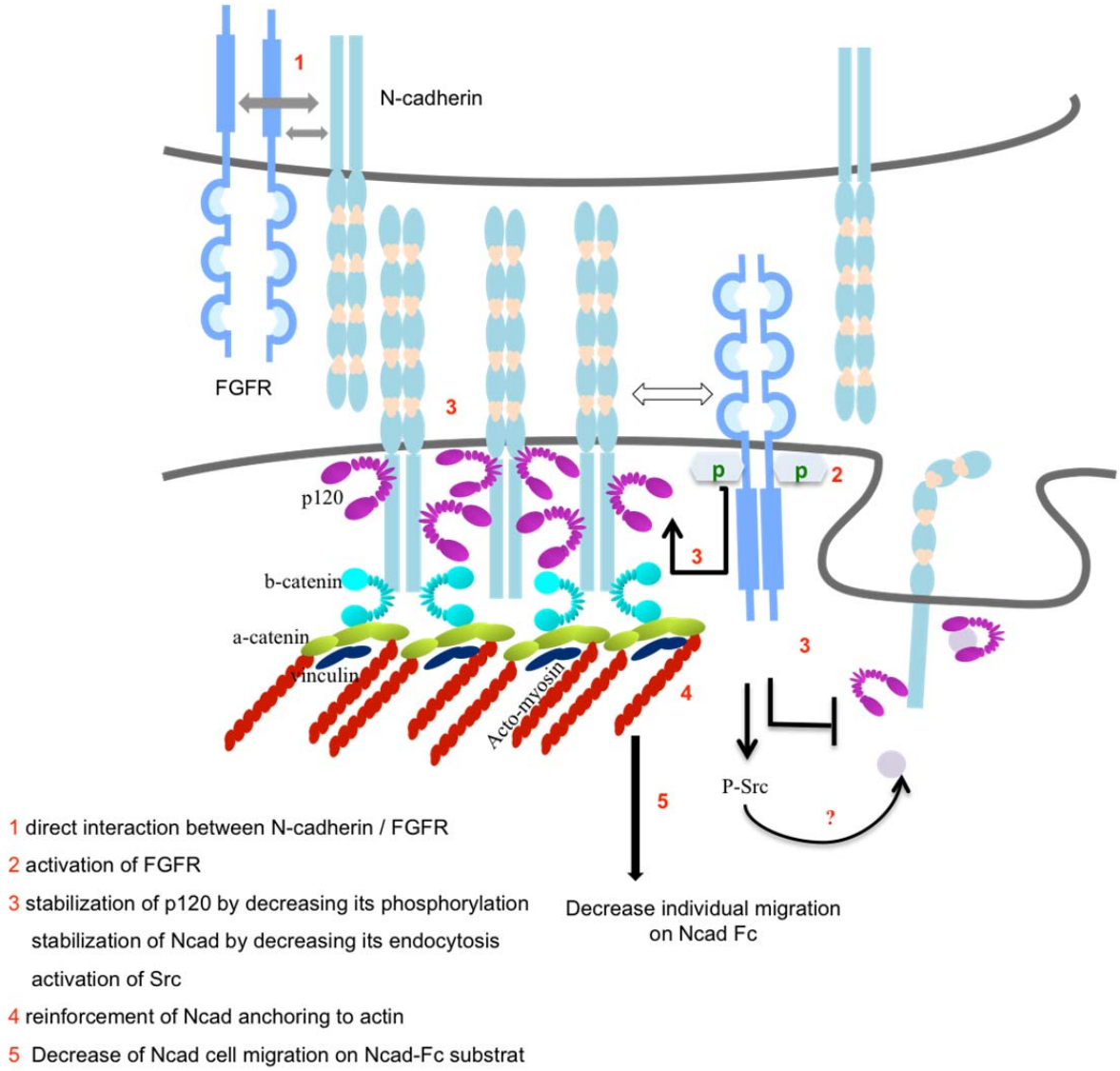


Figure 12: Proposed positive feed-back loop between N-cadherin and FGFR at adhesion sites limiting N-cadherin-based single cell migration. FGFR recruited by N-cadherin-mediated-adhesion is activated thanks to local crowding. FGFR then activates Src and induces p120 dephosphorylation. FGFR or Src activates subsequent partners leading to p120 dephosphorylation. Dephosphorylated p120 remains bound to N-cadherin which stabilizes it at the membrane by blocking its endocytosis. This stabilization strengthens cell-cell interactions, which together with Src activation, limits single cell N-cadherin-dependent migration.

Figure S1

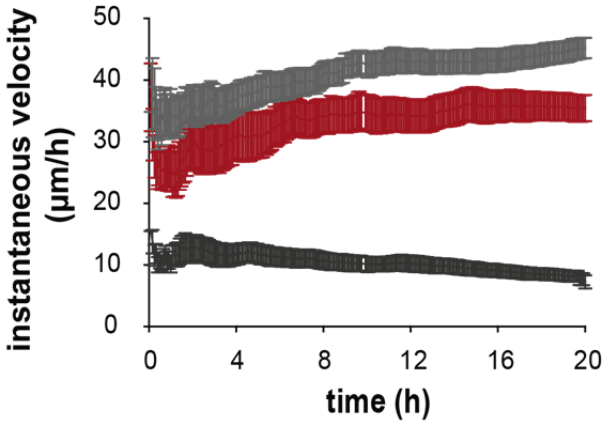


Figure S1: FGFR decreases instantaneous velocity of N-cadherin expressing HEK cells migrating on N-cad-Fc coated surfaces. Plot shows the evolution of instantaneous velocity during time of Ncad (grey), Ncad / FGFR (black) and Ncad / FGFR + inh (red) cells. FGFR negatively controls Ncad cells velocity on Ncad-Fc coated substrates.

Figure S2

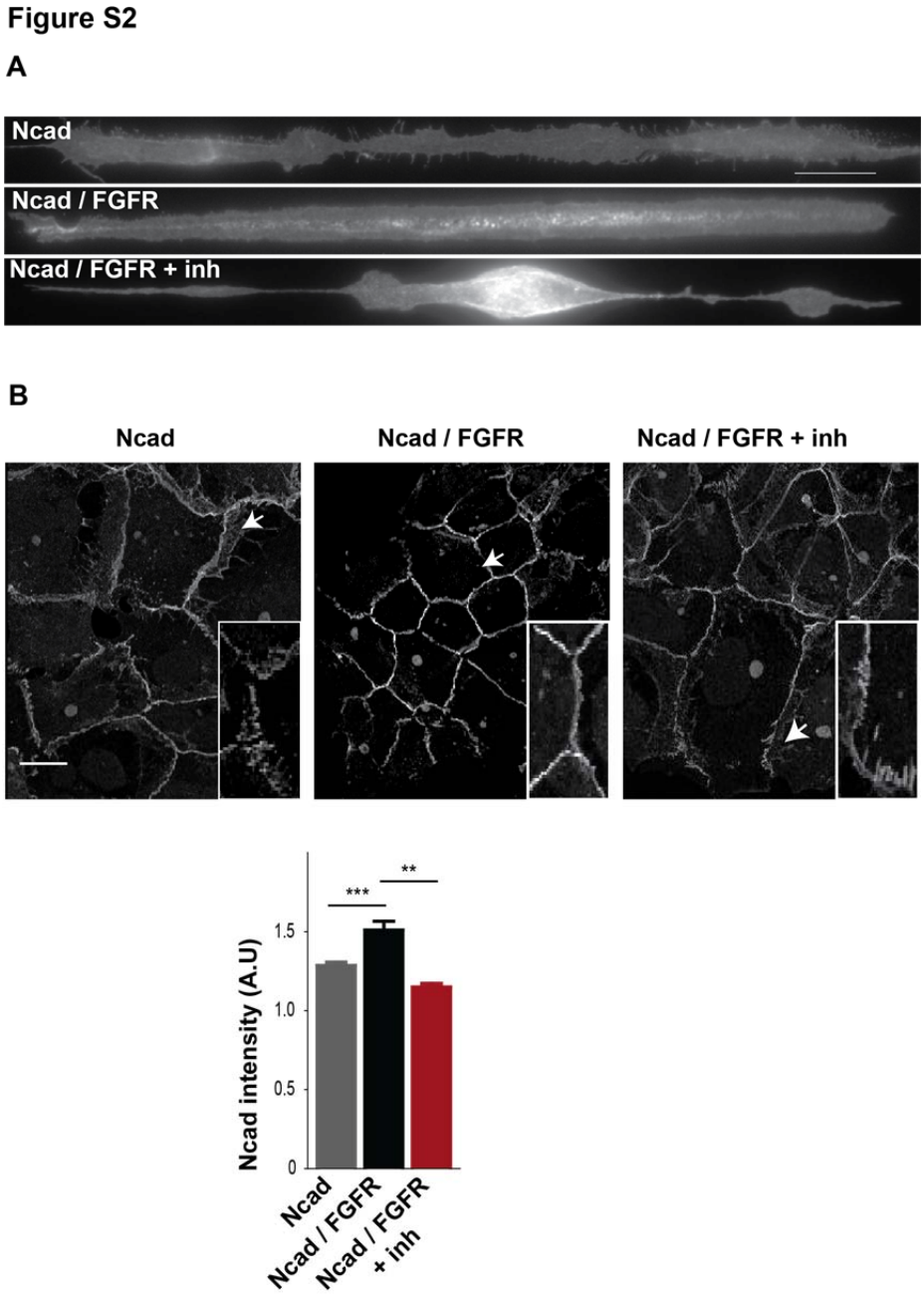


Figure S2: FGFR enhances clustering of N-cadherin at cell-cell and cell-substrate interface. (A) Example of DsRed-Ncad signal in fixed Ncad cells, Ncad / FGFR cells and Ncad / FGFR + inh cells. Bar = 20 μm. (A) Ncad cell (upper), Ncad / FGFR cell (middle) and Ncad / FGFR + inh cells (lower) seeded on Ncad-Fc coated lines. Ncad / FGFR cell was more elongated and displayed Ncad foci along the cell-substrate interface compared to Ncad cell. Inhibition of FGFR in Ncad / FGFR cell (lower lane) reduced cell elongation and Ncad foci appearance. (B) Example of DsRed-Ncad signal in fixed Ncad cell (left), Ncad / FGFR cells (middle) and Ncad / FGFR + inh cells (right) seeded on coverslip. Boxes are zooms of heads arrows. Histogram shows DsRed-Ncad intensity measured at the cell-cell contacts using Imaris in three conditions. Cell-cell contacts in Ncad / FGFR cells appeared more robust and straight than those in Ncad cells and Ncad / FGFR cells + inh (head arrows, boxes).

Figure S3

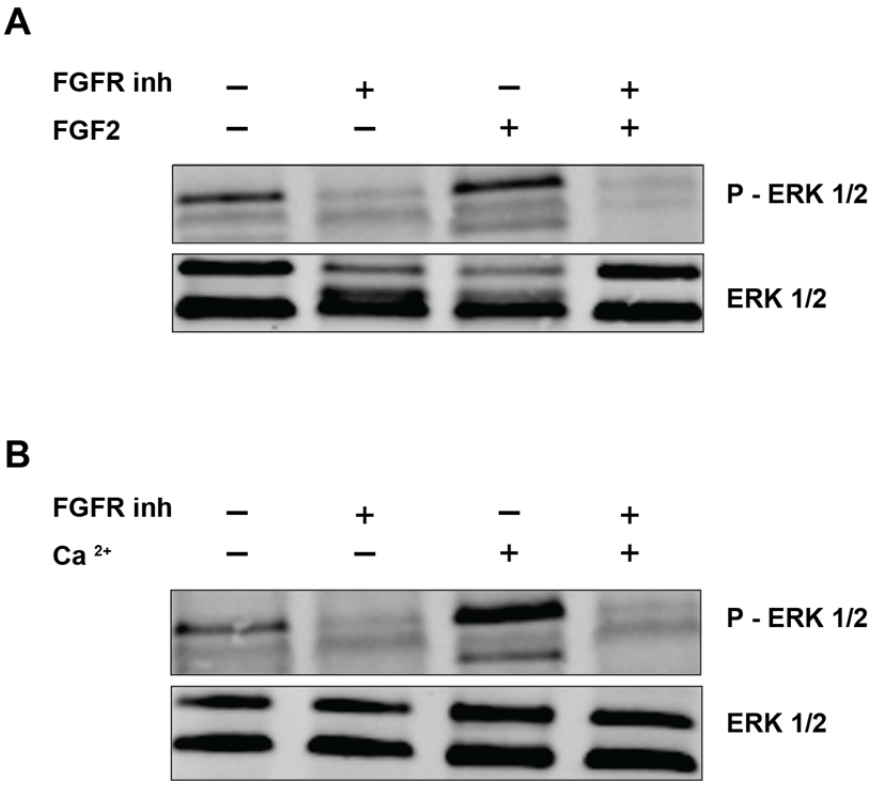


Figure S3: Enhanced N-cadherin clustering sustains activation of FGFR downstream pathway. C2C12 treated with FGFR inhibitor or/and FGF2 (A) or/and EGTA (B) were lysed and total extractions were subjected to immunoblotting with antibody anti P-ERK1/2 and anti ERK1/2. (A) Activated FGFR activates ERK 1/2 pathway. (B) Enhanced Ncad clustering induced by Ca^{2+} sustains ERK1/2 activation.

Figure S4

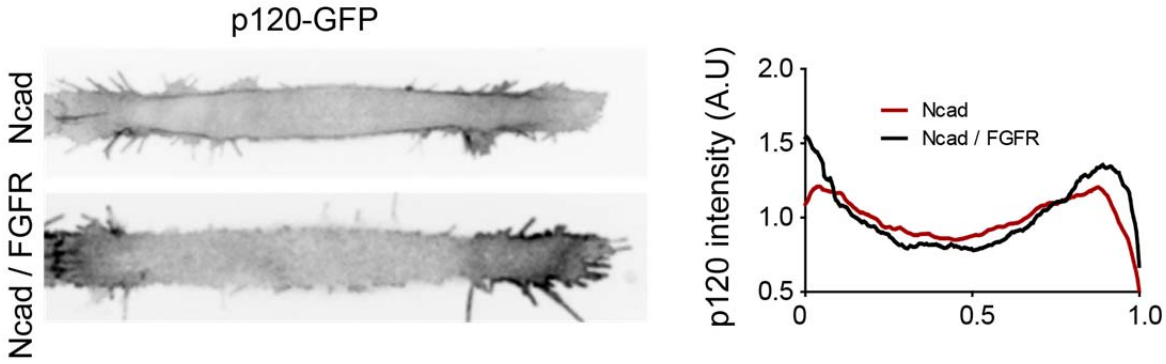


Figure S4: FGFR increases p120 recruitment at the cell-cell contacts. Ncad and Ncad / FGFR cells were transfected with p120-GFP and seeded on fibronectin coated lines. Doublets of cell were imaged. p120 intensity was calculated by Matlab along the cell with 0 value as junctional end and 1 value as free end. p120 intensity at the junctional end in Ncad / FGFR cell is higher than in Ncad cells.

Figure S5

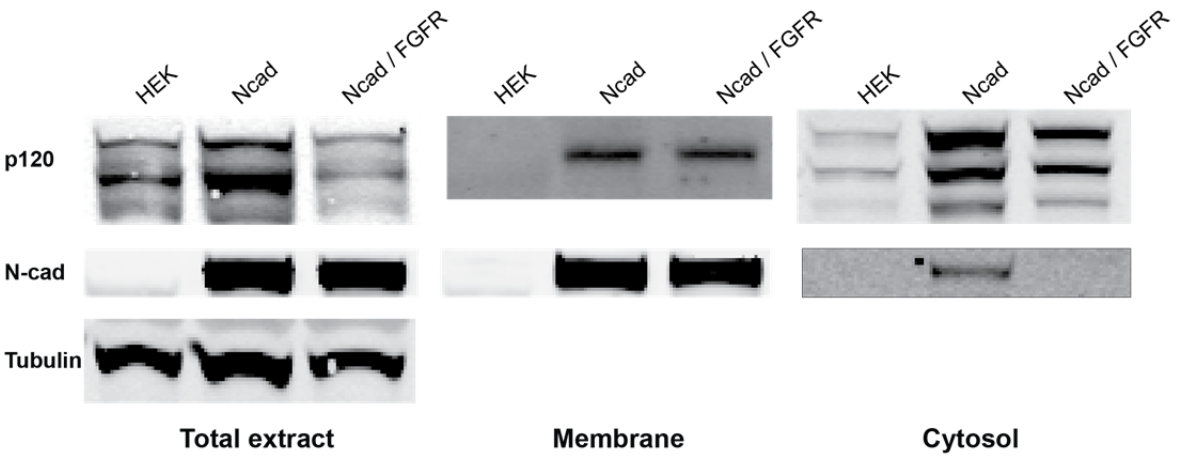


Figure S5: FGFR reduces cytosolic distribution of N-cadherin and p120. HEK or Transfected HEK cells were lysed without detergent, followed by subsequent centrifugation to separate cytosolic and membranous fractions in Ncad and Ncad / FGFR HEK cells. The different fractions were blotted with antibodies anti-p120, Ncad and tubulin. FGFR1 expression decreases N-cadherin as well as p120 levels in the cytosol without affecting their distribution at the membrane.

Materials and Methods

Cell culture, transient cell transfection and generation of stable cell lines

Human Embryonic Kidney (HEK) cells and Myoblast C2C12 cells were both grown in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 100 IU of penicillin, 100 µg/mL streptomycin at 37°C in 5% CO2. HEK cells were transiently transfected with the plasmids encoding wild type chicken N-cadherin fused to dsRed (Ncad-dsRed)(Bard et al., 2008) or dsRed-tagged N-cadherin 3A mutated in the p120 binding site (Bard et al., 2008) and Flag or GFP tagged FGFR1 via electroporation thanks to the Amaxa Cell Line Nucleofector (kit V, program X-032), resulting in more than 80% transfection efficiency. To generate Ncad-dsRed, FGFR-GFP and -Ncad-dsRed/FGFR-GFP stable HEK cell lines, transfected cells were grown under a selection pressure of 200 µg/mL of Hygromycin B and 1 mg/mL of Geneticin or both. Drug resistant cells were then sorted out by FACS, subcloned and further cultured and maintained with half of concentration of antibiotic pressure. Cells were always used at passages ≤ 20.

Reagents:

Inhibitor of FGFRs PD173074 (Sigma) was used at 20nM, 30 minutes. Inhibitor of Src family PP2 (Abcam) proteins was used at 100nM, 30 minutes. Hydroxy-dynasore (Sigma) was used at 100 nM, 1 hour.

Protein extraction and co-immunoprecipitation

Proteins were extracted from 5-6 x 10^6 cells. Cell cultures were rinsed in ice-cold PBS, detached with non-enzymatic detaching solution (Cell Dissociation Solution Non-enzymatic 1x, Sigma) and centrifuged at 1000 rpm for 7 minutes. Cell pellets were suspended in lysis buffer (10mM TrisCl pH 7.5; 150mM Nacl; 0.5 mM EDTA; 0.5% Triton) on ice. Cells were then passed slowly 10 times through a 27-gauge needle and left on ice for 1hour with extensive pipetting every 10 minutes. Lysates were cleared by centrifugation at 13000 rpm for 10 min at 4°C and protein concentration estimated by micro BCA assay (Pierce). GFP-tagged proteins were then immunoprecipitated using magnetic GFP-Trap®-M accordingly to the instructions of the manufacturer (Chromotek). Briefly, 25 µl of GFP-Trap®-M beads were washed 3 times with wash buffer (10mM Tris H-Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA). 15 µl of beads were added to 300 µl samples diluted with lysis buffer and tumbled end-over-

end for 1 hour at 4°C. Beads were then magnetically separated, washed 3 times with wash buffer, suspended in 100 µl 2x sample buffer (NuPAGEr LDS sample buffer 4X mixed with NuPAGEr Sample Reducing Agent 10X, ThermoFisher) and boiled at 95°C for 10 minutes to recover bound proteins. Proteins from the input and bound proteins were subjected to SDS-PAGE electrophoresis using Bis-Trisacrylamide 4%-12% gels (Invitrogen) then transferred on nitrocellulose membranes. Membranes were blocked with 5% nonfat milk and incubated with the adequate primary antibodies and then with IRDye-coupled secondary antibodies (Rockland). The blots were developed using Odyssey Imaging System (LY-COR Biosciences).

FGFR activation and Ca^{2+} switch assay

C2C12 cells were cultured in serum-free medium 24 hours before. Cells were then treated with FGF2 (1ng/ml, 5 minutes), inhibitor of FGFR PD17430 (20 µM, 20 minutes) or EGTA (4 mM, 20 minutes). After three washes with PBS 0 Ca^{2+} 0 Mg^{2+}, cells were incubated for 10 minutes in normal culture medium supplemented with 5 mM of Ca^{2+}. Cells were then subjected to protein extraction followed with immunoblot to detect MAPK pathway proteins using antibody anti-ERK1/2 (Sigma, 1:1000) and anti-P-ERK 1/2 (Sigma, 1/1000).

Surface biotinylation and endocytosis assay

For cell surface biotinylation, cell cultures were chilled down to 4°C by three washes with cold PBS/Mg^{2+}/Ca^{2+} then labelled with 1mg/ml of NHS-SS biotin (Pierce) diluted in PBS/Mg^{2+}/Ca^{2+} for 12 minutes at 4°C with gentle rocking. Biotinylation reagent was quenched by two washes with PBS/Mg^{2+}/Ca^{2+} containing 50 mM Glycine and 0.5% BSA at 4°C. Cells were then washed twice in cold PBS/Mg^{2+}/Ca^{2+} and lysed. Biotinylated plasma membrane proteins were then separated by precipitation with streptavidin-coated magnetic bead (Pierce™).

For endocytosis biochemical assays, cell surface biotinylation and quenching at 4°C in PBS/Mg^{2+}/Ca^{2+} at 4°C were performed as above. Then pre-warmed culture medium was returned to the dishes that were returned in the incubator at 37°C to allow endocytosis to resume for 40 minutes. Cells were then chilled down with cold PBS/Mg^{2+}/Ca^{2+} and bound biotin remaining at the cell surface was cleaved by incubating with 50 mM Glutathione in 75 mM NaCl, 10 mM EDTA, pH 7.5 for 15 minutes at 4°C under gentle agitation. After three

washes with PBS/Mg^{2+}/Ca^{2+}, cells were lysed and protein extracts subjected to precipitation with streptavidin-coated beads to obtain the endocytosed protein fractions.

Immunofluorescent staining

Cells were fixed at room temperature PBS 2 % formaldehyde for 15 minutes using, rinsed with PBS and permeabilized with PBS 0.15 % Triton X-100, 1 % BSA, then blocked for 1hour in PBS-1% BSA. Cells were then incubated overnight at 4°C with rabbit polyclonal anti-p120 (Santa-Cruz), anti-Phospho p120 (Abcam), and mouse monoclonal anti-vinculin (Marina Glukowa, Institut Pasteur, Paris) antibodies. Preparations were then washed 3 times with PBS and incubated with cy5-conjugated anti-rabbit IgGs or FITC-conjugated anti-mouse IgGs (Jackson ImmunoResearch Laboratories, Inc.) or cy5-conjugated phalloidin (1:400, Molecular Probes) at a 1:400 dilution for 1hour at room temperature. Preparations were then mounted in Mowiol, 90% glycerol. Images were acquired with a Leica TCS sp5 inverted confocal microscope AOBS tandem, equipped with a 63x oil objective (N.A=1.4), controlled by LAS AF (Leica System).

Ncad-Fc line guided cell migration

Patterned silicon stamps bearing 10 µm width lines spaced of 70 µm were prepared by soft lithography according to (Vedula et al., 2014). Patterned microcontact stamps were incubated with 1 µg/cm^2 human-IgG, pressed on non-culture treated petri dishes or on sonicated coverslip previously activated by deep UV (Jelight, 4 X 60W, 15 minutes). Microcontact printed surfaces were passivated by incubation with 1% Pluronics F-127 diluted in distilled water (Sigma) for 1 hour followed by 3 washes with PBS. hNcad-Fc (1µg/ μm^2) was allowed to bind for 2 hours at room temperature. Surfaces were washed three times with PBS and kept at 4°C before cells seeding (conservation maximum during72 hours).

Ncad and Ncad/FGFR expressing cells, treated or not with the FGFR inhibitor (PD 17340) were seeded over arrays of Ncad-Fc-coated10 µm width lines, prepared as described above, and allowed to adhere for 1-2 hours in culture medium containing 1 µg/mL of mytomycin. Non-adhesive cells were gently washed off. Cells were imaged live or fixed 18 hours after seeding.

For live cell imaging, images were acquired with a 10 X objective, every 6 minutes during 24 hours under controlled environment (37° C, 5% CO2, Biostation Nikon). Manual tracking of individual cells over 20 hours was performed with MTrackJ plugin. Individual

trajectories were positioned on an orthonormal axis with the coordinates of the cells at t0 = (0;0). The displacements and mean body speed of migration were then extracted for each condition and plotted versus time and cell area, respectively

Molecular intensity cell-cell contacts assay

Ncad and Ncad FGFR HEK cells transiently transfected with p120-GFP or lifeact-GFP were seeded on fibronectine-coated-10 µm width lines and let adhering for 1-2 hours. Samples were gently washed, returned to the incubator over nigh. Doublets of cells were chosen to image in red-phenol-free DMEM supplemented with 10% serum every minutes during 1 hour under a controlled temperature and CO2 environment (37°C, 5% CO_2, 40x oil objectives (N.A = 1.4), Spinning disk CSU22). Localization of fluorescent probe relative to the junction end was analysed using ImageJ for mask creating and Matlab for probe intensity calculation. All the fluorescence images were background-subtracted before quantification. The cells shape were detected by segmenting the fluorescence intensity image using Otsu method and converted into binary mask images with values outside the cell set to zero. The cell lengths were normalized to unity in the x direction (strip or horizontal direction). For each individual cell, the fluorescence intensity within the cell mask along the x direction was projected and averaged in the y direction (perpendicular to the strip direction), and the average intensity curve was normalized by the whole cell intensity and plotted against the normalized distance to the junction end. The average intensities in the x-direction from multiple cells with the same experimental condition were calculated and an average curve was then created using Matlab function smooth (Mathworks, Natick, MA) by filtering with locally quadratic regression utilizing a moving window of size 5. The overall behaviour of each group of multiple cells can then be represented by one single curve.

Fluorescence Recovery after Photobleaching

Fluorescence recoveries after photobleaching (FRAP) was performed at 37°C on stably transfected Ncad, FGFR or Ncad/FGFR expressing cells, treated or not with indicated drugs as well as on transiently transfected Ncad and Ncad/FGFR expressing cells. FRAP was performed using a Leica TCS SP5 confocal microscope equipped with a 40 X immersion objective (N.A=1.4) and carried out by setting the double scanning mode at 560 nm for dsRed and 480 nm for GFP and the image format to 256 X256 pixels. The cell-cell contact was photobleached by performing repeated scans. After 3 prebleach scans (0.347 sec), the

rectangular ROI (white rectangular) was bleached with laser at full power. Recovery was recorded by imaging with low laser power every 0.347 sec (20 scans) then every 2 sec (20 scans) and finally every 10 sec (20 scans). The normalized recovery of fluorescence was expressed as a ratio of prebleach fluorescence after correction for photobleaching as reported previously. Normalized fluorescence recovery in function of time curves were fitted with a one-term exponential equation using GraphPad Prism 5.01 software (one-phase decay non-linear regression function), allowing to extract a plateau value representing the fraction of diffusion-limited molecules (mobile fraction) and a recovery half-time ($t_{1/2}$) a proxy the apparent diffusion coefficient of diffusion-limited molecules (Thoumine et al., 2006).

Cell-cell contact disruption assay

Glass or plastic surfaces were microcontact printed with silicon stamps bearing 200 µm fibronectin-coated squares. Briefly the stamps were prepared according to ref, incubated with 50 µg/mL fibronectin, and microcontact printed on the surfaces, which were then blocked with Pluronics® F-127 1% and washed three times with PBS/Mg^{2+}/Ca^{2+} and kept at 4°C before cells seeding (conservation maximum during72 hours).

Cells were seeded on fibronectin-coated patterned surfaces in the presence of 10 µg/mL mitomycine. After 1 h, unattached cells and mitomycin were washed out and preparations were returned to incubator overnight. Samples were directly processed for live image after addition of 5mM of EGTA solution or fixed after 15 minutes of EGTA treatment. Live images were acquired at 20 x objective, every 30 seconds for 30 minutes under a controlled environment (37°C, 5% CO_2, type Inverted Olympus IX81, camera CoolSnap HQ^2); fixed samples were acquired using 20 x and 60 x objectives, using MetaMorph.

Flow Cytometry

Cells were detached using non-enzymatic detaching solution (Cell Dissociation Solution Non-enzymatic 1x, Sigma), centrifuged at 1000 rpm during 3 minutes, resuspended in culture medium and returned to the incubator for 10 minutes favoring moderate cell-cell adhesion in suspension. Cells were centrifuged at 1000 rpm for 4 minutes, fixed in 4% formaldehyde for 15 minutes, washed 3 times in PBS, incubated in the presence of 0.1% Dapi in PBS-BSA 0.1 % for 5 minutes, washed again then imaged under flow using ImageStream X (Amnis) set with the 405, 488, 560-nm laser and 480-560 filter. Data were analyzed using

the IDEAS software (Amnis) focusing on singulet cells for endocytosis quantification and on doublets for cell-cell accumulation quantifications.

For endocytosis, regions corresponding to the total cell surface, the internal cell area and the cell membrane area were extracted from bright field images of singulets. Briefly, the morphology mask was applied to bright field images. Then, 4 pixels were evenly eroded from the border of the mask in order to exclude the cell membrane from the mask. The resulting mask was applied to the fluorescence channel. Internalization score is defined as the ration of fluorescence intensity and surface of the mask.

The Ncad interface recruitment was determined on doublets were regions corresponding to the total cell surface and the cell-cell interface were extracted from bright field images and dapi staining, respectively. The 4 pixels interface mask was determined as a region centered at the dimmest pixel between the 2 nuclei (dapi). The interface mask was applied to the bright field channel to determine the surface area of the cell-cell contacts in the doublet populations of cells, then to the fluorescence channel to count the intensity of Ncad staining at the cell-cell contacts. Results were expressed as fluorescence intensity per surface unit. The Bright Detail Intensity (BDI) was determined by a feature of IDEAS analysis assessing for the intensity of bright spots that have radii of 4 pixels after neglecting background staining. Data acquisition was performed for 150 000 cells for each condition and repeated 4 times.

Magnetic tweezers

A homemade magnetic microneedle device was the source of the magnetic field gradient used to pull Ncad-Fc coated paramagnetic microbeads attached to the cells. The magnetic tweezers was made of a 5-cm long stainless steel sewing needle glued to the top of a permanent neodymium iron boron (NeFeB) surface surrounding an aluminium rod. The whole was mounted on a micromanipulator (MP-285, Sutter Instrument) at a 30°C vertical angle, and the tip initially aligned at 600 μm from the centre of the observation field. The magnitude of the horizontal magnetic force applied to each bead was a function of the distance between the needle tip and the bead. The device was mounted on an inverted microscope (Olympus) equipped with a 40x phase contrast dry objective and a CCD camera (CoolSnap HQ^2) operating in the burst mode (frequency of 15 frames/s for 2 minutes). Distance between the tip of needle and detached bead was measured with imageJ.

To calibrate the magnetic force, single beads ware placed in a 100 % Polyethylenglycol solution (Mn 700, Sigma) near the needle and the bead motion was tracked by video microscopy. The instantaneous horizontal velocity of single beads was obtained through analysing video images, and the viscous force on a bead was calculated on Stokes Law: $F= 6\pi\eta R\nu$ where F was the total viscous force on the beads, η the dynamic viscosity of the PEG (η = 0.103 Pa.s at 25°C), R the radius of the beads (4,8 μm), and ν the beads velocity. The calibration was performed ten times and the forces versus distance data were regressed to an exponential equation. Magnetic force decayed as a function of distance from the needle tip (Fig. 5 A, right).

Acknowledgement

We thank N. Boggetto for her help with flow imaging and G. Wentzingerand for her help with FACS. We thank Ch.Murade and M.Yao for held with magnetic tweezer experiment. We thank O.Thoumine, R. Horwitz and M.Glukova for their kind gift of Ncad + Ncad3A plasmid, FGFR plasmid and anti-vinculin antibodies, respectively. We thank ImagoSeine for use of microscope platform technique.

This work was supported by grants from CNRS, ARC foundation, Human Frontier Science Program (HFSP grant RPG0040/2012), and Agence Nationale de la Recherche (ANR 2010 Blan1515). TN was supported by a HFSP grant RPG0040/2012 then by FRM (Fondation pour la Recherche Médicale). We would like also to thank all present and past member of the Cell Adhesion & Mechanics lab at the Institute Jacques Monod for constant support and exchange.

References

Arboleda-Estudillo, Y., Krieg, M., Stühmer, J., Licata, N. a., Muller, D.J., Heisenberg, C.P., 2010. Movement Directionality in Collective Migration of Germ Layer Progenitors. Curr. Biol. 20, 161–169.

Bard, L., Boscher, C., Lambert, M., Mege, R.-M., Choquet, D., Thoumine, O., Mège, R.-M., Choquet, D., Thoumine, O., Mege, R.-M., Choquet, D., Thoumine, O., Mège, R.-M., Choquet, D., Thoumine, O., 2008. A Molecular Clutch between the Actin Flow and N-Cadherin Adhesions Drives Growth Cone Migration. J. Neurosci. 28, 5879–5890. doi:10.1523/JNEUROSCI.5331-07.2008

Borghi, N., Sorokina, M., Shcherbakova, O.G., Weis, W.I., Pruitt, B.L., Nelson, W.J., Dunn, A.R., 2012. E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. Proc. Natl. Acad. Sci. U. S. A. 109, 12568–73. doi:10.1073/pnas.1204390109

Boscher, C., Mège, R.-M.M., 2008. Cadherin-11 interacts with the FGF receptor and induces neurite outgrowth through associated downstream signalling. Cell. Signal. 20, 1061–1072. doi:10.1016/j.cellsig.2008.01.008

Brittis, P.A., Silver, J., Walsh, F.S., Doherty, P., 1996. Fibroblast growth factor receptor function is required for the orderly projection of ganglion cell axons in the developing mammalian retina. Mol. Cell. Neurosci. 8, 120–8.

Buckley, C.D., Tan, J., Anderson, K.L., Hanein, D., Volkmann, N., Weis, W.I., Nelson, W.J., Dunn, A.R., 2014. Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. Science 346, 1254211. doi:10.1126/science.1254211

Cai, D., Montell, D.J., 2014. Diverse and dynamic sources and sinks in gradient formation and directed migration. Curr. Opin. Cell Biol. 30, 91–98. doi:10.1016/j.ceb.2014.06.009

Chazeau, A., Garcia, M., Czondor, K., Perrais, D., Tessier, B., Giannone, G., Thoumine, O., 2015. Mechanical coupling between transsynaptic N-cadherin adhesions and actin flow stabilizes dendritic spines. Mol. Biol. Cell 26, 859–873. doi:10.1091/mbc.E14-06-1086

Chen, X., Kojima, S.I., Borisy, G.G., Green, K.J., 2003. P120 Catenin Associates With Kinesin and Facilitates the Transport of Cadherin-Catenin Complexes To Intercellular Junctions. J. Cell Biol. 163, 547–557.

Chu, W.-C., Lee, Y.-M., Henry Sun, Y., 2013. FGF /FGFR signal induces trachea extension in the drosophila visual system. PLoS One 8, e73878.

Chu, Y.-S.S., Thomas, W.A., Eder, O., Pincet, F., Perez, E., Thiery, J.P., Dufour, S., 2004. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. J. Cell Biol. 167, 1183–94. doi:10.1083/jcb.200403043

Ciruna, B., Rossant, J., 2001. FGF Signaling Regulates Mesoderm Cell Fate Specification and Morphogenetic Movement at the Primitive Streak. Dev. Cell 1, 37–49.

Ciruna, B.G., Schwartz, L., Harpal, K., Yamaguchi, T.P., Rossant, J., 1997. Chimeric analysis

of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenetic movement through the primitive streak. Development 124, 2829–2841.

Coleman, S.J., Chioni, A., Ghallab, M., Anderson, R.K., Lemoine, N.R., Kocher, H.M., Grose, R.P., 2014. Nuclear translocation of FGFR1 and FGF2 in pancreatic stellate cells facilitates pancreatic cancer cell invasion. EMBO Mol. Med. 6, 467–81.

Davis, M.A., Ireton, R.C., Reynolds, A.B., 2003. A core function for p120-catenin in cadherin turnover. J. Cell Biol. 163, 525–534. doi:10.1083/jcb.200307111

Debiais, F., Lemonnier, J., Hay, E., Delannoy, P., Caverzasio, J., Marie, P.J., 2001. Fibroblast growth factor-2 (FGF-2) increases N-cadherin expression through protein kinase C and Src-kinase pathways in human calvaria osteoblasts. J. Cell. Biochem. 81, 68–81.

Deng, C.X., Wynshaw-Boris, A., Shen, M.M., Daugherty, C., Ornitz, D.M., Leder, P., 1994. Murine FGFR-1 is required for early postimplantation growth and axial organization. Genes Dev. 8, 3045–3057.

Derycke, L.D.M., Bracke, M.E., 2004. N-cadherin in the spotlight of cell-cell adhesion, differentiation, invasion and signalling. Int. J. Dev. Biol. 48, 463–476.

Dumortier, J.G., Martin, S., Meyer, D., Rosa, F.M., David, N.B., 2012. Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. Proc. Natl. Acad. Sci. U. S. A. 109, 16945–16950.

Franco, S.J., Martinez-Garay, I., Gil-Sanz, C., Harkins-Perry, S.R., Müller, U., 2011. Reelin regulates cadherin function via Dab1/Rap1 to control neuronal migration and lamination in the neocortex. Neuron 69, 482–97.

Fung, S., Wang, F., Chase, M., Godt, D., Hartenstein, V., 2008. Expression profile of the cadherin family in the developing Drosophila brain. J. Comp. Neurol. 506, 469–88. doi:10.1002/cne.21539

Giannone, G., Mège, R.-M., Thoumine, O., 2009. Multi-level molecular clutches in motile cell processes. Trends Cell Biol. 19, 475–86.

Hazan, R.B., Kang, L., Whooley, B.P., Borgen, P.I., 1997. N-cadherin promotes adhesion between invasive breast cancer cells and the stroma. Cell Adhes. Commun. 4, 399–411.

Hazan, R.B., Phillips, G.R., Qiao, R.F., Norton, L., Aaronson, S.A., 2000. Exogenous Expression of N-Cadherin in Breast Cancer Cells Induces Cell Migration, Invasion, and Metastasis. J. Cell Biol. 148, 779–790. doi:10.1083/jcb.148.4.779

Hong, E., Brewster, R., 2006. N-cadherin is required for the polarized cell behaviors that drive neurulation in the zebrafish. Development 133, 3895–3905.

Huttenlocher, A., Horwitz, A.R., 2011. Integrins in Cell Migration. Cold Spring Harb. Perspect. Biol. 3, a005074–a005074. doi:10.1101/cshperspect.a005074

Ireton, R.C.R.C.R.C.R.C., Davis, M.A., Hengel, J. Van, Mariner, D.J., Barnes, K., Thoreson, M.A., Anastasiadis, P.Z., Matrisian, L., Bundy, L.M., Sealy, L., Gilbert, B., Roy, F. Van, Reynolds, A.B., Van Hengel, J., Mariner, D.J., Barnes, K., Thoreson, M.A., Anastasiadis, P.Z., Matrisian, L., Bundy, L.M., Sealy, L., Gilbert, B., Van Roy, F.,

Reynolds, A.B., 2002. A novel role for p120 catenin in E-cadherin function. J. Cell Biol. 159, 465–76. doi:10.1083/jcb.200205115

Jossin, Y., Cooper, J.A., 2011a. Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. 14, 697–703.

Jossin, Y., Cooper, J.A., 2011b. Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. 14, 697–703. doi:10.1038/nn.2816

Kashima, T., Nakamura, K., Kawaguchi, J., Takanashi, M., Ishida, T., Aburatani, H., Kudo, A., Fukayama, M., Grigoriadis, A.E., 2003. Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo. Int. J. cancer 104, 147–54. doi:10.1002/ijc.10931

Kolijn, K., Verhoef, E.I., van Leenders, G.J.L.H., 2015. Morphological and immunohistochemical identification of epithelial-to-mesenchymal transition in clinical prostate cancer. Oncotarget 5.

Kontaridis, M.I., Liu, X., Zhang, L., Bennett, A.M., 2002. Role of SHP-2 in Fibroblast Growth Factor Receptor-Mediated Suppression of Myogenesis in C2C12 Myoblasts. Mol. Cell. Biol. 22, 3875–3891. doi:10.1128/MCB.22.11.3875-3891.2002

Kowalczyk, A.P., Nanes, B.A., 2012. Adherens Junction Turnover: Regulating Adhesion Through Cadherin Endocytosis, Degradation, and Recycling, in: Sub-Cellular Biochemistry. pp. 197–222. doi:10.1007/978-94-007-4186-7_9

Künstlinger, H., Fassunke, J., Schildhaus, H.-U., Brors, B., Heydt, C., Angelika Ihle, M., Mechtersheimer, G., Wardelmann, E., Büttner, R., Merkelbach-Bruse, S., 2015. FGFR2 is overexpressed in myxoid liposarcoma and inhibition of FGFR signaling impairs tumor growth in vitro. Oncotarget 5.

Lambert, M., Thoumine, O., Brevier, J., Choquet, D., Riveline, D., Mège, R.-M., 2007. Nucleation and growth of cadherin adhesions. Exp. Cell Res. 313, 4025–40. doi:10.1016/j.yexcr.2007.07.035

Lamszus, K., Brockmann, M.A., Eckerich, C., Bohlen, P., May, C., Mangold, U., Filibrandt, R., Westphal, M., 2005. Inhibition of glioblastoma angiogenesis and invasion by combined treatments directed against vascular endothelial growth factor receptor-2, epidermal growth factor receptor, and vascular endothelial-cadherin. Clin. Cancer Res. 11, 4934–4940.

Lebreton, G., Casanova, J., 2016. Ligand-binding and constitutive FGF receptors in single Drosophila tracheal cells: Implications for the role of FGF in collective migration. Dev. Dyn. 245, 372–8.

Lebreton, G., Casanova, J., 2014. Specification of leading and trailing cell features during collective migration in the Drosophila trachea. J. Cell Sci. 127, 465–74.

Lemmon, M. a, Schlessinger, J., 2011. NIH Public Access. Biochemistry 141, 1117–1134.

Li, G., Satyamoorthy, K., Herlyn, M., 2001. N-cadherin-mediated intercellular interactions

promote survival and migration of melanoma cells. Cancer Res. 61, 3819–3825.

Luccardini, C., Hennekinne, L., Viou, L., Yanagida, M., Murakami, F., Kessaris, N., Ma, X., Adelstein, R.S., Mège, R.-M., Métin, C., 2013. N-cadherin sustains motility and polarity of future cortical interneurons during tangential migration. J. Neurosci. 33, 18149–60.

Martin, A.C., Kaschube, M., Wieschaus, E.F., 2009. Pulsed contractions of an actin-myosin network drive apical constriction. Nature 457, 495–9.

Matsunaga, M., Hatta, K., Takeichi, M., 1988. Role of N-cadherin cell adhesion molecules in the histogenesis of neural retina. Neuron 1, 289–95.

Mège, R.-M., Gavard, J., Lambert, M., 2006. Regulation of cell-cell junctions by the cytoskeleton. Curr. Opin. Cell Biol. 18, 541–8.

Mitchison, T., Kirschner, M., 1988. Cytoskeletal dynamics and nerve growth. Neuron 1, 761–72.

Miyashita, Y., Ozawa, M., 2007. Increased internalization of p120-uncoupled E-cadherin and a requirement for a dileucine motif in the cytoplasmic domain for endocytosis of the protein. J. Biol. Chem. 282, 11540–8. doi:10.1074/jbc.M608351200

Nakashima, T., Huang, C., Liu, D., Kameyama, K., Masuya, D., Kobayashi, S., Kinoshita, M., Yokomise, H., 2003. Neural-cadherin expression associated with angiogenesis in non-small-cell lung cancer patients. Br. J. Cancer 88, 1727–1733.

Nguyen, T., Mège, R.M., 2016. N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. doi:10.1016/j.ejcb.2016.05.002

Nomura, S., Yoshitomi, H., Takano, S., Shida, T., Kobayashi, S., Ohtsuka, M., Kimura, F., Shimizu, H., Yoshidome, H., Kato, A., Miyazaki, M., 2008. FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer. Br. J. Cancer 99, 305–13.

Ouyang, M., Lu, S., Kim, T., Chen, C.-E., Seong, J., Leckband, D.E., Wang, F., Reynolds, A.B., Schwartz, M.A., Wang, Y., 2013. N-cadherin regulates spatially polarized signals through distinct p120ctn and β-catenin-dependent signalling pathways. Nat. Commun. 4, 1589. doi:10.1038/ncomms2560

Parish, a, Schwaederle, M., Daniels, G., Piccioni, D., Fanta, P., Schwab, R., Shimabukuro, K., Parker, B. a, Helsten, T., Kurzrock, R., 2015. Fibroblast Growth Factor Family Aberrations in Cancers: Clinical and Molecular Characteristics. Cell Cycle 00–00.

Partanen, J., Schwartz, L., Rossant, J., 1998. Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for Fgfr1 in anteroposterior patterning of mouse embryos. Genes Dev. 12, 2332–2344.

Peterson, S.J., Krasnow, M.A., 2015. Subcellular trafficking of FGF controls tracheal invasion of Drosophila flight muscle. Cell 160, 313–23. doi:10.1016/j.cell.2014.11.043

Plestant, C., Strale, P.-O.P.-O., Seddiki, R., Nguyen, E., Ladoux, B., Mege, R.-M., Mège, R.-M., 2014. Adhesive interactions of N-cadherin limit the recruitment of microtubules to cell-cell contacts through organization of actomyosin. J. Cell Sci. 127, 1660–71.

doi:10.1242/jcs.131284

Reynolds, A.B., Carnahan, R.H., 2004. Regulation of cadherin stability and turnover by p120ctn: Implications in disease and cancer. Semin. Cell Dev. Biol. 15, 657–663.

Reynolds, A.B., Roczniak-Ferguson, A., 2004. Emerging roles for p120-catenin in cell adhesion and cancer. Oncogene 23, 7947–7956.

Rieger-Christ, K.M., Lee, P., Zagha, R., Kosakowski, M., Moinzadeh, A., Stoffel, J., Ben-Ze'ev, A., Libertino, J. a, Summerhayes, I.C., 2004. Novel expression of N-cadherin elicits in vitro bladder cell invasion via the Akt signaling pathway. Oncogene 23, 4745–4753.

Roura, S., Miravet, S., Piedra, J.J., García de Herreros, A., Duñach, M., Garcia de Herreros, A., Dunach, M., Garc??a De Herreros, A., Du??achl, M., 1999. Regulation of E-cadherin/catenin association by tyrosine phosphorylation. J. Biol. Chem. 274, 36734–36740. doi:10.1074/jbc.274.51.36734

Strale, P.-O., Duchesne, L., Peyret, G., Montel, L., Nguyen, T., Png, E., Tampe, R., Troyanovsky, S., Henon, S., Ladoux, B., Mege, R.-M., 2015. The formation of ordered nanoclusters controls cadherin anchoring to actin and cell-cell contact fluidity. J. Cell Biol. 210, 333–346. doi:10.1083/jcb.201410111

Suyama, K., Shapiro, I., Guttman, M., Hazan, R.B., 2002. A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2, 301–14.

Taeger, J., Moser, C., Hellerbrand, C., Mycielska, M.E., Glockzin, G., Schlitt, H.J., Geissler, E.K., Stoeltzing, O., Lang, S. a., 2011. Targeting FGFR/PDGFR/VEGFR Impairs Tumor Growth, Angiogenesis, and Metastasis by Effects on Tumor Cells, Endothelial Cells, and Pericytes in Pancreatic Cancer. Mol. Cancer Ther. 10, 2157–2167.

Takehara, T., Teramura, T., Onodera, Y., Frampton, J., Fukuda, K., 2015. Cdh2 stabilizes FGFR1 and contributes to primed-state pluripotency in mouse epiblast stem cells. Sci. Rep. 5, 14722.

Thoumine, O., Lambert, M., Mège, R.-M., Choquet, D., 2006. Regulation of N-cadherin dynamics at neuronal contacts by ligand binding and cytoskeletal coupling. Mol. Biol. Cell 17, 862–75. doi:10.1091/mbc.E05-04-0335

Trolice, M.P., Pappalardo, A., Peluso, J.J., 1997. Basic fibroblast growth factor and N-cadherin maintain rat granulosa cell and ovarian surface epithelial cell viability by stimulating the tyrosine phosphorylation of the fibroblast growth factor receptors. Endocrinology 138, 107–13. doi:10.1210/endo.138.1.4836

Utton, M.A., Eickholt, B., Howell, F. V, Wallis, J., Doherty, P., 2001. Soluble N-cadherin stimulates fibroblast growth factor receptor dependent neurite outgrowth and N-cadherin and the fibroblast growth factor receptor co-cluster in cells. J. Neurochem. 76, 1421–30.

Van Roy, F., Berx, G., 2008. The cell-cell adhesion molecule E-cadherin. Cell. Mol. Life Sci. 65, 3756–3788. doi:10.1007/s00018-008-8281-1

Vedula, S.R.K., Ravasio, A., Anon, E., Chen, T., Peyret, G., Ashraf, M., Ladoux, B., 2014.

Microfabricated environments to study collective cell behaviors. Methods Cell Biol. 120, 235–52.

Williams, E.J., Furness, J., Walsh, F.S., Doherty, P., 1994. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. Neuron 13, 583–94.

Williams, E.J., Williams, G., Howell, F. V, Skaper, S.D., Walsh, F.S., Doherty, P., 2001. Identification of an N-cadherin motif that can interact with the fibroblast growth factor receptor and is required for axonal growth. J. Biol. Chem. 276, 43879–86.

Yamaguchi, T.P., Harpal, K., Henkemeyer, M., Rossant, J., 1994. fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. Genes Dev. 8, 3032–3044.

Zaromytidou, A.-I., 2014. Mechanotransduction in collective cell migration. Nat. Cell Biol. 16, 628–628. doi:10.1038/ncb3008

Manuscrit 2

Role of FGFR and regulation N-cadherin cross talk in collective cell migration of myogenic cells

Authors Thao Nguyen, René-Marc Mège

Institut Jacques Monod CNRS, Université Paris Diderot, Paris.

Abstract

Collective cell migration, required for proper development, organogenesis and tissue homeostasis refers to the movement of groups of cells attaching to each other. The efficacy of collective migration is controlled by the cohesion between member cells inside the monolayer. Too tight adhesion between cells hinders the cluster to migrate into narrow space whereas too loose interactions lead to cells dispersion and loss of tissue integrity. N-cadherin mediates dynamic cell-cell adhesion and is thus considered as a migration enhancer for cancer cells or neural crest cells. FGFR has been shown to be either enhanced or repressed N-cadherin-mediated adhesion dependent on cellular contexts. The two proteins are upregulated in invasive cancer cells. Moreover, FGFR and N-cadherin share signaling partners involved in cell migration. Surprisingly, their synergistic action in cell migration is poorly studied. Here, we report that FGFR1 increases the collective migration of myogenic C2C12 cells expressing N-cadherin. . FGFR1 expression confers a leader role to N-cadherin expressing cells at the migration front. FGFR1 also reinforces cell-cell contacts, especially of front cells in migrating sheet. This increases the cohesion of the monolayer at the front of migration and reduces the fluidity of the monolayer therefore promote the whole sheet to migrate as a unique entity.

Introduction

Cadherin based cell-cell adhesion is essential for maintaining tissue integrity but also for cell migration, implying the existence of mechanisms for coordinating cell adhesion and cell migration (Camand et al., 2012; Luo and Radice, 2005b). The coordinated movement of groups of cells, known as collective cell migration, happens during morphogenetic, physiological and pathological processes ranging from gastrulation to organogenesis, from tissue repair to tissue invasion in cancer. In this type of migration, cells remain associated to neighbors through intercellular junctions, which ensure cell-cell cohesion, mechanical integrity, cell polarity and cell-cell signaling. Increasing cell-cell adhesion converts dispersed cells towards collective migratory ensembles (Duband et al., 2009). On the contrary, reducing cell-cell adhesion by blocking cadherin function causes the disruption of cell collectives towards individual migratory cells (Vedula et al., 2012).

Dependent on cell type and cellular environment, the neuronal cadherin (N-cadherin) can support cell migration or participate in contact inhibition of cell migration (Broders-bondon et al., 2016; Kuriyama and Mayor, 2008). For instance, whereas N-cadherin expression facilities loose interaction of migrating epithelial cells, it inhibits the motility of endothelial cells and astrocytes (Camand et al., 2012; Luo and Radice, 2005b). During development, inactivation of N-cadherin in mice and zebrafish results in severe neural tube defects (Lele et al., 2002; Radice et al., 1997) and it was shown that those defects are correlated with aberrant cell migration (Taniguchi et al., 2006a). In the chick, inhibition of N-cadherin by injection of antibody disrupts somites and impairs cranial development (Duband et al., 1987). It alters the directed migration of neural crest cells, which accumulate outside the neural tube. The latter is distorted, overgrown and folded (Akitaya and Bronner-Fraser, 1992). During *Drosophila* wing development, overexpression of N-cadherin delays initiation and decreases efficiency of glial chain migration whereas N-cadherin knockout using RNAi increases the speed of migration. Thus, N-cadherin levels control glial chain collective migration (Kumar et al., 2015). In agreement, altered expression of N-cadherin in *Xenopus* causes dramatic effects on neural crest cell collective migration. Inhibition of N-cadherin using an antisense morpholino increased the motility of neural crest cell clusters (Scarpa et al., 2015). These cells disperse more rapidly than control cells and produced numerous protrusions on top of each other with wide membranous overlapping. At the opposite, overexpression of N-cadherin blocked completely neural crest migration (Scarpa et al., 2015).

N-cadherin controls neural crest cells collective migration by regulating cell-cell adhesion(Kuriyama et al., 2014b). Indeed, disruption of N-cadherin-mediated cell-cell adhesion leads to cell dispersion and loss of cell cooperation causing loss of directional migration. On the contrary, strongly cohesive sheet of neural crest cells can undergo directional migration but fail to invade narrow spaces because of their incapability to exchange neighbors (Kuriyama et al., 2014b). This indicates that the levels of N-cadherin must be tightly regulated for cell-cell contacts between neural crest cells to achieve an optimal plasticity and cells migrate properly.

This is also true in cancer cell metastasis where N-cadherin controls the balance between suppression and promotion of cancer cells migration and invasion. For example, transfection of exogenous N-cadherin into breast cancer cells increases cell migration, invasion and metastasis by regulating the adhesion of clusters of cancer cells to the stroma (Hazan et al., 1997). In prostate cancer development, N-cadherin by mediating weak cell-cell contacts is critical for efficient prostate cancer cells migration and invasion (Cui and Yamada, 2013). In contrast, complete disruption of N-cadherin-mediated cell-cell junctions is critical in the pulmonary metastasis of osteosarcoma cells (Kashima et al., 2003).

To decipher the diversity in the role of N-cadherin in cell migration, we looked at FGFR as a candidate regulator of N-cadherin-mediated cell-cell contacts and collective cell migration. Indeed, FGFR and N-cadherin have similar feature of subcellular localization in similar physiologic or pathologic conditions; they are both upregulated in cancers. N-cadherin and FGFRs are known to control cell adhesion, cell migration and metastasis while sharing common partners and signaling pathways. Inhibition of FGFR leads to the decreased expression of N-cadherin and consequently to the reduction of cell motility in pancreatic cancer (Taeger et al., 2011). Hazan's lab showed a mutual contribution of N-cadherin and FGFR to the invasion of breast cancer or lung cancer cells into matrigel (Suyama et al., 2002) and this, through the promotion of EMT(Qian et al., 2013). These authors suggest that N-cadherin sensitize tumor cells to resist against FGFR inhibition. Their study in 2002 concludes that N-cadherin enhances FGFR signaling by attenuating ligand-induced internalization (Suyama et al., 2002). Interestingly, in pituitary cancer, loss of membranous N-cadherin is correlated with cytoplasmic FGFR expression and invasive characters of these cells(Ezzat et al., 2006). Selective inhibition of FGFR with PD173074 results in recovery of

membranous N-cadherin and a significant reduction in tumor volume with less invasive growth.

Although these roles of FGFR and N-cadherin in cell migration have been studied, their combined effects remain poorly understood. Here, we used the myogenic C2C12 cell model to study (1) how FGFR regulates cell-cell junctions in expending monolayers and (2) to assess the affect of this regulation on collective migration. C2C12 cells express endogenously N-cadherin and are highly motile. They can migrate as monolayer with very dynamic cell-cell adhesion but also easily escape from the cluster to migrate as single cells. Thus, C2C12 cells monolayer is valuable tool to study the changes in cell migration correlated with cell-cell junction.

In this study, we showed that FGFR1 expression enhances the collective migration by regulating N-cadherin mediated cell-cell junctions. FRAP experiments revealed an increased stability of N-cadherin at the cell-cell contacts at the front of migration whereas this stability was not affected at the rear. The increased stability of N-cadherin at the front induced by FGFR1 expression leads to the reduction of N-cadherin retrograde flow at the cell-cell contacts. This could reinforce the cohesion between cells in the monolayer. Accordingly, immunostainings showed an increased recruitment of N-cadherin-associated proteins in cells expression FGFR1. Moreover, the co-culture of non-transfected cells and cells expressing FGFR1 revealed the pioneer role of FGFR1 expressing cells. We conclude that FGFR1 enhances the capacity of N-cadherin expressing cells to migrate collectively by reinforcing cell-cell contacts inside the cell sheet and therefore favors the migration of the collective as a unique element.

Results

FGFR1 enhances collective migration of N-cadherin expressing cells by increasing cohesion of the cell sheet

Our previous study (manuscript 1) showed that FGFR1 decreases the migration of individual N-cadherin expressing cell over a N-cadherin-coated surface by increasing the strength of N-cadherin-mediated adhesion. We questioned here the role of FGFR in the collective migration of cluster of cells adhering to each other by N-cadherin-mediated

contacts. We used cell layer expansion after release of confinement as migration test (Vedula et al., 2012). Microfabrication techniques were adopted to produce 200 µm width fibronectin-coated lines. One part of the lines array was covered by a block PMDS and cells were seeded over the uncovered zone. When confluence was reached, the block was removed inducing cell expansion in the lines to the free surface. Experiments were performed with myogenic C2C12 cells stably expressing exogenous FGFR1 (C2C12 / FGFR) allowing the study of FGFR1 effects on endogenous N-cadherin. This model provides well-defined initial conditions as well as precise spatial control of the size and geometry of free space that the cell sheet invades. The migration front was followed over 21 hours and analysed for its progression and roughness. Both C2C12 and C2C12 / FGFR cells monolayers expended linearly. However, FGFR expression increased the migratory capacity of C2C12 cells as revealed by the faster progression (Fig. 1A) and the higher speed of front progression (Fig. 1B, left). At the front, C2C12 cells had tendency to escape from the sheet to migrate individually (Movie 1 and 2) resulting in a higher value of roughness (Fig. 1B, right). These observations suggest that C2C12 / FGFR cells attach more to their neighbours while C2C12 cells escape from the sheet as a result of reduced cell-cell cohesion.

In order to see if the higher migratory capacity of C2C12 / FGFR comes from an autonomous migratory behaviour, we tracked individual C2C12 and C2C12 / FGFR cells seeded at low density on fibronectin substrates in unconstrained conditions (Fig. S1). Both cell types when migrating as isolated cells displayed comparable migration, suggesting that the higher migration of C2C12 / FGFR cells was a result of collective cell behaviour. We also observed that cell division was frequent during migration for both cell types. Since cell proliferation may affect directly the monolayer expansion, we asked whether the division rate was different in two cells types. We counted the number of division in function of time in both cases and confirmed that the two cell types divide at the same rate (data not shown). Thus the faster migration of C2C12 cells induced by FGFR1 expression is specific of collective behaviour.

During collective cell migration, cells in the cluster could either frequently exchanges their neighbours or retain their relative position inside the cell sheet(Trepat et al., 2009). To further analyse this aspect, we tracked the trajectories of cells located either at the front or at the back (at least five rows behind the leading edge) of the monolayer. C2C12 cells at the front changed frequently neighbours as well as migratory direction resulting in tortuous trajectories (Fig. 1C, front; Movie 1). On contrary, C2C12 / FGFR cells at the front behave

more collectively with less neighbours exchange and straighter movements (Fig. 1C, front; Movie 2).

The behaviours of cells at the rear were similar as those observed at the front for each cell type. C2C12 cells at the rear had twisted trajectories while C2C12 / FGFR showed straight tracks (Fig. 1C, rear). Thus, the trajectories of C2C12 / FGFR cells appeared straighter at the front and at the rear compared to those of C2C12 cells. To confirm these observations, we analysed the linearity of cell trajectories in each type of monolayer. This parameter was defined as the ratio L/l, with l = straight distance between final position and initial cell position one, L = cumulative distance covered by the cell during the same time (Fig. 1D). C2C12 / FGFR cells migrate more persistently than C2C12 cells both at the front and at the rear with less exchange of neighbours. These results suggest FGFR1 expression increases the cohesion between cells at the front and at the rear of the migrating sheet. To put aside an effect of the constraint on cell migration, we performed with the same cells, migration experiments in an unconstrained environment. Cells were seeded on a Petri dish (d=5 cm) surface on which was previously deposited a 0,3 by 4,5 cm PDMS block, then induced to migrate by releasing the PDMS block. Both C2C12 and C2C12 / FGFR cells aligned at the front before migrating (Movie 3 and 4). However, as observed in the 200 µm-width lines migration assay, FGFR1 expression increased the displacement speed of the front of migration of the monolayer (Fig. S2). Furthermore the front appeared smoother, suggesting that C2C12 / FGFR cells are more cohesive than C2C12 cells.

Taken together, these results suggest that the collective cell migration of C2C12 cells is enhanced by FGFR1 expression, which may stabilize N-cadherin-mediated cell-cell contacts formed between neighbouring cells (see manuscript 1).

FGFR increases the stability of N-cadherin at the cell-cell contacts

We test if FGFR1 also enhances N-cadherin-meditated cell-cell contacts stability in collectively migrating cells, we performed FRAP experiments on DsRed-N-cadherin in collectively migrating Ncad and Ncad / FGFR expressing HEK cells (Fig. 2A). FRAP was performed on two types of cell-cell contact regions: one type located in the first row of cells at the front of migration and the second situated at least 5 rows of cell behind (Fig. 2A).

First, we compared the junctional mobility of N-cadherin at the front versus the back of Ncad or Ncad / FGFR expressing cell sheet, separately. In N-cadherin expressing cell monolayers, the mobile fraction of N-cadherin in junctions situated at the front was

significantly higher than the mobility fraction of those situated at the back (Fig. 2D, grey and dot grey). In Ncad / FGFR expressing cell monolayer, N-cadherin had the same dynamic at the front and at the back (Fig. 2 D, black and black dot). These observations suppose that Ncad expressing monolayer have intrinsically a decrease of N-cadherin mobility at cell-cell contacts at the front. At the opposite, Ncad / FGFR cell sheet cohesion is more homogenous front the front to the back.

Next, we compared the mobility of N-cadherin at cell-cell contacts either at the front or at the back of N-cadherin expressing cell sheet to Ncad / FGFR expressing one. FGFR expression induced an important decrease in the N-cadherin mobile fraction at the cell-cell contacts formed between cells of the front of migration (Fig. 2B,D). However, it did not affect the dynamics of N-cadherin in cell-cell contacts located in the back of the migrating sheet (Fig. 2C,D). Taken with the previous observations, these results suggest that FGFR1 strongly enhances the cohesion between N-cadherin expressing cells specifically at the front.

The analysis of DsRed-Ncad recovery half-times further confirmed that FGFR1 expression significantly decreased the mobility of N-cadherin molecules at the front (Fig. 2E; $t_{1/2}$= 2 ± 0.4 s for Ncad cells versus $t_{1/2}$= 5 ± 0.5 s for Ncad / FGFR cells. This effect was not observed at the rear (Fig. 2E). Taken together, FGFR1 decreases N-cadherin mobility at the cell-cell contacts at the front of migrating sheet. This results in reinforced the cohesion of cell sheet at the front may promote efficient collective migration.

FGFR decreases the N-cadherin retrograde flow in cell-cell junctions forming at the front of migration

Initiation of cell sheet migration was correlated in the astrocyte cell model with the initiation of cadherin retrograde flow at the junction between cells positioned in the front zone of moving cell sheet (Kametani and Takeichi, 2007; Peglion et al., 2014). This retrograde flow is controlled by the prevalence as well as the mobility of cadherins (Peglion et al., 2014). Since our previous experiments (manuscript 1) revealed the role of FGFR1 in regulating N-cadherin mobility at the cell-cell contacts, we studied its role in the control of the retrograde intercellular flow of N-cadherin at the front of migration. C2C12 and C2C12 / FGFR cells transiently overexpressing DsRed-Ncad were seeded on fibronectin-coated 200 µm lines. When cells reached confluency, the constraint was released and cell monolayer were let expand during 2 hours before imaging. Cell-cell junctions of the two first rows of cell and

parallel to the direction of migration were analyzed (Fig. 3, left). Kymograph analysis showed that both cell types showed a front-rear flow of N-cadherin at the cell-cell contacts. However, FGFR1 expression decreased significantly this retrograde flow (Fig. 3, right). Thus, we conclude that FGFR1 expression stabilizes N-cadherin cell-cell contacts leading to decreased dynamics of cell-cell junctions during migration. This probably helps maintaining a linear front for an efficient migration.

FGFR responsive cells act as pioneer cells to drag the sheet movement

At this point, our experiments show that FGFR1 expression has a positive effect on the directed cell movement and it stabilizes N-cadherin-mediated cell-cell contact at the migration front. Moreover, we also observed that C2C12 / FGFR cells displayed large protrusions at the free edge but remained cohesive with neighbours at the back. As a consequence, C2C12 / FGFR cells shape at the front is more stretched than in C2C12 cells (Movie 1 and 2). Therefore, we hypothesized that the effect of FGFR1 expression is more important at the front therefore could confer a leader roles for cells at this level to guide and drag the whole cell sheet. To test this hypothesis, we performed coculture experiments by mixing native C2C12 cells with C2C12 / FGFR1-GFP cells on the fibronectine-coated lines. The two populations of cells were homogenously distributed inside the confluent monolayer (data not shown) before releasing the constraint. Cells were fixed and stained for F-actin and N-cadherin after 8 hours of migration and the spatial relationship between C2C12 and C2C12 / FGFR-GFP cells was analyzed. Images showed that the front of migration was constituted nearly exclusive of C2C12 / FGFR cells (yellow=merged between mCherry-Ncad and GFP-FGFR signal) whereas C2C12 cells (red) localized at few rows behind the leading edge (Fig. 4). We noticed that C2C12 / FGFR cells at the sheet margin behave as pioneer cells, projecting large lamellipodial protrusions into the open space while maintaining contacts with their neighbors (Fig. 4, black head arrows). Some C2C12 cells situated behind were oriented in the same direction of pioneers C2C12 / FGFR cells. F-actin-staining showed that actin stress fibers were oriented in a front-rear direction in C2C12 / FGFR cells as well as in C2C12 cells already engaged in the direction of migration. On the contrary, follower C2C12 cells not aligned with the C2C12 / FGFR pioneer cells showed a multidirectional actin meshwork (Fig. 3). This raises the hypothesis that FGFR1 expression favors the alignment of cells and their actin meshwork at the front to promote the directed movement of the C2C12 monolayer.

Moreover, we observed that cell-cell contacts of the C2C12 / FGFR pioneer cells were more mature presenting thinner and straighter aspects than that of followers C2C12 cells (blue arrows). The latters exhibit cryptic lamellipodia that protrude underneath neighbor cells (red arrows).

Together, these observations suggest that FGFR1 expressing cells function as pioneer, moving into cell-free space. With enhanced cell-cell cohesion with neighbors, these cells guide or pull onto neighbors to create a unique directed sheet movement.

FGFR enhances junctional proteins and acto-myosin network recruitment sealing cells sheet at the front of migration

C2C12 / FGFR cells displayed important protrusions at the front and their cell-cell contacts were likely more mature than those of C2C12 follower cells. Thus, FGFR1 seems to promote pioneer cells protrusion at the leading edges and increase cell-cell contact robustness. We confirmed these observations by immunostaining of p120 and vinculin in migrating C2C12 and C2C12 / FGFR cell sheets (Fig. 5B). The cytoplasmic partner of N-cadherin, p120, is acknowledged to stabilize the latter at the cell-cell junctions (Reynolds and Carnahan, 2004), while vinculin has been reported to accumulate in a force-dependent manner at cell-cell contacts during cadherin adhesions maturation (le Duc et al., 2010). However, vinculin is recruited both at cell-cell and cell-substrate contacts and its junctional repartition depends on the maturity of cadherin mediated cell-cell contact (Twiss and de Rooij, 2013). In our experiment, p120 was recruited to the cell-cell junctions in both cell populations (Fig. 5 B). However, its recruitment was more important in the C2C12 / FGFR cells (red arrows). Vinculin was recruited both at cell-cell and junctions and cell-substratum adhesions. Interestingly, FGFR1 expression increases vinculin recruitment at the cell-cell contacts compared to C2C12 cells without FGFR1.

The actomyosin network was also stained since it is the machinery controlling cell shape and migration (Le Clainche and Carlier, 2008). C2C12 cells displayed moderate protrusions at the front of migration whereas C2C12 / FGFR displayed large protrusions as expected for leader cells (Fig. 5A). Actin fibers at the front of migration in both cell types were aligned with cell's long axes in the monolayer. However, actin fibers in C2C12 / FGFR cells were more intense and much longer than that of C2C12 cells. Moreover, the distribution of actin fibers was not similar between cells at the front. C2C12 cells displayed mixed

populations of stress fibers containing dorsal (arrows) and arc (red head arrows) fibers. In contrast, C2C12 / FGFR cells at the front displayed only dorsal stress fibers (arrows) which are similarly directed from one cell to another (Fig. 5 A). In both cell types, the distribution of Myosin IIB had the same feature as that of F-actin with higher intensity of Myosin IIB in C2C12 / FGFR (Fig. 5 A). Merged images showed that Myosin IIB is excluded from the cell-cell junctions and protrusions of C2C12 cells (white head arrows).

Taken together, FGFR1 increases lamellipodia extension, recruitment of p120 and vinculin at the cell-cell contact, actomyosin overall distribution at the front of migrating sheet. Interestingly, FGFR1 induces actin alignment of cells at the front in the migratory direction, comforting the idea of leader that FGFR1 expressing cells behave as leader cells. Thus FGFR1 may coordinate cell-cell adhesion, cell-substratum adhesion and the actomyosin network.

Discussion

N-cadherin and FGFR1 have been separately shown to modulate cell migration by regulating cell-cell contacts, especially in the case of cancer migration where both proteins are up-regulated. Here, we studied the synergistic roles of FGFR1 and N-cadherin in the control of cell-cell adhesion as a mechanism to regulate collective cell migration. Indeed, collective cell migration requires coordination of cell-cell adhesion and of motile and protrusive activities of cells inside the cluster. To study collective cell behaviors in well-defined and reproducible conditions, we analyzed expansion of C2C12 cells or stably FGFR1 expressing C2C12 cells seeded on confined fibronectin space after release of a physical barrier. This model keeps the cells intact as they encounter an empty space. C2C12 cells were chosen for their endogenous expression of N-cadherin and their dynamic cell-cell contacts. Overall our results indicate that FGFR1 expression enhances collective cell migration of N-cadherin expressing cells by stabilizing cell-cell interactions. This results in cell moving straighter with less exchange of neighbors. Thus, FGFR1 expression seems to reduce the fluidity of the C2C12 monolayer leading to an enhanced collective migration as a unique entity.

Capacity of cells to exchange neighbors is favored by loosen contacts. We evaluated the dynamics of N-cadherin mediated cell-cell contacts either at the front or at the back of the cell monolayers using FRAP. Interestingly, in C2C12 cell sheets, N-cadherin mobility at the

cell-cell contacts is higher at the front than at the back of the migrating sheet, suggesting an intrinsic mechanism of collective cell migration, as showed by (Peglion et al., 2014). This may explain why C2C12 cells at the front can disrupt their junctions from neighbors, escape from the monolayer and migrate individually into the free space. When FGFR1 was expressed, the mobility of N-cadherin at the cell-cell contacts was similar at the front as at the back. Indeed, FGFR1 expression specifically reinforces cell-cell contacts at the front of the monolayer. This may increases the cohesion of the front of the monolayer. Thus, the capacity to exchange neighbors and escape were hindered in cells at the edge of C2C12 / FGFR migrating sheet, compared to C2C12 one. This result is consistent with our previous study that showed that FGFR1 stabilizes cell-cell contacts by decreasing N-cadherin mobility (see manuscript 1). In this first study, FRAP experiments were performed on small cluster of cells where all cells were facing cell-free surface, similarly to migrating front. This could explain why we did not observed, in this first study, cell-cell contacts in which N-cadherin mobility was not affected by FGFR1 expression.

The decreased mobility of N-cadherin at the front induced by FGFR1 expression resulted in a decreased retrograde flow of N-cadherin at the cell-cell contacts at the front. This front-to-rear flow has been described previously for astrocytes (Peglion et al., 2014). The front to back treadmilling of N-cadherin was ensured by a reversed flow of N-cadherin recycling from the back to front, allowing to maintain N-cadherin availability at the front of migration necessary to buit new interecellular junctions. This N-cadherin treadmilling cadherin is also observed here in C2C12 cells. It is significantly decreased by FGFR1 expression. We showed previously that FGFR1 reduces N-cadherin endocytosis to enhance cell-cell contact strength (see manuscript 1). In this model, it is highly conceivable that FGFR1 trapped N-cadherin at the cell-cell junctions therefore decrease directly the N-cadherin retrograde flow. Moreover, it may equally reduces N-cadherin trafficking speed, therefore slows down the N-cadherin mediated cell-cell contacts dynamic. This dynamics may promote strong attachment of cells within the monolayer that favor collective migration. The role of FGFR1 in tightening cell-cell contacts through mediation of N-cadherin endocytosis is partly confirmed by p120 and vinculin staining. Indeed, FGFR1 increases p120 signal at the cell-cell contacts.

FGFR1 expression confers a leader role to C2C12 cells at the front of migrating sheets. In co-cultures of C2C12 cells and C2C12 / FGFR cells, migrating sheet contained quasi-exclusive C2C12 / FGFR cells. They display very large protrusive membranes and

abundant stress fibers aligned to the migratory direction, well stretched and establish robust contacts with the trailing cells. Interestingly, FGFR1 non-expressing cells become oriented even when FGFR expressing cells are positioned to their sides. Therefore, FGFR1 may increase the efficiency of collective migration of N-cadherin expressing cell sheet by guiding and dragging the whole cell sheet persistently into a directed movement. In the future, live-imaging study could be done to see whether leader(s) cell(s) persist(s) during long-term migration.

During collective migration, cluster of cells move due to the traction force induced by the individual cells pulling on the surrounding matrix (Trepat et al., 2009)The first row of cells at the migrating edge exert outward traction forces to direct the migration of the whole cell sheet (tug-on-war mechanisms). The anterior traction forces generated by cell-substratum adhesion are balanced by the tensile forces at cell-cell junctions of follower cells at the rear. Follower cells can also engage in cell-substrate traction forces, possibly as a consequence of 'cryptic lamellipodia' and transmit forces across a longer distance an multiple cell bodies within moving cell sheets (Bazellières et al., 2015; Trepat et al., 2009). Thus, both leader and, to a lesser extent, follower cells generate traction forces toward the substrate, which are balanced with the forces extending across cell-cell bodies. Therefore, cells generate long-range gradients of intercellular tension by engaging in a multi-cellular tug of war to drive collective movement. Our preliminary results showed that C2C12 / FGFR cells recruited more vinculin to their cell-cell junctions at the edge of the migrating sheet, compared to C2C12 cells. Thus, FGFR could possible enhance the imbalance between cell-substrat and cell-cell adhesion to arise a superior net force then promote the migration.

In sum, our work pointed out the positive cross talk between FGFR1 and Ncadherin in promoting collective cell migration. Thus, FGFR1 expression may increase the efficiency of collective cell migration by 1) increasing intercellular contacts 2) coordinating cell-cell adhesion and actomyosin organization 3) conferring enhanced leader cells characteristic to cells at the migrating front.

Materials and Methods

Cell culture, transient cell transfection and generation of stable cell lines

Mouse myoblasts C1C12 cells and Human Embryonic Kidney (HEK) cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 100 IU of penicillin, 100 µg/mL streptomycin, at 37°C in 5% CO^2. C2C12 cells were transiently transfected with the plasmids encoding GFP tagged hFGFR1 (Saffell et al., 1997), via electroporation thanks to the Amaxa Cell Line Nucleofector (kit V, program X-032), resulting in more than 60% transfection efficiency. To generate FGFR-GFP stable C2C12 cell lines, transfected cells were grown under a selection pressure of 1 mg/mL of Geneticin. HEK cells were transiently transfected with the plasmids encoding wild type chicken N-cadherin fused to dsRed (Ncad-dsRed)(Bard et al., 2008) or / and GFP tagged FGFR1 using the same method. To generate Ncad-dsRed, FGFR-GFP and Ncad-dsRed / FGFR-GFP stable HEK cell lines, transfected cells were grown under a selection pressure of 200 µg/mL of Hygromycin B and 1 mg/mL of Geneticin or both. Drug resistant cells were then sorted out by FACS, subcloned and further cultured and maintained with half of concentration of antibiotic pressure. Cells were always used at passages ≤ 20.

Immunofluorescent staining

Cells were fixed at room temperature in PBS 2 % formaldehyde for 15 minutes, rinsed with PBS and permeabilized with PBS 0.15 % Triton X-100, 1 % BSA, then blocked for 1 hour in PBS-1% BSA. Cells were then incubated overnight at 4°C with rabbit polyclonal anti-p120 (Santa-Cruz, 1:100 dilution), anti-Phospho p120 (Abcam, 1:100 dilution), and mouse monoclonal anti-vinculin ready-to-use (obtained from Marina Klukova, Institut Curie, Paris) antibodies. Preparations were then washed 3 times with PBS and incubated with cy5-conjugated anti-rabbit IgGs or FITC-conjugated anti-mouse IgGs (Jackson ImmunoResearch Laboratories, Inc.) or cy5-conjugated phalloidin (1:400, Molecular Probes) for 1 hour at room temperature. Preparations were then mounted in Mowiol, 90% glycerol. Images were acquired with a Leica TCS sp5 confocal microscope, HCX PL APO 40X oil objective (NA=1,3) with 20 % laser power of Diode (405), Argon (488), Diode (560) and Helium (633), pinhole=1,2.

Fibronectin coated line guided cell migration

Patterned silicon stamps bearing 200 µm width lines spaced of 70 µm were prepared by soft lithography according to (Vedula et al., 2014). Patterned microcontact stamps were incubated with Fibronectin at 5 µg/cm^2, pressed on non-culture treated Petri dishes or on sonicated coverslip previously activated by deep UV (Jelight, 4 X 60W, 15 minutes). Microcontact printed surfaces were passivated by incubation with 1% Pluronics F-127 (Sigma) diluted in distilled water for 1 hour followed by 3 washes with PBS. hNcad-Fc (1µg/ µm^2) was allowed to bind for 2 hours at room temperature. Surfaces were washed three times with PBS then half of the printed zone was covered with a PDMS block and kept at 4°C before cell seeding.

C2C12 and C2C12 / FGFR expressing cells were seeded over arrays of Fibronectin-coated-200 µm width lines bearing the PDMS block, and allowed to adhere for 20 minutes in culture medium containing 1 µg/mL mytomycin. Non-adhesive cells were gently washed off and attached cell let grow in the incubator overnight. The day after, the PDMS block was lifted off and samples were either directly imaged or to let migrating for 24 hours then fixed for immuno-staining.

For live cell imaging, images were acquired with a 10 X objective, every 6 minutes during 24 hours under controlled environment (37° C, 5% CO2, Biostation Nikon). Manual tracking of individual cells over 20 hours was performed with MTrackJ plugin. Individual trajectories were positioned on an orthonormal axis with the coordinates of the cells at t_0 = (0;0). The displacements and mean body cell speed were then extracted for each condition and plotted versus time.

Fluorescence Recovery after Photobleaching

Cells stably expressing Ncad, FGFR or Ncad / FGFRwere seeded over arrays of Fibronectin-coated-200 µm width lines as described above. Four hours after the PDMS block was lifted off inducing cell migration, cells were subjected to Fluorescence recoveries after photobleaching (FRAP), performed at 37°C in a red-phenol-free DMEM supplemented with 10% serum, using a Leica TCS SP5 confocal microscope equipped with a 40 X immersion objective. FRAP was carried out by setting the double scanning mode at 560 nm for dsRed and 480 nm for GFP and the image format to 256 X256 pixels. Cell-cell contacts either at the front or at the rear (at least 5 ranges of cell behind the edge of cell sheet) of migration were photobleached. After 3 prebleach scans (0.2 sec), the circular ROI (red circular) was bleached with laser at full power. Recovery was recorded by imaging with low laser power every 0.2

sec (20 scans) then every 2 sec (20 scans) and finally every 5 sec (8 scans). The normalized recovery of fluorescence was expressed as a ratio of prebleach fluorescence after correction for photobleaching as reported previously (Lambert et al., 2007). Normalized fluorescence recovery in function of time curves were fitted with a one-term exponential equation using GraphPad Prism 5.01 software (one-phase decay non-linear regression function), allowing to extract a plateau value representing the fraction of diffusion-limited molecules (mobile fraction) and a recovery half-time ($t_{1/2}$) as a proxy the apparent diffusion coefficient of diffusion-limited molecules (Thoumine et al., 2006).

N-cadherin retrograde flow quantification

N-cadherin-dsRed nucleofected C2C12 or C2C12 / FGFR cells were seeded in 200 µm PDMS -free Fibronectin-coated-lines and grown to confluence. PDMS block was lifted off and cells were let migrate in a red-phenol-free DMEM supplemented with 10% serum. Cells were monitored 2 hours later, allowing them to grow a polarized protrusion. Live imaging was performed with an inverted microscope (Olympus) equipped with a high-sensitive cooled CCD camera (XM10, Olympus) and an oil-immersion X40 1.4 NA Objective, within an incubation chamber (5% $C0_2$, 37°C). Images were recorded every minute during 1 hour. The N-cadherin retrograde flow was quantified by kymograph analysis using ImageJ. Junctions of the second cell row from the edge of migration were drawn with segmented line.

Statistical analysis, curve fitting and image processing

Statistical analysis and curves fitting were performed with Prism 5.0 software. Differences were considered for p-value ≤ 0.05. Image processing was done using ImageJ, and then panels were assembled using Photoshop and Illustrator (Adobe).

Figure 1

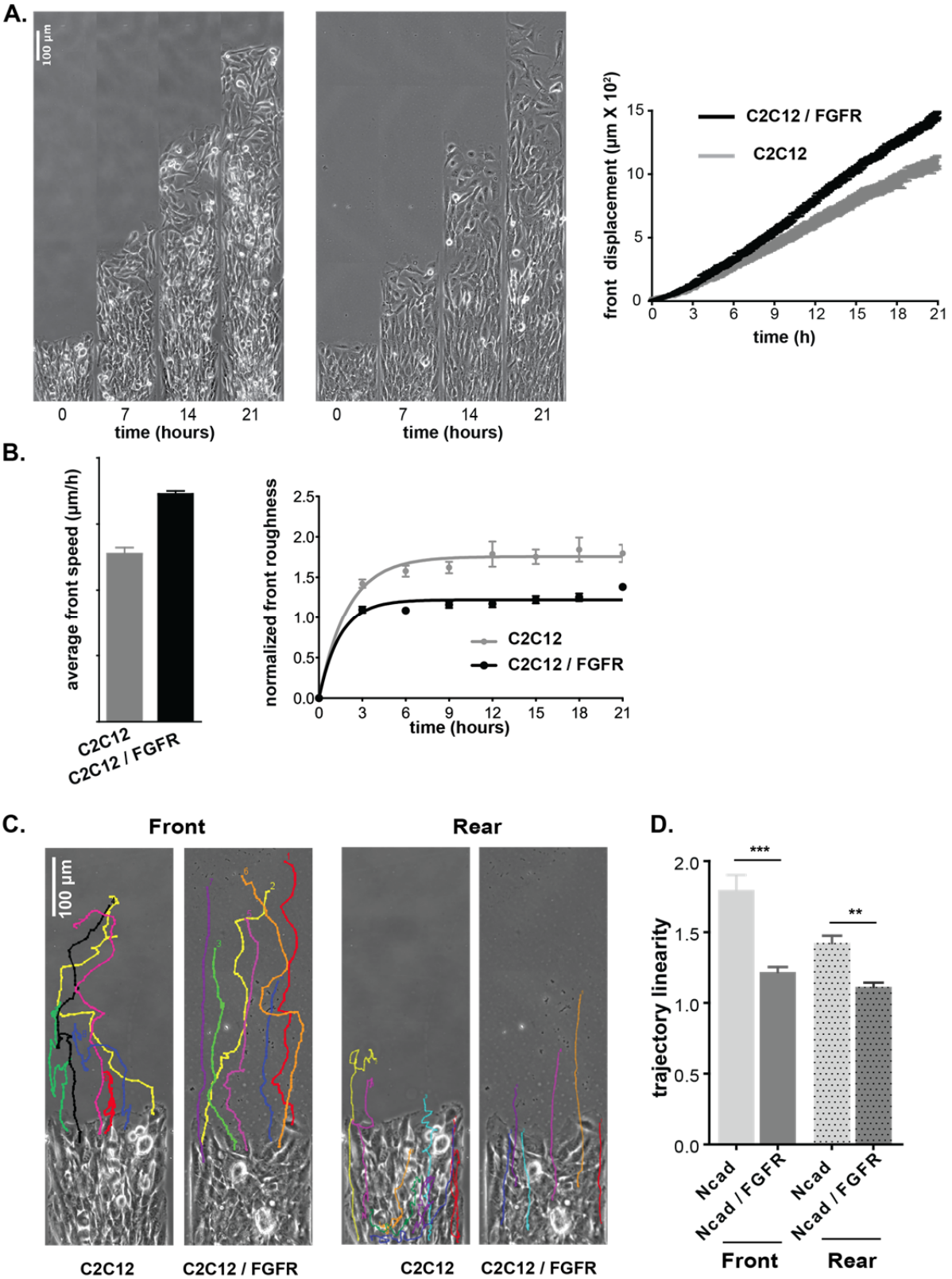

A.
100 µm
0 7 14 21
time (hours)
0 7 14 21
time (hours)
front displacement (µm X 10²)
C2C12 / FGFR
C2C12
time (h)
B.
average front speed (µm/h)
C2C12
C2C12 / FGFR
normalized front roughness
C2C12
C2C12 / FGFR
time (hours)
C.
Front
Rear
100 µm
C2C12
C2C12 / FGFR
C2C12
C2C12 / FGFR
D.
trajectory linearity
***
**
Ncad
Ncad / FGFR
Ncad
Ncad / FGFR
Front
Rear

Figure 1: FGFR increases N-cadherin expressing cells collective migration by enhancing the cohesion of the cell sheet. Cells were phase-contrast imaged starting at the time the PDMS block was removed (t_0) and for 21 h. (A) Example of the evolution of the migration front in function of time for C2C12 and C2C12 / FGFR cells. Plot shows the front displacement in function of time (n = 18 lines) for C2C12 and C2C12 / FGFR cells. C1C12 / FGFR migrate faster than C2C12 cells. (B) Histograms show the speed of migration (right) and the normalized front roughness in function of time (right) for both cases. (C) Single cell trajectories at the front and at the rear of C2C12 (left) and C2C12 / FGFR (right) cells. (D) Single cell trajectory linearity of C2C12 and C2C12 / FGFR monolayer at the front and the rear of migration. Trajectory linearity is defined as the ratio L/l, with l = straight distance between final position and initial cell position one, L = cumulative distance covered by the cell during the same time C2C12 / FGFR cells migrate on significant larger distances than C2C12 cells both at the front and at the rear with lesser exchanges of neighbours. ***, $P < 0.0001$ (paired Student's t test, n = 18).

Figure 2

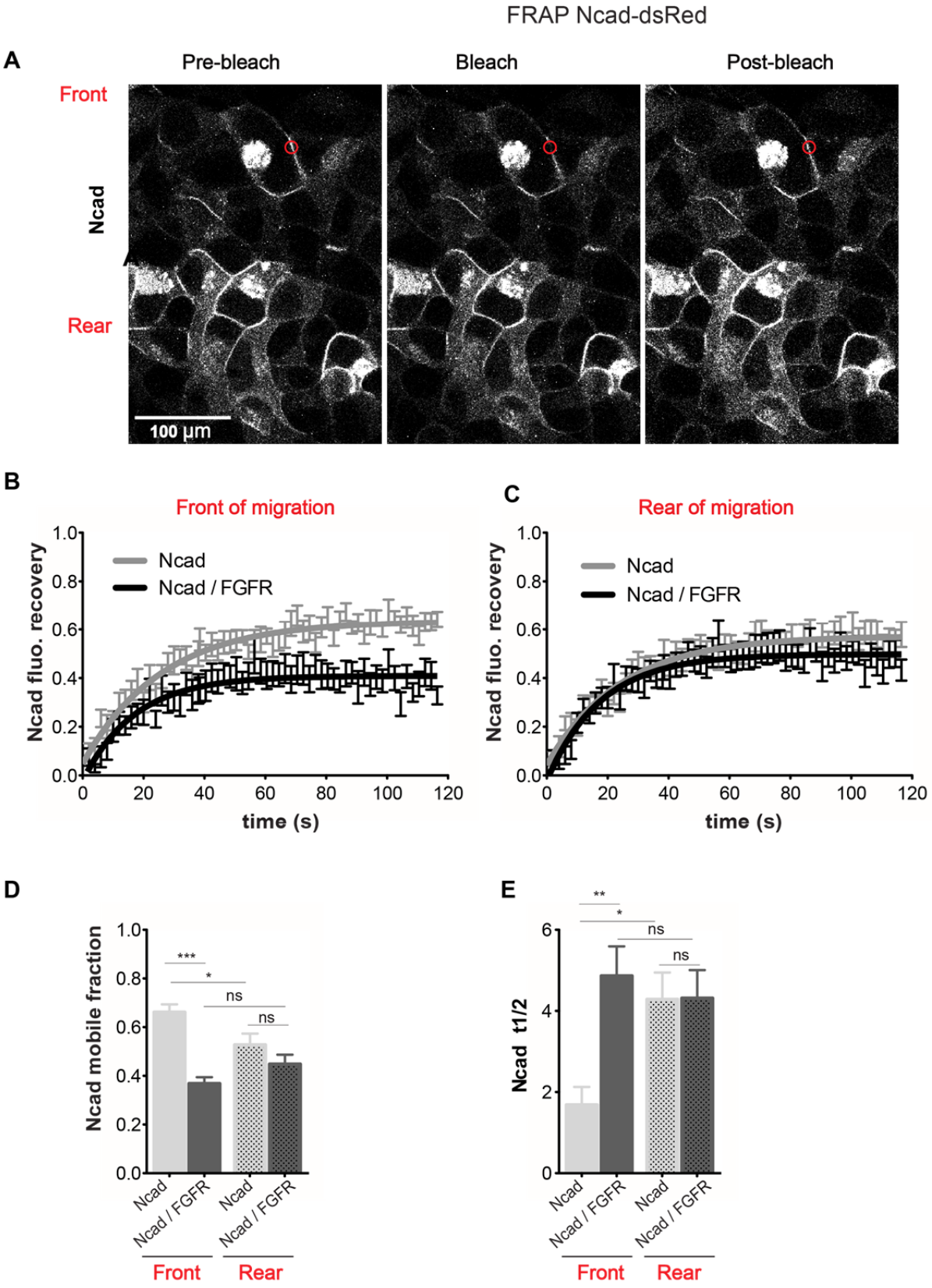

FRAP Ncad-dsRed
A
Pre-bleach
Bleach
Post-bleach
Front
Ncad
Rear
100 µm
B
Front of migration
Ncad
Ncad / FGFR
Ncad fluo. recovery
time (s)
C
Rear of migration
Ncad
Ncad / FGFR
Ncad fluo. recovery
time (s)
D
Ncad mobile fraction
***
*
ns
ns
Ncad
Ncad / FGFR
Ncad
Ncad / FGFR
Front
Rear
E
Ncad t1/2
**
*
ns
ns
Ncad
Ncad / FGFR
Ncad
Ncad / FGFR
Front
Rear

Figure 2: FGFR stabilizes N-cadherin at cell-cell contacts in HEK cells. Ncad-dsRed or Ncad-dsRed / FGFR-GFP stably expressing HEK cells were seeded on 200 μm fibronectin-coated-lines at the PDMS uncovered area and let grow overnight. PDMS block was lifted off 4h before FRAP experiments allowing cell sheets migration towards the free space. FRAP was realized either at cell-cell contacts in the first row of cell at the front of migration or at the back, at least 5 tows behind. (A) Characteristic images of N-cadherin signal before (Pre-bleach), immediately after bleaching (Bleach) and 120 s after the bleach (Post-bleach) performed on N-cad-dsRed / FGFR doubly expressing HEK cells. Red circles represent the bleached region. (B) Normalized fluorescence recovery curves versus time for Ncad-dsRed, Ncad-dsRed / FGFR-GFP expressing cells ($n \geq 40$ contacts in 18 lines) at the front (left) and rear (right) of the migrating sheet. (C) Histograms showing the mobile fraction (left) and the $t_{1/2}$ (right) extracted from a one-exponential decay fit of fluorescence recovery curves. The presence of FGFR reinforces N-cadherin stability at the cell-cell contacts at the front of migration and significantly increases the diffusion characteristic times. *, $P < 0.002$; **, $P < 0.005$; ***, $P < 0.0001$; ns, not significant (paired Student's *t* test, $n = 35$).

Figure 3

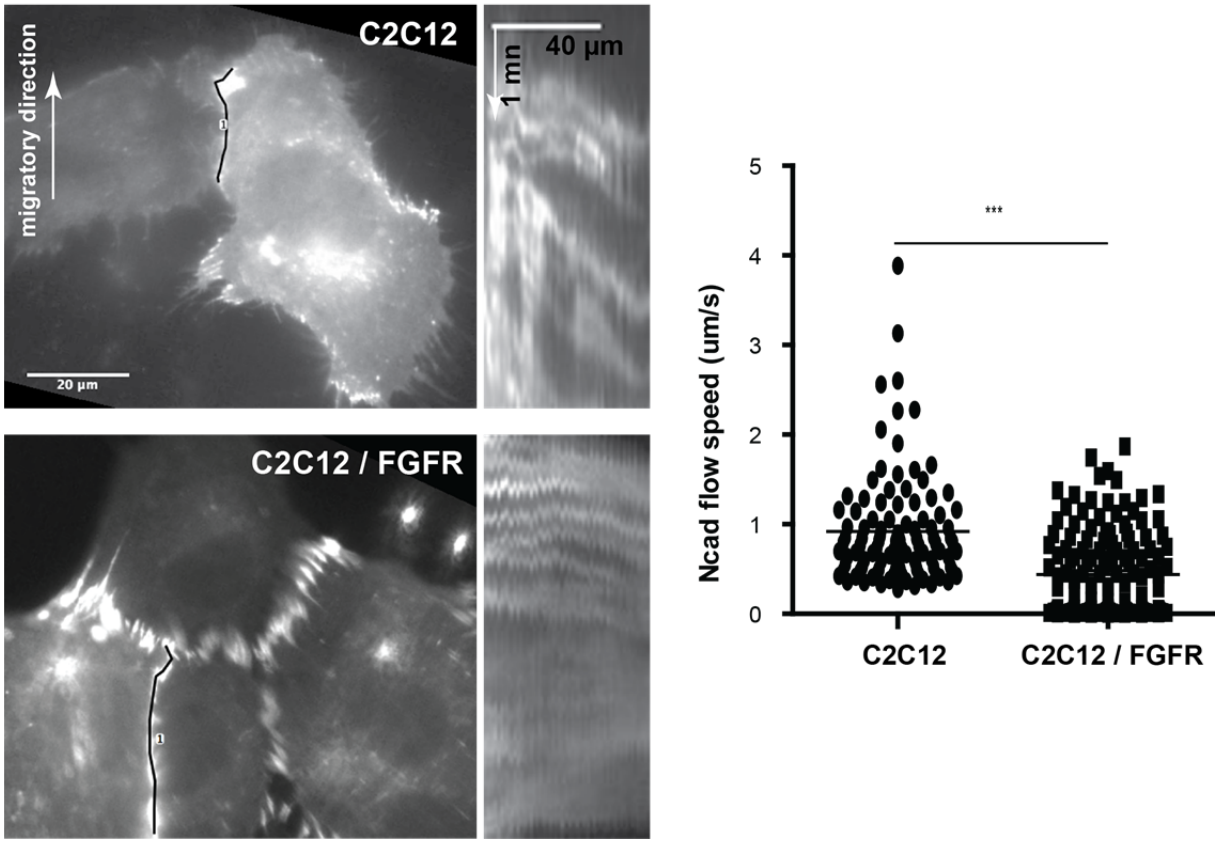


Figure 3: FGFR reduces N-cadherin retrograde flow of cell-cell contacts at the migration front. C2C12 or C2C12 / FGFR were overexpressed with N-cadherin-dsRed, seeded on PDMS free area of fibronectin-coated line. Cells were imaged after 2 hours of PDMS block release with an inverted microscope for 1 hour at a frequency of one image per second. (Left) Still images of Ncad-dsRed (upper) or Ncad-dsRed / FGFR cells (lower) at the edge of cell sheet. (Middle+Right) The N-cadherin retrograde flow was quantified by kymograph analysis (black segmented line, 3 pixel width, along the junctions, oriented in the direction of the migration (n= 120 kymographs from 12 lines). Histogram show the speed of N-cadherin flow at the cell-cell contacts for both cell types. The N-cadherin retrograde flow is significantly higher in C2C12 cells than in C2C12 / FGFR cells. ***, $P < 0.0001$ (paired Student's *t* test, n = 18).

Figure 4

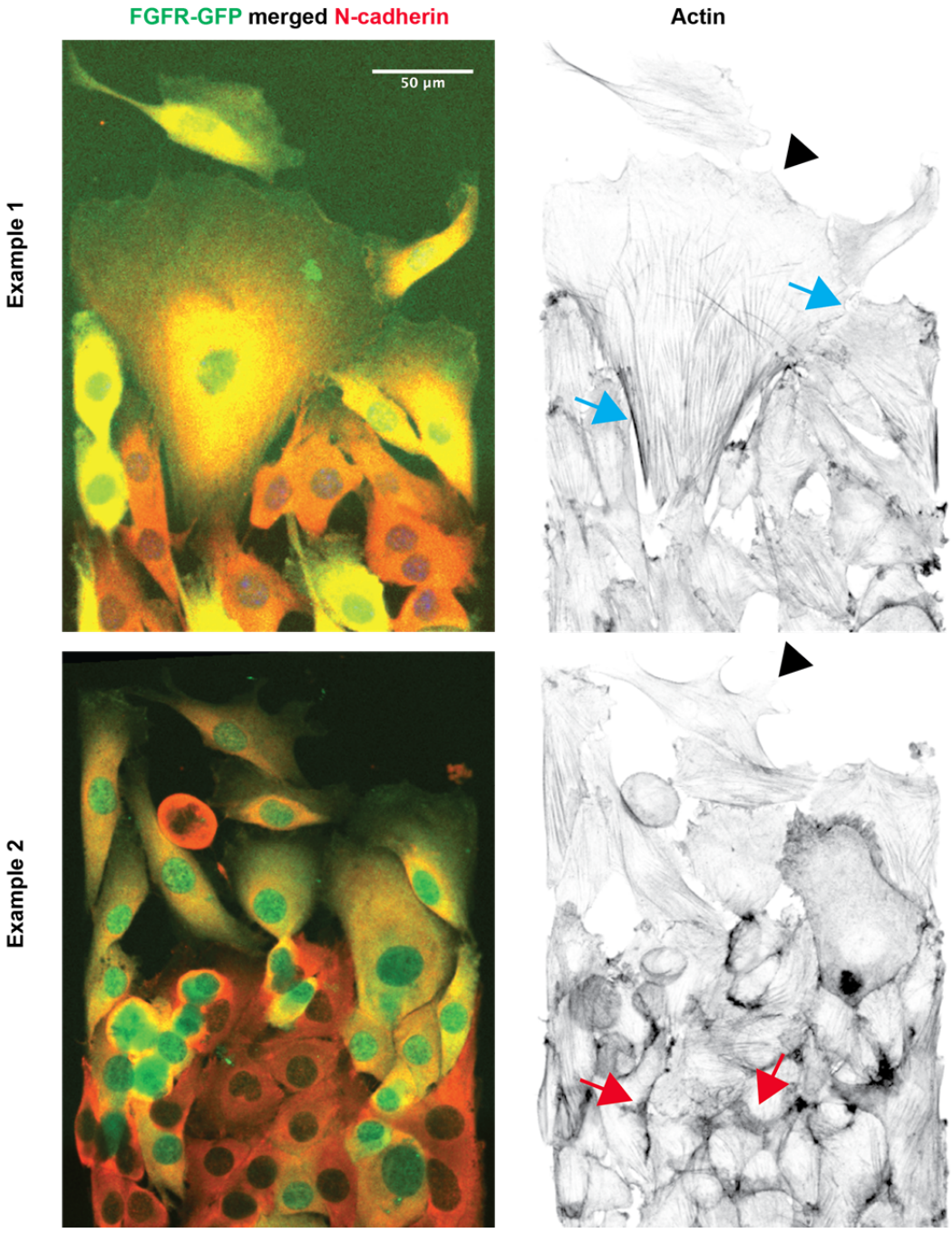


Figure 4: FGFR induces pioneer cell behaviors in mixed culture. Co-culture by mixing C2C12 cells with C2C12 / FGFR-GFP cells seeded on fibronectine-coated 200 µm width lines. Barrier was released to induce cells expansion to the free space during 8 hours. Cells were fixed and stained with F-actin and N-cadherin, two examples of the edge of migrating cell sheets. C2C12 / FGFR-GFP are localized at the first row of migrating sheets following by C2C12 cells. Actin fibers of front cells are aligned in the direction of cell migration.

Figure 5

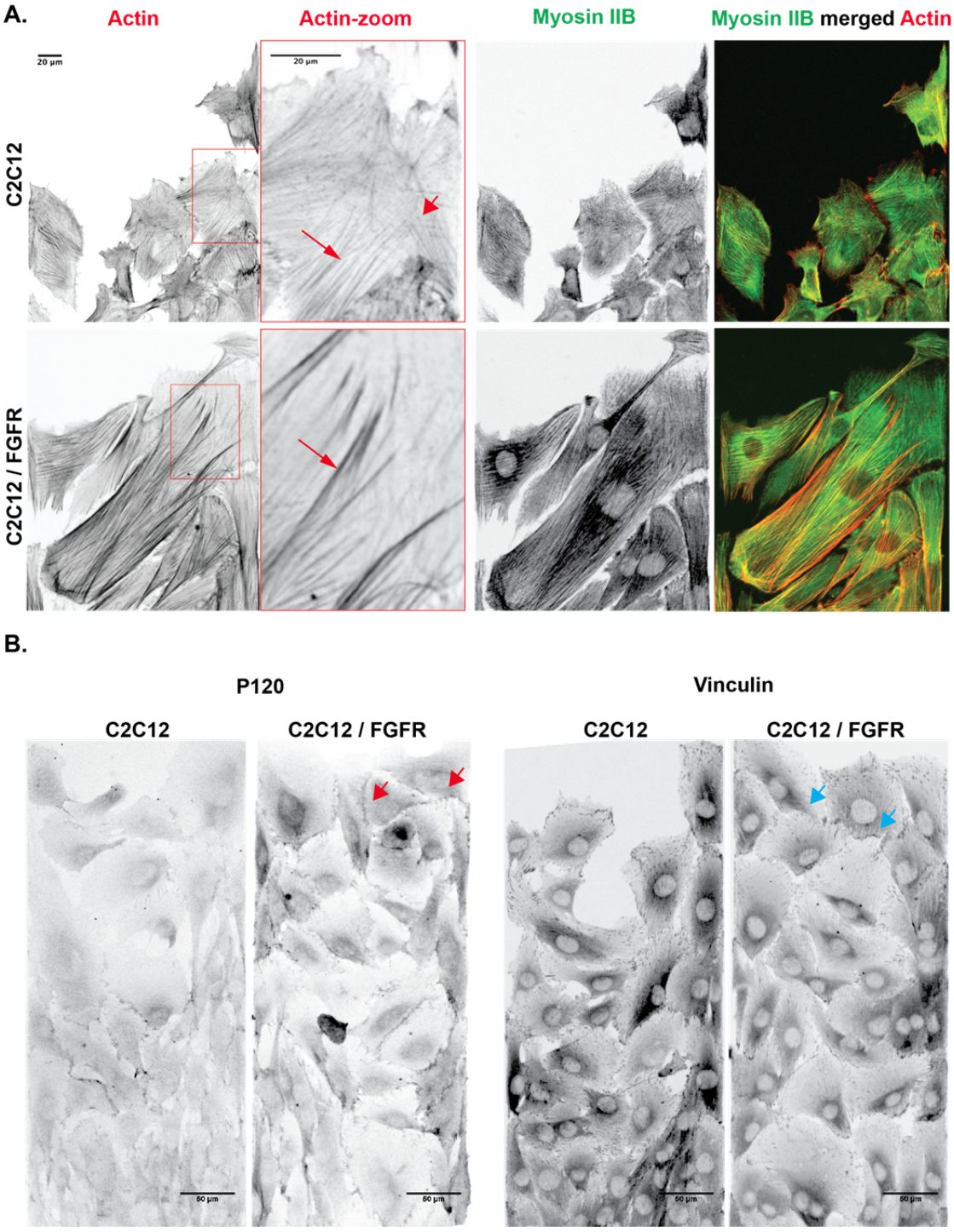


Figure 5: FGFR coordinates actomyosin network, cell-cell junction and cell-substratum adhesion. C2C12 and C2C12 / FGFR cells were fixed after 8 hours of migration and stained for F-actin, myosin, p120 and vinculin. (A) Acto-myosin staining and merge. (B) p120 (left) and Vinculin (right) staining. FGFR expression enhanced p120 and vinculin recruitment at the cell-cell junctions and oriented actin to the migrating direction.

Figure S1

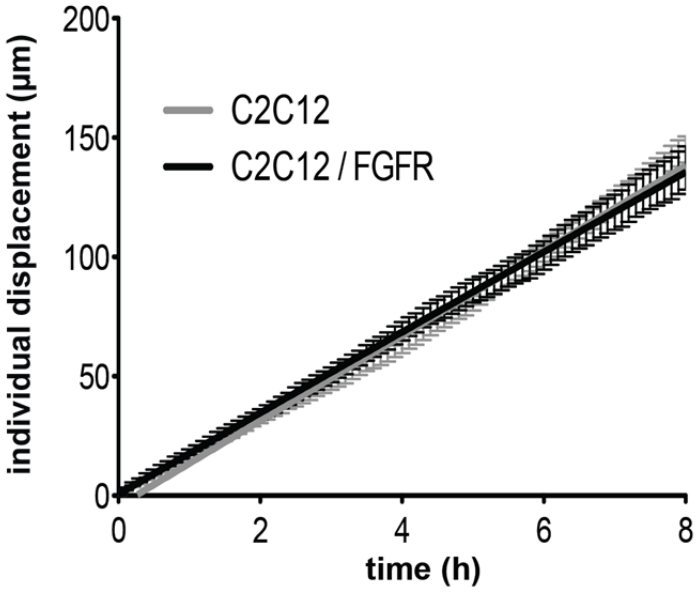


Figure S1: FGFR does not influence individual C2C12 migration on fibronectin. C2C12 or C2C12 / FGFR cells were seeded at low concentration on Petri dish (d = 5 cm), let grow overnight then phase contrast imaged for 8 hours. Plots show the displacement of single cell in function of time for C2C12 (grey) and C2C12 / FGFR (black) cells. The two cell types show similar capacity of isolated cell migration.

Figure S2

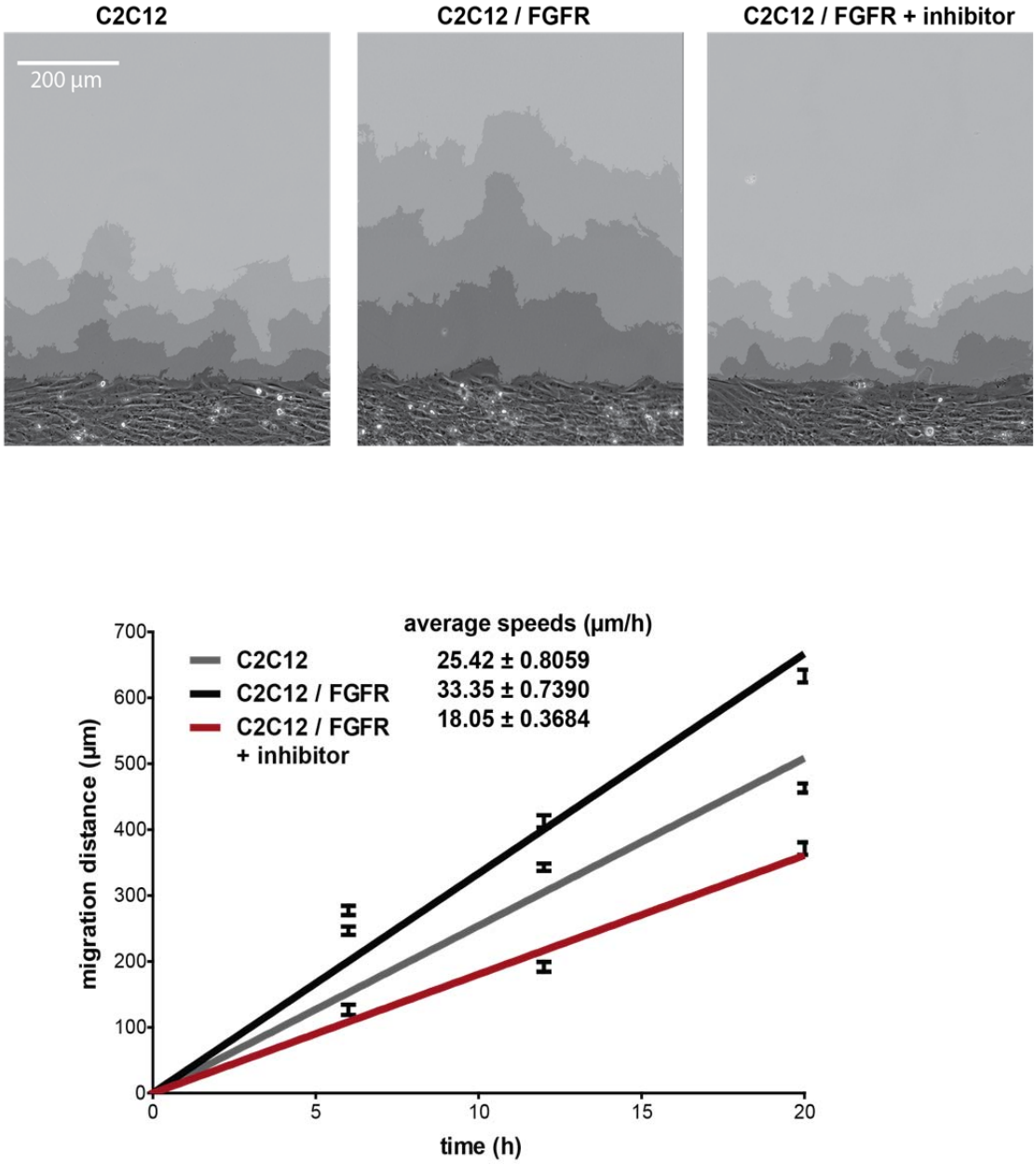


Figure S2: FGFR increases N-cadherin expressing cells collective migration in unconstraint environment. Cells were seeded on PDMS uncovered area of Petri dish (d=5 cm), and phase-contrast imaged starting at the time the PDMS block was removed (t_0) up to 21 h. (A) Example of the evolution of the migration front in function of time for C2C12, C2C12 / FGFR cells. Plot shows the front displacement in function of time (n = 18 lines) for C2C12 and C2C12 / FGFR cells. C1C12 / FGFR migrate faster than C2C12 cells. ***, $P < 0.0001$ (paired Student's *t* test, n = 18).

Acknowledgement

We thank G. Wentzingerand for her help with FACS. We thank O.Thoumine, R. Horwitz and M.Glukova for their kind gift of Ncad plasmid, FGFR plasmid and anti-vinculin antibodies, respectively. We thank ImagoSeine for use of microscope platform technique.

This work was supported by grants from CNRS, ARC foundation, Human Frontier Science Program (HFSP grant RPG0040/2012), and Agence Nationale de la Recherche (ANR 2010 Blan1515). TN was supported by HFSP then by FRM (Fédération pour la Recherche Médicale). We would like also to thank all present and past member of the Cell Adhesion & Mechanics lab at the Institute Jacques Monod for constant support and exchange.

References

Akitaya, T., Bronner-Fraser, M., 1992. Expression of cell adhesion molecules during initiation and cessation of neural crest cell migration. Dev. Dyn. 194, 12–20.

Bard, L., Boscher, C., Lambert, M., Mege, R.-M., Choquet, D., Thoumine, O., Mège, R.-M., Choquet, D., Thoumine, O., Mege, R.-M., Choquet, D., Thoumine, O., Mège, R.-M., Choquet, D., Thoumine, O., 2008. A Molecular Clutch between the Actin Flow and N-Cadherin Adhesions Drives Growth Cone Migration. J. Neurosci. 28, 5879–5890. doi:10.1523/JNEUROSCI.5331-07.2008

Bazellières, E., Conte, V., Elosegui-Artola, A., Serra-Picamal, X., Bintanel-Morcillo, M., Roca-Cusachs, P., Muñoz, J.J., Sales-Pardo, M., Guimerà, R., Trepat, X., 2015. Control of cell–cell forces and collective cell dynamics by the intercellular adhesome. Nat. Cell Biol. 17, 409–420. doi:10.1038/ncb3135

Broders-bondon, F., Paul-gilloteaux, P., Gazquez, E., Heysch, J., Piel, M., Mayor, R., Lambris, J.D., Dufour, S., 2016. Control of the collective migration of enteric neural crest cells by the Complement anaphylatoxin C3a and N-cadherin. Dev. Biol. 1–15. doi:10.1016/j.ydbio.2016.03.022

Camand, E., Peglion, F., Osmani, N., Sanson, M., Etienne-Manneville, S., 2012. N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration. J. Cell Sci. 125, 844–57. doi:10.1242/jcs.087668

Cui, Y., Yamada, S., 2013. N-Cadherin Dependent Collective Cell Invasion of Prostate Cancer Cells Is Regulated by the N-Terminus of α-Catenin. PLoS One 8, e55069. doi:10.1371/journal.pone.0055069

Duband, J.-L., Blavet, C., Jarov, A., Fournier-Thibault, C., 2009. Spatio-temporal control of neural epithelial cell migration and epithelium-to-mesenchyme transition during avian neural tube development. Dev. Growth Differ. 51, 25–44. doi:10.1111/j.1440-169X.2009.01076.x

Duband, J.L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G.M., Thiery, J.P., 1987. Adhesion molecules during somitogenesis in the avian embryo. J. Cell Biol. 104, 1361–74.

Ezzat, S., Zheng, L., Winer, D., Asa, S.L., 2006. Targeting N-cadherin through fibroblast growth factor receptor-4: distinct pathogenetic and therapeutic implications. Mol. Endocrinol. 20, 2965–75.

Hazan, R.B., Kang, L., Whooley, B.P., Borgen, P.I., 1997. N-cadherin promotes adhesion between invasive breast cancer cells and the stroma. Cell Adhes. Commun. 4, 399–411.

Kametani, Y., Takeichi, M., 2007. Basal-to-apical cadherin flow at cell junctions. Nat. Cell Biol. 9, 92–8. doi:10.1038/ncb1520

Kashima, T., Nakamura, K., Kawaguchi, J., Takanashi, M., Ishida, T., Aburatani, H., Kudo, A., Fukayama, M., Grigoriadis, A.E., 2003. Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo. Int. J. cancer 104, 147–54. doi:10.1002/ijc.10931

Kumar, A., Gupta, T., Berzsenyi, S., Giangrande, A., 2015. N-cadherin negatively regulates collective Drosophila glial migration through actin cytoskeleton remodeling. J. Cell Sci. 128, 900–912. doi:10.1242/jcs.157974

Kuriyama, S., Mayor, R., 2008. Molecular analysis of neural crest migration. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 1349–62. doi:10.1098/rstb.2007.2252

Kuriyama, S., Theveneau, E., Benedetto, A., Parsons, M., Tanaka, M., Charras, G., Kabla, A., Mayor, R., 2014. In vivo collective cell migration requires an LPAR2-dependent increase in tissue fluidity. J. Cell Biol. 206, 113–127. doi:10.1083/jcb.201402093

Lambert, M., Thoumine, O., Brevier, J., Choquet, D., Riveline, D., Mège, R.-M., 2007. Nucleation and growth of cadherin adhesions. Exp. Cell Res. 313, 4025–40. doi:10.1016/j.yexcr.2007.07.035

Le Clainche, C., Carlier, M.-F., 2008. Regulation of actin assembly associated with protrusion and adhesion in cell migration. Physiol. Rev. 88, 489–513. doi:10.1152/physrev.00021.2007

le Duc, Q., Shi, Q., Blonk, I., Sonnenberg, A., Wang, N., Leckband, D., de Rooij, J., 2010. Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. J. Cell Biol. 189, 1107–15. doi:10.1083/jcb.201001149

Lele, Z., Folchert, A., Concha, M., Rauch, G.G.-J.G.G.-J., Geisler, R., Rosa, F.F., Wilson, S.W., Hammerschmidt, M., Bally-cuif, L., 2002. Parachute/N-Cadherin Is Required for Morphogenesis and Maintained Integrity of the Zebrafish Neural Tube. Development 129, 3281–3294.

Luo, Y., Radice, G.L., 2005. N-cadherin acts upstream of VE-cadherin in controlling vascular morphogenesis. J. Cell Biol. 169, 29–34. doi:10.1083/jcb.200411127

Peglion, F., Llense, F., Etienne-Manneville, S., 2014. Adherens junction treadmilling during collective migration. Nat. Cell Biol. 16, 639–51.

Qian, X., Anzovino, A., Kim, S., Suyama, K., Yao, J., Hulit, J., Agiostratidou, G., Chandiramani, N., McDaid, H.M., Nagi, C., Cohen, H.W., Phillips, G.R., Norton, L., Hazan, R.B., 2013. N-cadherin/FGFR promotes metastasis through epithelial-to-mesenchymal transition and stem/progenitor cell-like properties. Oncogene 33, 1–11.

Radice, G.L., Rayburn, H., Matsunami, H., Knudsen, K.A., Takeichi, M., Hynes, R.O., 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev. Biol. 181, 64–78.

Reynolds, A.B., Carnahan, R.H., 2004. Regulation of cadherin stability and turnover by p120ctn: Implications in disease and cancer. Semin. Cell Dev. Biol. 15, 657–663.

Saffell, J.L., Williams, E.J., Mason, I.J., Walsh, F.S., Doherty, P., 1997. Expression of a dominant negative FGF receptor inhibits axonal growth and FGF receptor phosphorylation stimulated by CAMs [published erratum appears in Neuron 1998 Mar;20(3):619]. Neuron 18, 231–242.

Scarpa, E., Szabó, A., Bibonne, A., Theveneau, E., Parsons, M., Mayor, R., 2015. Cadherin Switch during EMT in Neural Crest Cells Leads to Contact Inhibition of Locomotion via Repolarization of Forces. Dev. Cell 34, 421–434. doi:10.1016/j.devcel.2015.06.012

Suyama, K., Shapiro, I., Guttman, M., Hazan, R.B., 2002. A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2, 301–14.

Taeger, J., Moser, C., Hellerbrand, C., Mycielska, M.E., Glockzin, G., Schlitt, H.J., Geissler, E.K., Stoeltzing, O., Lang, S. a., 2011. Targeting FGFR/PDGFR/VEGFR Impairs Tumor Growth, Angiogenesis, and Metastasis by Effects on Tumor Cells, Endothelial Cells, and Pericytes in Pancreatic Cancer. Mol. Cancer Ther. 10, 2157–2167.

Taniguchi, H., Kawauchi, D., Nishida, K., Development, F.M., Taniguchi, H., Kawauchi, D., Nishida, K., Murakami, F., 2006. Classic cadherins regulate tangential migration of precerebellar neurons in the caudal hindbrain. Development 133, 1923–1931. doi:10.1242/dev.02414

Thoumine, O., Lambert, M., Mège, R.-M., Choquet, D., 2006. Regulation of N-cadherin dynamics at neuronal contacts by ligand binding and cytoskeletal coupling. Mol. Biol. Cell 17, 862–75. doi:10.1091/mbc.E05-04-0335

Trepat, X., Wasserman, M.R., Angelini, T.E., Millet, E., Weitz, D.A., Butler, J.P., Fredberg, J.J., 2009. Physical forces during collective cell migration. Nat. Phys. 5, 426–430. doi:10.1038/nphys1269

Twiss, F., de Rooij, J., 2013. Cadherin mechanotransduction in tissue remodeling. Cell. Mol. Life Sci. 70, 4101–4116. doi:10.1007/s00018-013-1329-x

Vedula, S.R.K., Leong, M.C., Lai, T.L., Hersen, P., Kabla, A.J., Lim, C.T., Ladoux, B., 2012. Emerging modes of collective cell migration induced by geometrical constraints. Proc. Natl. Acad. Sci. U. S. A. 109, 12974–9.

Vedula, S.R.K., Ravasio, A., Anon, E., Chen, T., Peyret, G., Ashraf, M., Ladoux, B., 2014. Microfabricated environments to study collective cell behaviors. Methods Cell Biol. 120, 235–52.

5 GENERAL CONCLUSIONS AND PERSPECTIVES

My thesis project aimed to evaluate the impact of FGFR1 on the stability of N-cadherin mediated cell-cell contacts and their combined effects on individual cell migration as well as collective cell migration, using microfabrication techniques. This method is a powerful, reproducible tool to study cell migration in a controlled, micrometer scaled environment that mimics the native microenvironment of the cells. Here, we fabricated N-cadherin-Fc coated lines of 10 μm width and fibronectin coated stripes of 200 μm width to study single cell and collective cell migration, respectively.

In the first part (Manuscript 1), we showed a bidirectional communication between FGFR1 and N-cadherin in strengthening the N-cadherin meditated cell-cell contacts thus reducing individual N-cadherin expressing cell migration on N-cadherin coated surface. In the second part, we showed the crosstalk between FGFR1 and N-cadherin reduced the fluidity of the cell sheet, reorganized the cytoskeleton and conferred a leader role to front cells, allowing higher efficiency of collective movement. Taken together, we conclude that FGFR1 specifically strengthen N-cadherin/N-cadherin interaction leading to two opposite effects on two modes of migration: decreased migration of individual N-cadherin expressing cell on a N-cadherin coated surface while increased migration of N-cadherin expressing cell monolayer.

To decipher the role of FGFR1 on single cell migration, we analyzed the migration of N-cadherin or N-cadherin / FGFR expressing HEK cells (Ncad or Ncad / FGFR cells) on N-cadherin coated line. This model is relevant since it mimics the migration of single neural or cancer cell over neighboring cells. Moreover, HEK cells do not express any cadherins, which could bias our interpretation. In the first part of my thesis, I unraveled the negative role of FGFR1 on N-cadherin-mediated single cell migration. Indeed, FGFR1 expression decreased the migration of N-cadherin expressing HEK cells Ncad-Fc coated surfaces by increasing cell spreading over the N-cadherin surface. Therefore, Ncad / FGFR cells remained attached and rarely detach to/from the substrate leading drastic inhibition of cell motion.

N-cadherin and FGFR1 are co-recruited and co-stabilized at the cell-cell contacts through the direct interaction of their ectodomains. This may imply an increase of N-cadherin aggregation at the cell surface. As a consequence, N-cadherin mediated cell-cell contacts are strengthened

in presence of FGFR1. This was confirmed by Ca^{2+} switch and magnetic tweezer experiments. FGFR1 may also increase N-cadherin-mediated cell-cell adhesion by retaining N-cadherin at the membrane through a decrease of N-cadherin endocytosis. Moreover, FGFR1 reinforces N-cadherin anchoring to actin network. This may also contribute to the enhanced strength of N-cadherin-mediated cell-cell junction. At the present time, it is not clear whether FGFR1 activity primarily affect cadherin endocytosis, clustering or anchoring to F-actin. Indeed, the three processes have been reported to be interdependent and further analysis with mutant proteins will be required to answer this question. Although a negative effect of FGFR1 on N-cadherin endocytosis may account for the regulation of N-cadherin prevalence at the cell membrane, the endocytosis pathway remained unclear. More work need to be done to identify the FGFR1-dependent endocytosis pathway of N-cadherin. We would like also to study the effect of FGFR1 on Myosin expression and activation and on the contractility of N-cadherin expressing cells.

The increased association of cadherin-catenin complexes by FGFR1 could also explain the suppression of bleb formation. Indeed, we observed that during their migration N-cadherin expressing HEK cells displayed numerous blebs, which were not observed when FGFR1 was expressed. Bleb formation has been shown to drive cell migration (amoeboid). Their initiation depends on the detachment of the actin cortex from the plasma membrane (Charras and Paluch, 2008). Here, abundant formation of bleb in migrating-cadherin expressing cells is concordant with the work of Bergert in which they showed instantaneous switch between bleb formation on low adhesion micropatterned substrate and lamellipodia formation on highly adhesive substrate (Bergert et al., 2012). The enhanced anchoring of N-cadherin to actin network induced by FGFR1 may account for the strong inhibition of blebs formation in cells expressing the two receptors as a result of an increased coupling of the actin cortex to the plasma membrane.

All these cells responses upon FGFR1 overexpression require FGFR activation. FGFR1 activation is induced by N-cadherin mobilization as previously reported (Boscher and Mège, 2008; Saffell et al., 1997). Importantly, this activation does not require exogenous FGF. Thus, we think that the increased prevalence and stabilization of FGFR1 at cell-cell contacts by itself induced FGFR activation independently of FGF ligand stimulation. In the future, to confirm this hypothesis, we could follow the activation of FGFR in cells co-expressing the kinase-death FGFR1 (KD-FGFR1) and N-cadherin. In this case, even N-cadherin and KD-

FGFR1 could co-stabilize and co-recruit at the cell surface; subsequent signaling events of FGFR1 would be less activated. EC domain of N-cadherin ectodomain has been shown to be responsible for the binding with FGFR1. Mutant N-cadherin lacking EC4 domain co-expressed with FGFR1 in cells would fail to activate the latter. We could also follow the activated degree of FGFR1 in function of N-cadherin concentration using Digital Micromirror Devices (DMD) microscope. This technique allows the fabrication of N-cadherin coated surface with different concentration of protein grafted.

Downstream of FGR1 activation we observed a decreasing of the phosphorylation of p120 and (2) an activation of Src that are bot regulating single cell migration of N-cadherin substrates. To see whether there is a direct relation between Src and p120, we will need to follow the phosphorylated state of p120 in cells expressing FGFR unphosphorylable for FRS2 docking, which was reported to recruit Src upon FGFR activation. In the second part of the thesis, we studied the effect of this reinforcement of cell-cell contacts by FGFR1 on the collective migration of C2C12 cells. We used cell layer expansion on 200 μm width fibronectin-coated strips after release of confinement as migration test. This model provides well-defined initial conditions as well as precise spatial control of the size and geometry of free space that the cell sheet invades. The impact of FGFR1 on the reinforcement of N-cadherin-mediated cell-cell contacts is more preeminent at the leading edge of the migrating monolayer.. This leads to decreased retrograde flow of N-cadherin at the cell-cell contacts at the front in the presence of FGFR. Importantly, we found that FGFR1 expressing cells function as leader cells. They display large protrusions, and the presence of stress fibers aligned in the displacement direction. Furthermore, they establish robust contacts with trailing cells. Therefore, FGFR increases the efficiency of collective migration of N-cadherin expressing cells by increasing the cohesion of the monolayer, especially at the front.

To further understand the effect of FGFR1 expression on collective cell migration, we will analyze of the map of traction forces applied by N-cadherin or N-cadherin / FGFR1 expressing cells on the substratum. From these traction force maps, one could extract the map of the stress transmitted at cell-cell contacts. To better characterize the dynamics of leader cells and to confirm if these leader cells persist during long-term migration, we will perform live cell imaging. Finally, more work needs to be done to inquire about actomyosin dynamics at the front of migration, in order to characterize the mechanotransduction response of cells to FGFR1.

The first part of thesis demonstrated that all the actions of FGFR1 on cell-cell contact dynamic and individual cell migration require the signaling function of the receptor. Thus, we would like to enquire about the activation of FGFR1 in the C2C12 model and its impact on the regulation of cell-cell contact and cytoskeleton dynamics.

Overall of our studies confirm that cellular responses to same proteins are diverse. N-cadherin and FGFR1 mediated cell-cell contacts could enhance or repress cell migration dependent on cellular context. For cancer cells that upregulated both N-cadherin and FGFR1, experiments aim to reduce metastasis and invasion playing on cell adhesion must consider their migration mode to better target the treatment.

6 Bibliography

Akitaya, T., Bronner-Fraser, M., 1992. Expression of cell adhesion molecules during initiation and cessation of neural crest cell migration. Dev. Dyn. 194, 12–20.

Al Moustafa, A.-E., Yen, L., Benlimame, N., Alaoui-Jamali, M.A., 2002. Regulation of E-cadherin/catenin complex patterns by epidermal growth factor receptor modulation in human lung cancer cells. Lung Cancer 37, 49–56.

Amaya, E., Stein, P.A., Musci, T.J., Kirschner, M.W., 1993. FGF signalling in the early specification of mesoderm in Xenopus. Development 118, 477–87.

Angst, B.D., Marcozzi, C., Magee, A.I., 2001. The cadherin superfamily: diversity in form and function. J. Cell Sci. 114, 629–41.

Aono, S., Nakagawa, S., Reynolds, A.B., Takeichi, M., 1999. p120(ctn) acts as an inhibitory regulator of cadherin function in colon carcinoma cells. J. Cell Biol. 145, 551–62.

Arboleda-Estudillo, Y., Krieg, M., Stühmer, J., Licata, N. a., Muller, D.J., Heisenberg, C.P., 2010. Movement Directionality in Collective Migration of Germ Layer Progenitors. Curr. Biol. 20, 161–169.

Bachler, M., Neubüser, A., 2001. Expression of members of the Fgf family and their receptors during midfacial development. Mech. Dev. 100, 313–316. doi:10.1016/S0925-4773(00)00518-9

Bard, L., Boscher, C., Lambert, M., Mege, R.-M., Choquet, D., Thoumine, O., Mège, R.-M., Choquet, D., Thoumine, O., Mege, R.-M., Choquet, D., Thoumine, O., Mège, R.-M., Choquet, D., Thoumine, O., 2008. A Molecular Clutch between the Actin Flow and N-Cadherin Adhesions Drives Growth Cone Migration. J. Neurosci. 28, 5879–5890. doi:10.1523/JNEUROSCI.5331-07.2008

Bazellières, E., Conte, V., Elosegui-Artola, A., Serra-Picamal, X., Bintanel-Morcillo, M., Roca-Cusachs, P., Muñoz, J.J., Sales-Pardo, M., Guimerà, R., Trepat, X., 2015. Control of cell–cell forces and collective cell dynamics by the intercellular adhesome. Nat. Cell Biol. 17, 409–420. doi:10.1038/ncb3135

Beiman, M., Shilo, B.Z., Volk, T., 1996. Heartless, a Drosophila FGF receptor homolog, is essential for cell migration and establishment of several mesodermal lineages. Genes Dev. 10, 2993–3002.

Bergert, M., Chandradoss, S.D., Desai, R.A., Paluch, E., 2012. Cell mechanics control rapid transitions between blebs and lamellipodia during migration. Proc. Natl. Acad. Sci. U. S. A. 109, 14434–9. doi:10.1073/pnas.1207968109

Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., Plastino, J., 2014. Actin dynamics, architecture, and mechanics in cell motility. Physiol. Rev. 94, 235–263.

Boggon, T.J., Murray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B.M., Shapiro, L., 2002. C cadherin ectodomain structure and implications for cell adhesion mechanisms. Science (80-.). 296, 1308–1313.

Borghi, N., Sorokina, M., Shcherbakova, O.G., Weis, W.I., Pruitt, B.L., Nelson, W.J., Dunn, A.R., 2012. E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. Proc. Natl. Acad. Sci. U. S. A. 109, 12568–73. doi:10.1073/pnas.1204390109

Borrmann, C., Grund, C., Kuhn, C., Hofmann, I., Pieperhoff, S., Franke, W., 2006. The area composita of adhering junctions connecting heart muscle cells of vertebrates. II. Colocalizations of desmosomal and fascia adhaerens molecules in the intercalated disk. Eur. J. Cell Biol. 85, 469–485. doi:10.1016/j.ejcb.2006.02.009

Boscher, C., Mège, R.-M.M., 2008. Cadherin-11 interacts with the FGF receptor and induces neurite outgrowth through associated downstream signalling. Cell. Signal. 20, 1061–1072. doi:10.1016/j.cellsig.2008.01.008

Boyden, S., 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J. Exp. Med. 115, 453–66.

Bozdagi, O., Shan, W., Tanaka, H., Benson, D.L., Huntley, G.W., 2000. Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. Neuron 28, 245–59.

Brittis, P.A., Silver, J., Walsh, F.S., Doherty, P., 1996. Fibroblast growth factor receptor function is required for the orderly projection of ganglion cell axons in the developing mammalian retina. Mol. Cell. Neurosci. 8, 120–8.

Broders-bondon, F., Paul-gilloteaux, P., Gazquez, E., Heysch, J., Piel, M., Mayor, R., Lambris, J.D., Dufour, S., 2016. Control of the collective migration of enteric neural crest cells by the Complement anaphylatoxin C3a and N-cadherin. Dev. Biol. 1–15. doi:10.1016/j.ydbio.2016.03.022

Bryant, D.M., Wylie, F.G., Stow, J.L., 2005. Regulation of endocytosis, nuclear translocation, and signaling of fibroblast growth factor receptor 1 by E-cadherin. Mol. Biol. Cell 16, 14–23.

Buckley, C.D., Tan, J., Anderson, K.L., Hanein, D., Volkmann, N., Weis, W.I., Nelson, W.J., Dunn, A.R., 2014. Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. Science 346, 1254211. doi:10.1126/science.1254211

Cai, D., Montell, D.J., 2014. Diverse and dynamic sources and sinks in gradient formation and directed migration. Curr. Opin. Cell Biol. 30, 91–98. doi:10.1016/j.ceb.2014.06.009

Camand, E., Peglion, F., Osmani, N., Sanson, M., Etienne-Manneville, S., 2012. N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration. J. Cell Sci. 125, 844–57. doi:10.1242/jcs.087668

Carmeliet, P., Lampugnani, M.G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oosthuyse, B., Dewerchin, M., Zanetti, A., Angellilo, A., Mattot, V., Nuyens, D., Lutgens, E., Clotman, F., De Ruiter, M.C., Groot, A.G. De, Poelmann, R., Lupu, F., Herbert, J.M., Collen, D., Dejana, E., 1999. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell 98, 147–157.

Cavallaro, U., Niedermeyer, J., Fuxa, M., Christofori, G., 2001. N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. Nat. Cell Biol. 3, 650–657.

Cavallaro, U., Schaffhauser, B., Christofori, G., 2002. Cadherins and the tumour progression: is it all in a switch? Cancer Lett. 176, 123–8.

Charras, G., Paluch, E., 2008. Blebs lead the way: how to migrate without lamellipodia. Nat. Rev. Mol. Cell Biol. 9, 730–6. doi:10.1038/nrm2453

Chazeau, A., Garcia, M., Czondor, K., Perrais, D., Tessier, B., Giannone, G., Thoumine, O., 2015. Mechanical coupling between transsynaptic N-cadherin adhesions and actin flow stabilizes dendritic spines. Mol. Biol. Cell 26, 859–873. doi:10.1091/mbc.E14-06-1086

Chen, X., Kojima, S.I., Borisy, G.G., Green, K.J., 2003. P120 Catenin Associates With Kinesin and Facilitates the Transport of Cadherin-Catenin Complexes To Intercellular Junctions. J. Cell Biol. 163, 547–557.

Chen, Y.T., Stewart, D.B., Nelson, W.J., 1999. Coupling assembly of the E-cadherin/beta-catenin complex to efficient endoplasmic reticulum exit and basal-lateral membrane targeting of E-cadherin in polarized MDCK cells. J. Cell Biol. 144, 687–99.

Chiasson-MacKenzie, C., Morris, Z.S., Baca, Q., Morris, B., Coker, J.K., Mirchev, R., Jensen, A.E.,

Carey, T., Stott, S.L., Golan, D.E., McClatchey, A.I., 2015. NF2/Merlin mediates contact-dependent inhibition of EGFR mobility and internalization via cortical actomyosin. J. Cell Biol. 211, 391–405.

Chiasson, C.M., Wittich, K.B., Vincent, P.A., Faundez, V., Kowalczyk, A.P., 2009. p120-catenin inhibits VE-cadherin internalization through a Rho-independent mechanism. Mol. Biol. Cell 20, 1970–80. doi:10.1091/mbc.E08-07-0735

Chioni, A.M., Grose, R., 2012. FGFR1 cleavage and nuclear translocation regulates breast cancer cell behavior. J. Cell Biol. 197, 801–817.

Chrétien, M.L., Zhang, M., Jackson, M.R., Kapus, A., Langille, B.L., 2010. Mechanotransduction by endothelial cells is locally generated, direction-dependent, and ligand-specific. J. Cell. Physiol. 224, 352–61. doi:10.1002/jcp.22125

Chu, W.-C., Lee, Y.-M., Henry Sun, Y., 2013. FGF /FGFR signal induces trachea extension in the drosophila visual system. PLoS One 8, e73878.

Chu, Y.-S.S., Thomas, W.A., Eder, O., Pincet, F., Perez, E., Thiery, J.P., Dufour, S., 2004. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. J. Cell Biol. 167, 1183–94. doi:10.1083/jcb.200403043

Chu, Y.S., Thomas, W.A., Eder, O., Pincet, F., Perez, E., Thiery, J.P., Dufour, S., 2004. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. J. Cell Biol. 167, 1183–1194. doi:10.1083/jcb.200403043

Cinnamon, Y., Ben-Yair, R., Kalcheim, C., 2006. Differential effects of N-cadherin-mediated adhesion on the development of myotomal waves. Development 133, 1101–12. doi:10.1242/dev.02291

Ciruna, B., Rossant, J., 2001. FGF Signaling Regulates Mesoderm Cell Fate Specification and Morphogenetic Movement at the Primitive Streak. Dev. Cell 1, 37–49.

Ciruna, B.G., Schwartz, L., Harpal, K., Yamaguchi, T.P., Rossant, J., 1997. Chimeric analysis of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenetic movement through the primitive streak. Development 124, 2829–2841.

Coleman, S.J., Chioni, A., Ghallab, M., Anderson, R.K., Lemoine, N.R., Kocher, H.M., Grose, R.P.,

2014. Nuclear translocation of FGFR1 and FGF2 in pancreatic stellate cells facilitates pancreatic cancer cell invasion. EMBO Mol. Med. 6, 467–81.

Coon, B.G., Baeyens, N., Han, J., Budatha, M., Ross, T.D., Fang, J.S., Yun, S., Thomas, J.L., Schwartz, M.A., 2015. Intramembrane binding of VE-cadherin to VEGFR2 and VEGFR3 assembles the endothelial mechanosensory complex. J. Cell Biol. 208, 975–986.

Cooper, J.A., 2013. Cell biology in neuroscience: mechanisms of cell migration in the nervous system. J. Cell Biol. 202, 725–34. doi:10.1083/jcb.201305021

Corell, M., Wicher, G., Limbach, C., Kilimann, M.W., Colman, D.R., Svenningsen, Å.F., 2010. Spatiotemporal distribution and function of N-cadherin in postnatal Schwann cells: A matter of adhesion? J. Neurosci. Res. n/a–n/a. doi:10.1002/jnr.22398

Cui, Y., Yamada, S., 2013. N-Cadherin Dependent Collective Cell Invasion of Prostate Cancer Cells Is Regulated by the N-Terminus of α-Catenin. PLoS One 8, e55069. doi:10.1371/journal.pone.0055069

Daniel, J.M., Reynolds, A.B., 1995. The Tyrosine Kinase Substrate p120 cas Binds Directly to E-Cadherin but Not to the Adenomatous Polyposis Coli Protein or α-Catenin. Microbiology 15, 4819–4824.

Davis, M.A., Ireton, R.C., Reynolds, A.B., 2003. A core function for p120-catenin in cadherin turnover. J. Cell Biol. 163, 525–534. doi:10.1083/jcb.200307111

De Wever, O., 2004. Critical role of N-cadherin in myofibroblast invasion and migration in vitro stimulated by colon-cancer-cell-derived TGF- or wounding. J. Cell Sci. 117, 4691–4703. doi:10.1242/jcs.01322

Debiais, F., Lemonnier, J., Hay, E., Delannoy, P., Caverzasio, J., Marie, P.J., 2001. Fibroblast growth factor-2 (FGF-2) increases N-cadherin expression through protein kinase C and Src-kinase pathways in human calvaria osteoblasts. J. Cell. Biochem. 81, 68–81.

del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J.M., Sheetz, M.P., 2009. Stretching Single Talin Rod Molecules Activates Vinculin Binding. Science (80-.). 323, 638–641. doi:10.1126/science.1162912

Deng, C.X., Wynshaw-Boris, A., Shen, M.M., Daugherty, C., Ornitz, D.M., Leder, P., 1994. Murine FGFR-1 is required for early postimplantation growth and axial organization. Genes Dev. 8, 3045–3057.

Derycke, L.D.M., Bracke, M.E., 2004. N-cadherin in the spotlight of cell-cell adhesion, differentiation, invasion and signalling. Int. J. Dev. Biol. 48, 463–476.

Dey, J.H., Bianchi, F., Voshol, J., Bonenfant, D., Oakeley, E.J., Hynes, N.E., 2010. Targeting fibroblast growth factor receptors blocks PI3K/AKT signaling, induces apoptosis, and impairs mammary tumor outgrowth and metastasis. Cancer Res. 70, 4151–4162.

Di Benedetto, A., Watkins, M., Grimston, S., Salazar, V., Donsante, C., Mbalaviele, G., Radice, G.L., Civitelli, R., 2010. N-cadherin and cadherin 11 modulate postnatal bone growth and osteoblast differentiation by distinct mechanisms. J. Cell Sci. 123, 2640–8. doi:10.1242/jcs.067777

Doherty, P., Williams, G., Williams, E.J., 2000. CAMs and axonal growth: a critical evaluation of the role of calcium and the MAPK cascade. Mol. Cell. Neurosci. 16, 283–95. doi:10.1006/mcne.2000.0907

Dossenbach, C., Röck, S., Affolter, M., 2001. Specificity of FGF signaling in cell migration in Drosophila. Development 128, 4563–72.

Drees, F., Pokutta, S., Yamada, S., Nelson, W.J., Weis, W.I., 2005. a-Catenin Is a Molecular Switch that Binds E-Cadherin-b-Catenin and Regulates Actin-Filament Assembly. Assembly 903–915. doi:10.1016/j.cell.2005.09.021

Duband, J.-L., Blavet, C., Jarov, A., Fournier-Thibault, C., 2009. Spatio-temporal control of neural epithelial cell migration and epithelium-to-mesenchyme transition during avian neural tube development. Dev. Growth Differ. 51, 25–44. doi:10.1111/j.1440-169X.2009.01076.x

Duband, J.L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G.M., Thiery, J.P., 1987. Adhesion molecules during somitogenesis in the avian embryo. J. Cell Biol. 104, 1361–74.

Duchesne, L., Octeau, V., Bearon, R.N., Beckett, A., Prior, I.A., Lounis, B., Fernig, D.G., 2012. Transport of fibroblast growth factor 2 in the pericellular matrix is controlled by the spatial distribution of its binding sites in heparan sulfate. PLoS Biol. 10, 16.

Duguay, D., Foty, R.A., Steinberg, M.S., 2003. Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. Dev. Biol. 253, 309–23.

Dumortier, J.G., Martin, S., Meyer, D., Rosa, F.M., David, N.B., 2012. Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. Proc. Natl. Acad. Sci. U. S. A. 109, 16945–16950.

Engl, W., Arasi, B., Yap, L.L., Thiery, J.P., Viasnoff, V., 2014. Actin dynamics modulate

mechanosensitive immobilization of E-cadherin at adherens junctions. Nat. Cell Biol. 16, 587–594. doi:10.1038/ncb2973

Erez, N., Zamir, E., Gour, B.J., Blaschuk, O.W., Geiger, B., 2004. Induction of apoptosis in cultured endothelial cells by a cadherin antagonist peptide: Involvement of fibroblast growth factor receptor-mediated signalling. Exp. Cell Res. 294, 366–378.

Esser, S., Lampugnani, M.G., Corada, M., Dejana, E., Risau, W., 1998. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. J.Cell Sci. 111 (Pt 1, 1853–1865.

Ezzat, S., Zheng, L., Winer, D., Asa, S.L., 2006. Targeting N-cadherin through fibroblast growth factor receptor-4: distinct pathogenetic and therapeutic implications. Mol. Endocrinol. 20, 2965–75.

Farooqui, R., 2005. Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. J. Cell Sci. 118, 51–63. doi:10.1242/jcs.01577

Fedor-Chaiken, M., Hein, P.W., Stewart, J.C., Brackenbury, R., Kinch, M.S., 2003. E-Cadherin Binding Modulates EGF Receptor Activation. Cell Commun. Adhes. 10, 105–118.

Fedor-Chaiken, M., Meigs, T.E., Kaplan, D.D., Brackenbury, R., 2003. Two Regions of Cadherin Cytoplasmic Domains Are Involved in Suppressing Motility of a Mammary Carcinoma Cell Line. J. Biol. Chem. 278, 52371–52378. doi:10.1074/jbc.M310576200

Ferrari, S.L., Traianedes, K., Thorne, M., Lafage-Proust, M.H., Genever, P., Cecchini, M.G., Behar, V., Bisello, A., Chorev, M., Rosenblatt, M., Suva, L.J., 2000. A role for N-cadherin in the development of the differentiated osteoblastic phenotype. J. Bone Miner. Res. 15, 198–208. doi:10.1359/jbmr.2000.15.2.198

Foty, R.A., Steinberg, M.S., 2005. The differential adhesion hypothesis: A direct evaluation. Dev. Biol. 278, 255–263. doi:10.1016/j.ydbio.2004.11.012

Franco, S.J., Martinez-Garay, I., Gil-Sanz, C., Harkins-Perry, S.R., Müller, U., 2011. Reelin regulates cadherin function via Dab1/Rap1 to control neuronal migration and lamination in the neocortex. Neuron 69, 482–97.

Friedl, P., Gilmour, D., 2009. Collective cell migration in morphogenesis, regeneration and cancer. Nat. Rev. Mol. Cell Biol. 10, 445–457. doi:10.1038/nrm2720

Friedl, P., Hegerfeldt, Y., Tusch, M., 2004. Collective cell migration in morphogenesis and cancer. Int. J. Dev. Biol. 48, 441–449.

Friedl, P., Wolf, K., 2010. Plasticity of cell migration: a multiscale tuning model. J. Cell Biol. 188, 11–19.

Fujita, Y., Krause, G., Scheffner, M., Zechner, D., Leddy, H.E.M., Behrens, J., Sommer, T., Birchmeier, W., 2002. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. Nat. Cell Biol. 4, 222–31. doi:10.1038/ncb758

Fung, S., Wang, F., Chase, M., Godt, D., Hartenstein, V., 2008. Expression profile of the cadherin family in the developing Drosophila brain. J. Comp. Neurol. 506, 469–88. doi:10.1002/cne.21539

Gavard, J., Gutkind, J.S., 2006. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat. Cell Biol. 8, 1223–1234.

Gemmill, R.M., Roche, J., Potiron, V.A., Nasarre, P., Mitas, M., Coldren, C.D., Helfrich, B.A., Garrett-Mayer, E., Bunn, P.A., Drabkin, H.A., 2011. ZEB1-responsive genes in non-small cell lung cancer. Cancer Lett. 300, 66–78.

Gerhardt, H., Wolburg, H., Redies, C., 2000. N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken. Dev. Dyn. 218, 472–9.

Ghabrial, A.S., Krasnow, M.A., 2006. Social interactions among epithelial cells during tracheal branching morphogenesis. Nature 441, 746–9. doi:10.1038/nature04829

Giampietro, C., Taddei, A., Corada, M., Sarra-Ferraris, G.M., Alcalay, M., Cavallaro, U., Orsenigo, F., Lampugnani, M.G., Dejana, E., 2012. Overlapping and divergent signalling pathways of N- and VE-cadherin in endothelial cells. Blood 119, 2159–2170. doi:10.1182/blood-2011-09-381012

Giannone, G., Mège, R.-M., Thoumine, O., 2009. Multi-level molecular clutches in motile cell processes. Trends Cell Biol. 19, 475–86.

Gisselbrecht, S., Skeath, J.B., Doe, C.Q., Michelson, A.M., 1996. heartless encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the Drosophila embryo. Genes Dev. 10, 3003–17.

Griffin, K., Patient, R., Holder, N., 1995. Analysis of FGF function in normal and no tail zebrafish embryos reveals separate mechanisms for formation of the trunk and the tail. Development 121,

2983–94.

Guerra, M.M., Henzi, R., Ortloff, A., Lichtin, N., Vio, K., Jimenez, A.J., Dominguez-Pinos, M.D., Gonzalez, C., Jara, M.C., Hinostroza, F., Rodriguez, S., Jara, M., Ortega, E., Guerra, F., Sival, D.A., den Dunnen, W.F., Perez-Figares, J.M., McAllister, J.P., Johanson, C.E., Rodriguez, E.M., 2015. Cell Junction Pathology of Neural Stem Cells Is Associated With Ventricular Zone Disruption, Hydrocephalus, and Abnormal Neurogenesis. J.Neuropathol.Exp.Neurol. 74, 653–671.

Guillaume, E., Comunale, F., Do Khoa, N., Planchon, D., Bodin, S., Gauthier-Rouviere, C., 2013. Flotillin microdomains stabilize cadherins at cell-cell junctions. J. Cell Sci. 126, 5293–5304. doi:10.1242/jcs.133975

Gunduz, V., Kong, E., Bryan, C.D., Hinds, P.W., 2012. Loss of the Retinoblastoma Tumor Suppressor Protein in Murine Calvaria Facilitates Immortalization of Osteoblast-Adipocyte Bipotent Progenitor Cells Characterized by Low Expression of N-Cadherin. Mol. Cell. Biol. 32, 2561–2569. doi:10.1128/MCB.06453-11

Häger, A., Alexander, S., Friedl, P., 2013. Cancer invasion and resistance. Eur. J. Cancer Suppl. 11, 291–293.

Hansen, S.M., Berezin, V., Bock, E., 2008. Signaling mechanisms of neurite outgrowth induced by the cell adhesion molecules NCAM and N-Cadherin. Cell. Mol. Life Sci. 65, 3809–3821.

Harris, T.J.C., Tepass, U., 2010. Adherens junctions: from molecules to morphogenesis. Nat. Rev. Mol. Cell Biol. 11, 502–14. doi:10.1038/nrm2927

Hatta, K., Takeichi, M., 1986. Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. Nature 320, 447–449. doi:10.1038/320447a0

Haugsten, E.M., Sørensen, V., Brech, A., Olsnes, S., Wesche, J., 2005. Different intracellular trafficking of FGF1 endocytosed by the four homologous FGF receptors. J. Cell Sci. 118, 3869–3881.

Hazan, R.B., Kang, L., Whooley, B.P., Borgen, P.I., 1997. N-cadherin promotes adhesion between invasive breast cancer cells and the stroma. Cell Adhes. Commun. 4, 399–411.

Hazan, R.B., Phillips, G.R., Qiao, R.F., Norton, L., Aaronson, S.A., 2000. Exogenous Expression of N-Cadherin in Breast Cancer Cells Induces Cell Migration, Invasion, and Metastasis. J. Cell Biol. 148, 779–790. doi:10.1083/jcb.148.4.779

Hirano, S., Takeichi, M., 2012. Cadherins in brain morphogenesis and wiring. Physiol. Rev. 92, 597–634. doi:10.1152/physrev.00014.2011

Hirata, E., Park, D., Sahai, E., 2014. Retrograde flow of cadherins in collective cell migration. Nat. Cell Biol. 16, 621–623. doi:10.1038/ncb2995

Hirokawa, N., Keller, T.C.S., Chasan, R., Mooseker, M.S., 1983. Mechanism of brush border contractility studied by the quick-freeze, deep-etch method. J. Cell Biol. 96, 1325–1336. doi:10.1083/jcb.96.5.1325

Hong, E., Brewster, R., 2006. N-cadherin is required for the polarized cell behaviors that drive neurulation in the zebrafish. Development 133, 3895–3905.

Hu, X., Zhang, P., Xu, Z., Chen, H., Xie, X., 2013. GPNMB enhances bone regeneration by promoting angiogenesis and osteogenesis: Potential role for tissue engineering bone. J. Cell. Biochem. 114, 2729–2737. doi:10.1002/jcb.24621

Huang, R.Y., Wen, C., Liao, C., Wang, S., Chou, L., Wu, J., 2012. Lysophosphatidic acid modulates the association of PTP1B with N-cadherin/catenin complex in SKOV3 ovarian cancer cells. Cell Biol. Int. 36, 833–841. doi:10.1042/CBI20110687

Huber, A.H., Stewart, D.B., Laurents, D. V., Nelson, W.J., Weis, W.I., 2001. The cadherin cytoplasmic domain is unstructured in the absence of??-catenin. A possible mechanism for regulating cadherin turnover. J. Biol. Chem. 276, 12301–12309. doi:10.1074/jbc.M010377200

Hulit, J., Suyama, K., Chung, S., Keren, R., Agiostratidou, G., Shan, W., Dong, X., Williams, T.M., Lisanti, M.P., Knudsen, K., Hazan, R.B., 2007. N-Cadherin Signaling Potentiates Mammary Tumor Metastasis via Enhanced Extracellular Signal-Regulated Kinase Activation. Cancer Res. 67, 3106–3116.

Huttenlocher, A., Horwitz, A.R., 2011. Integrins in Cell Migration. Cold Spring Harb. Perspect. Biol. 3, a005074–a005074. doi:10.1101/cshperspect.a005074

Huveneers, S., Oldenburg, J., Spanjaard, E., van der Krogt, G., Grigoriev, I., Akhmanova, A., Rehmann, H., de Rooij, J., 2012. Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. J. Cell Biol. 196, 641–652. doi:10.1083/jcb.201108120

Inumaru, J., Nagano, O., Takahashi, E., Ishimoto, T., Nakamura, S., Suzuki, Y., Niwa, S., Umezawa, K., Tanihara, H., Saya, H., 2009. Molecular mechanisms regulating dissociation of cell-cell junction of epithelial cells by oxidative stress. Genes to Cells 14, 703–716. doi:10.1111/j.1365-

2443.2009.01303.x

Ireton, R.C.R.C.R.C.R.C., Davis, M.A., Hengel, J. Van, Mariner, D.J., Barnes, K., Thoreson, M.A., Anastasiadis, P.Z., Matrisian, L., Bundy, L.M., Sealy, L., Gilbert, B., Roy, F. Van, Reynolds, A.B., Van Hengel, J., Mariner, D.J., Barnes, K., Thoreson, M.A., Anastasiadis, P.Z., Matrisian, L., Bundy, L.M., Sealy, L., Gilbert, B., Van Roy, F., Reynolds, A.B., 2002. A novel role for p120 catenin in E-cadherin function. J. Cell Biol. 159, 465–76. doi:10.1083/jcb.200205115

Itoh, N., Mima, T., Mikawa, T., 1996. Loss of fibroblast growth factor receptors is necessary for terminal differentiation of embryonic limb muscle. Development 122, 291–300.

Jia, L., Cheng, L., Raper, J., 2005. Slit/Robo signaling is necessary to confine early neural crest cells to the ventral migratory pathway in the trunk. Dev. Biol. 282, 411–421. doi:10.1016/j.ydbio.2005.03.021

Johnson, D.E., Williams, L.T., 1993. Structural and functional diversity in the FGF receptor multigene family. Adv. Cancer Res. 60, 1–41.

Jones, M., Sabatini, P.J.B., Lee, F.S.H., Bendeck, M.P., Langille, B.L., 2002. N-cadherin upregulation and function in response of smooth muscle cells to arterial injury. Arterioscler. Thromb. Vasc. Biol. 22, 1972–7.

Jossin, Y., Cooper, J.A., 2011a. Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. 14, 697–703. doi:10.1038/nn.2816

Jossin, Y., Cooper, J.A., 2011b. Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. 14, 697–703.

Kadowaki, M., Nakamura, S., Machon, O., Krauss, S., Radice, G.L., Takeichi, M., 2007. N-cadherin mediates cortical organization in the mouse brain. Dev. Biol. 304, 22–33. doi:10.1016/j.ydbio.2006.12.014

Kametani, Y., Takeichi, M., 2007. Basal-to-apical cadherin flow at cell junctions. Nat. Cell Biol. 9, 92–8. doi:10.1038/ncb1520

Kanner, S.B., Reynolds, A.B., Parsons, J.T., 1991. Tyrosine phosphorylation of a 120-kilodalton pp60_src_ substrate upon epidermal growth factor and platelet-derived growth factor receptor stimulation and in polyomavirus middle-T-antigen- transformed cells. Mol. Cell. Biol. 11, 713–720.

Kashima, T., Nakamura, K., Kawaguchi, J., Takanashi, M., Ishida, T., Aburatani, H., Kudo, A.,

Fukayama, M., Grigoriadis, A.E., 2003. Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo. Int. J. cancer 104, 147–54. doi:10.1002/ijc.10931

Kawauchi, T., 2012. Cell adhesion and its endocytic regulation in cell migration during neural development and cancer metastasis. Int. J. Mol. Sci. 13, 4564–90. doi:10.3390/ijms13044564

Kim, D.-H., Kwak, Y., Kim, N.D., Sim, T., 2015. Antitumor effects and molecular mechanisms of ponatinib on endometrial cancer cells harboring activating FGFR2 mutations. Cancer Biol. Ther.

Kim, J.B., Islam, S., Kim, Y.J., Prudoff, R.S., Sass, K.M., Wheelock, M.J., Johnson, K.R., 2000. N-Cadherin extracellular repeat 4 mediates epithelial to mesenchymal transition and increased motility. J. Cell Biol. 151, 1193–206.

Kim, N., Kim, J.M., Lee, M., Kim, C.Y., Chang, K., Heo, W. Do, 2014. Resource Spatiotemporal Control of Fibroblast Growth Factor Receptor Signals by Blue Light. Chem. Biol. 21, 903–912. doi:10.1016/j.chembiol.2014.05.013

Kl, C., Glazer, L., Klambt, C., Glazer, L., Shilo, B.Z., Klämbt, C., Glazer, L., Shilo, B.Z., 1992. breathless, A Drosophila FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. Genes Dev. 6, 1668–1678.

Klambt, C., Glazer, L., Shilo, B.Z., 1992. breathless, A Drosophila FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. Genes Dev. 6, 1668–1678. doi:10.1101/gad.6.9.1668

Klingener, M., Chavali, M., Singh, J., McMillan, N., Coomes, A., Dempsey, P.J., Chen, E.I., Aguirre, A., 2014. N-cadherin promotes recruitment and migration of neural progenitor cells from the SVZ neural stem cell niche into demyelinated lesions. J. Neurosci. 34, 9590–606. doi:10.1523/JNEUROSCI.3699-13.2014

Knuchel, S., Anderle, P., Werfelli, P., Diamantis, E., Rüegg, C., 2015. Fibroblast surface-associated FGF-2 promotes contact-dependent colorectal cancer cell migration and invasion through FGFR-SRC signaling and integrin αvβ5-mediated adhesion. Oncotarget 6, 14300–17. doi:10.18632/oncotarget.3883

Kobielak, A., Fuchs, E., 2004. Alpha-catenin: at the junction of intercellular adhesion and actin dynamics. Nat. Rev. Mol. Cell Biol. 5, 614–25. doi:10.1038/nrm1433

Kolijn, K., Verhoef, E.I., van Leenders, G.J.L.H., 2015. Morphological and immunohistochemical identification of epithelial-to-mesenchymal transition in clinical prostate cancer. Oncotarget 5.

Kontaridis, M.I., Liu, X., Zhang, L., Bennett, A.M., 2002. Role of SHP-2 in Fibroblast Growth Factor Receptor-Mediated Suppression of Myogenesis in C2C12 Myoblasts. Mol. Cell. Biol. 22, 3875–3891. doi:10.1128/MCB.22.11.3875-3891.2002

Kourtidis, A., Yanagisawa, M., Huveldt, D., Copland, J. a., Anastasiadis, P.Z., 2015. Pro-Tumorigenic Phosphorylation of p120 Catenin in Renal and Breast Cancer. PLoS One 10, e0129964.

Kovacs, E.M., Goodwin, M., Ali, R.G., Paterson, A.D., Yap, A.S., 2002. Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. Curr. Biol. 12, 379–382. doi:10.1016/S0960-9822(02)00661-9

Kowalczyk, A.P., Nanes, B.A., 2012. Adherens Junction Turnover: Regulating Adhesion Through Cadherin Endocytosis, Degradation, and Recycling, in: Sub-Cellular Biochemistry. pp. 197–222. doi:10.1007/978-94-007-4186-7_9

Kubota, Y., Ito, K., 2000. Chemotactic migration of mesencephalic neural crest cells in the mouse. Dev. Dyn. 217, 170–9. doi:10.1002/(SICI)1097-0177(200002)217:2<170::AID-DVDY4>3.0.CO;2-9

Kumar, A., Gupta, T., Berzsenyi, S., Giangrande, A., 2015. N-cadherin negatively regulates collective Drosophila glial migration through actin cytoskeleton remodeling. J. Cell Sci. 128, 900–912. doi:10.1242/jcs.157974

Künstlinger, H., Fassunke, J., Schildhaus, H.-U., Brors, B., Heydt, C., Angelika Ihle, M., Mechtersheimer, G., Wardelmann, E., Büttner, R., Merkelbach-Bruse, S., 2015. FGFR2 is overexpressed in myxoid liposarcoma and inhibition of FGFR signaling impairs tumor growth in vitro. Oncotarget 5.

Kuriyama, S., Mayor, R., 2008. Molecular analysis of neural crest migration. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 1349–62. doi:10.1098/rstb.2007.2252

Kuriyama, S., Theveneau, E., Benedetto, A., Parsons, M., Tanaka, M., Charras, G., Kabla, A., Mayor, R., 2014a. In vivo collective cell migration requires an LPAR2-dependent increase in tissue fluidity. J. Cell Biol. 206, 113–27. doi:10.1083/jcb.201402093

Kuriyama, S., Theveneau, E., Benedetto, A., Parsons, M., Tanaka, M., Charras, G., Kabla, A., Mayor, R., 2014b. In vivo collective cell migration requires an LPAR2-dependent increase in tissue fluidity. J. Cell Biol. 206, 113–127. doi:10.1083/jcb.201402093

Ladoux, B., Mège, R.-M., Trepat, X., 2016. Front–Rear Polarization by Mechanical Cues: From

Single Cells to Tissues. Trends Cell Biol.

Lambert, M., Thoumine, O., Brevier, J., Choquet, D., Riveline, D., Mège, R.-M., 2007. Nucleation and growth of cadherin adhesions. Exp. Cell Res. 313, 4025–40. doi:10.1016/j.yexcr.2007.07.035

Lampugnani, M.G., Orsenigo, F., Gagliani, M.C., Tacchetti, C., Dejana, E., 2006. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. J. Cell Biol. 174, 593–604. doi:10.1083/jcb.200602080

Lamszus, K., Brockmann, M.A., Eckerich, C., Bohlen, P., May, C., Mangold, U., Filibrandt, R., Westphal, M., 2005. Inhibition of glioblastoma angiogenesis and invasion by combined treatments directed against vascular endothelial growth factor receptor-2, epidermal growth factor receptor, and vascular endothelial-cadherin. Clin. Cancer Res. 11, 4934–4940.

Le Clainche, C., Carlier, M.-F., 2008. Regulation of actin assembly associated with protrusion and adhesion in cell migration. Physiol. Rev. 88, 489–513. doi:10.1152/physrev.00021.2007

le Duc, Q., Shi, Q., Blonk, I., Sonnenberg, A., Wang, N., Leckband, D., de Rooij, J., 2010. Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. J. Cell Biol. 189, 1107–15. doi:10.1083/jcb.201001149

Lebreton, G., Casanova, J., 2016. Ligand-binding and constitutive FGF receptors in single Drosophila tracheal cells: Implications for the role of FGF in collective migration. Dev. Dyn. 245, 372–8.

Lebreton, G., Casanova, J., 2014. Specification of leading and trailing cell features during collective migration in the Drosophila trachea. J. Cell Sci. 127, 465–74.

Lele, Z., Folchert, A., Concha, M., Rauch, G.G.-J.G.G.-J., Geisler, R., Rosa, F.F., Wilson, S.W., Hammerschmidt, M., Bally-cuif, L., 2002. Parachute/N-Cadherin Is Required for Morphogenesis and Maintained Integrity of the Zebrafish Neural Tube. Development 129, 3281–3294.

Lemmon, M. a, Schlessinger, J., 2011. NIH Public Access. Biochemistry 141, 1117–1134.

Li, G., Satyamoorthy, K., Herlyn, M., 2001. N-cadherin-mediated intercellular interactions promote survival and migration of melanoma cells. Cancer Res. 61, 3819–3825.

Lindner, V., Reidy, M.A., 1993. Expression of basic fibroblast growth factor and its receptor by smooth muscle cells and endothelium in injured rat arteries. An en face study. Circ. Res. 73, 589–95.

Luccardini, C., Hennekinne, L., Viou, L., Yanagida, M., Murakami, F., Kessaris, N., Ma, X., Adelstein, R.S., Mège, R.-M., Métin, C., 2013. N-cadherin sustains motility and polarity of future cortical interneurons during tangential migration. J. Neurosci. 33, 18149–60.

Lugo-Martínez, V.-H., Petit, C.S., Fouquet, S., Le Beyec, J., Chambaz, J., Pinçon-Raymond, M., Cardot, P., Thenet, S., 2009. Epidermal growth factor receptor is involved in enterocyte anoikis through the dismantling of E-cadherin-mediated junctions. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G235–G244.

Luo, Y., High, F.A., Epstein, J.A., Radice, G.L., 2006. N-cadherin is required for neural crest remodeling of the cardiac outflow tract. Dev. Biol. 299, 517–528. doi:10.1016/j.ydbio.2006.09.003

Luo, Y., Radice, G.L., 2005a. N-cadherin acts upstream of VE-cadherin in controlling vascular morphogenesis. J. Cell Biol. 169, 29–34. doi:10.1083/jcb.200411127

Luo, Y., Radice, G.L., 2005b. N-cadherin acts upstream of VE-cadherin in controlling vascular morphogenesis. J. Cell Biol. 169, 29–34. doi:10.1083/jcb.200411127

Lyon, C.A., Koutsouki, E., Aguilera, C.M., Blaschuk, O.W., George, S.J., 2010. Inhibition of N-cadherin retards smooth muscle cell migration and intimal thickening via induction of apoptosis. J. Vasc. Surg. 52, 1301–9. doi:10.1016/j.jvs.2010.05.096

Maeda, M., Johnson, E., Mandal, S.H., Lawson, K.R., Keim, S. a, Svoboda, R. a, Caplan, S., Wahl, J.K., Wheelock, M.J., Johnson, K.R., 2006. Expression of inappropriate cadherins by epithelial tumor cells promotes endocytosis and degradation of E-cadherin via competition for p120(ctn). Oncogene 25, 4595–604. doi:10.1038/sj.onc.1209396

Mandal, L., Dumstrei, K., Hartenstein, V., 2004. Role of FGFR signaling in the morphogenesis of theDrosophila visceral musculature. Dev. Dyn. 231, 342–348. doi:10.1002/dvdy.20088

Martin, A.C., Kaschube, M., Wieschaus, E.F., 2009. Pulsed contractions of an actin-myosin network drive apical constriction. Nature 457, 495–9.

Martínez-Morales, P.L., Diez del Corral, R., Olivera-Martínez, I., Quiroga, A.C., Das, R.M., Barbas, J.A., Storey, K.G., Morales, A. V, 2011. FGF and retinoic acid activity gradients control the timing of neural crest cell emigration in the trunk. J. Cell Biol. 194, 489–503. doi:10.1083/jcb.201011077

Masai, I., Lele, Z., Yamaguchi, M., Komori, A., Nakata, A., Nishiwaki, Y., Wada, H., Tanaka, H.,

Nojima, Y., Hammerschmidt, M., Wilson, S.W., Okamoto, H., 2003. N-cadherin mediates retinal lamination, maintenance of forebrain compartments and patterning of retinal neurites. Development 130, 2479–2494.

Mateus, A.R., Seruca, R., Machado, J.C., Keller, G., Oliveira, M.J., Suriano, G., Luber, B., 2007. EGFR regulates RhoA-GTP dependent cell motility in E-cadherin mutant cells. Hum. Mol. Genet. 16, 1639–47.

Matsunaga, M., Hatta, K., Takeichi, M., 1988. Role of N-cadherin cell adhesion molecules in the histogenesis of neural retina. Neuron 1, 289–95.

Mayor, R., Etienne-Manneville, S., 2016. The front and rear of collective cell migration. Nat. Rev. Mol. Cell Biol. 17, 97–109. doi:10.1038/nrm.2015.14

McAndrews, K.M., Yi, J., McGrail, D.J., Dawson, M.R., 2015. Enhanced Adhesion of Stromal Cells to Invasive Cancer Cells Regulated by Cadherin 11. ACS Chem. Biol. 10, 1932–1938.

McCormack, J., Welsh, N.J., Braga, V.M.M., 2013. Cycling around cell-cell adhesion with Rho GTPase regulators. J. Cell Sci. 126, 379–91. doi:10.1242/jcs.097923

McCrea, P.D., Gumbiner, B.M., 1991. Purification of a 92-kDa cytoplasmic protein tightly associated with the cell-cell adhesion molecule E-cadherin (uvomorulin). Characterization and extractability of the protein complex from the cell cytostructure. J. Biol. Chem. 266, 4514–20.

Mège, R.-M., Gavard, J., Lambert, M., 2006. Regulation of cell-cell junctions by the cytoskeleton. Curr. Opin. Cell Biol. 18, 541–8.

Mege, R.M., Goudou, D., Diaz, C., Nicolet, M., Garcia, L., Geraud, G., Rieger, F., 1992. N-cadherin and N-CAM in myoblast fusion: compared localisation and effect of blockade by peptides and antibodies. J. Cell Sci. 103 (Pt 4, 897–906.

Mendez, P., De Roo, M., Poglia, L., Klauser, P., Muller, D., 2010. N-cadherin mediates plasticity-induced long-term spine stabilization. J. Cell Biol. 189, 589–600. doi:10.1083/jcb.201003007

Mitchison, T., Kirschner, M., 1988. Cytoskeletal dynamics and nerve growth. Neuron 1, 761–72.

Miyashita, Y., Ozawa, M., 2007. Increased internalization of p120-uncoupled E-cadherin and a requirement for a dileucine motif in the cytoplasmic domain for endocytosis of the protein. J. Biol. Chem. 282, 11540–8. doi:10.1074/jbc.M608351200

Moiseeva, E., 2001. Adhesion receptors of vascular smooth muscle cells and their functions.

Cardiovasc. Res. 52, 372–386. doi:10.1016/S0008-6363(01)00399-6

Montell, D.J., 2008. Morphogenetic Cell Movements: Diversity from Modular Mechanical Properties. Science (80-.). 322, 1502–1505. doi:10.1126/science.1164073

Muhamed, I., Wu, J., Sehgal, P., Kong, X., Tajik, A., Wang, N., Leckband, D.E., 2016. E-Cadherin-mediated force transduction signals regulate global cell mechanics. J. Cell Sci. 129, 1843–54. doi:10.1242/jcs.185447

Murakami, M., Nguyen, L.T., Zhang, Z.W., Moodie, K.L., Carmeliet, P., Stan, R. V., Simons, M., 2008. The FGF system has a key role in regulating vascular integrity. J. Clin. Invest. 118, 3355–3366.

Murakami, M., Simons, M., 2009. Regulation of vascular integrity. J. Mol. Med. (Berl). 87, 571–82. doi:10.1007/s00109-009-0463-2

Nagafuchi, A., Takeichi, M., 1988. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. EMBO J. 7, 3679–84.

Nakamura, T., Mochizuki, Y., Kanetake, H., Kanda, S., 2001. Signals via FGF receptor 2 regulate migration of endothelial cells. Biochem. Biophys. Res. Commun. 289, 801–6.

Nakashima, T., Huang, C., Liu, D., Kameyama, K., Masuya, D., Kobayashi, S., Kinoshita, M., Yokomise, H., 2003. Neural-cadherin expression associated with angiogenesis in non-small-cell lung cancer patients. Br. J. Cancer 88, 1727–1733.

Nanes, B.A., Chiasson-MacKenzie, C., Lowery, A.M., Ishiyama, N., Faundez, V., Ikura, M., Vincent, P.A., Kowalczyk, A.P., 2012. P120-Catenin Binding Masks an Endocytic Signal Conserved in Classical Cadherins. J. Cell Biol. 199, 365–380.

Navarro, P., Ruco, L., Dejana, E., 1998. Differential localization of VE- and N-cadherins in human endothelial cells: VE-cadherin competes with N-cadherin for junctional localization. J. Cell Biol. 140, 1475–1484.

Nelson, W.J., 2008. Regulation of cell–cell adhesion by the cadherin–catenin complex. Biochem. Soc. Trans. 36, 149–155. doi:10.1042/BST0360149

Nguyen, P.T., Tsunematsu, T., Yanagisawa, S., Kudo, Y., Miyauchi, M., Kamata, N., Takata, T., 2013. The FGFR1 inhibitor PD173074 induces mesenchymal-epithelial transition through the transcription factor AP-1. Br. J. Cancer 109, 2248–58.

Nguyen, T., Mège, R.M., 2016. N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. doi:10.1016/j.ejcb.2016.05.002

Nieman, M.T., Prudoff, R.S., Johnson, K.R., Wheelock, M.J., 1999. N-Cadherin Promotes Motility in Human Breast Cancer Cells Regardless of Their E-Cadherin Expression. J. Cell Biol. 147, 631–644. doi:10.1083/jcb.147.3.631

Niessen, C.M., Leckband, D., Yap, A.S., 2011. Tissue Organization by Cadherin Adhesion Molecules: Dynamic Molecular and Cellular Mechanisms of Morphogenetic Regulation. Physiol. Rev. 91, 691–731. doi:10.1152/physrev.00004.2010

Niessen, C.M., Leckband, D., Yap, A.S., 2011. Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation. Physiol. Rev. 91, 691–731. doi:10.1152/physrev.00004.2010

Nomura, S., Yoshitomi, H., Takano, S., Shida, T., Kobayashi, S., Ohtsuka, M., Kimura, F., Shimizu, H., Yoshidome, H., Kato, A., Miyazaki, M., 2008. FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer. Br. J. Cancer 99, 305–13.

Nourse, M.B., Rolle, M.W., Pabon, L.M., Murry, C.E., 2007. Selective control of endothelial cell proliferation with a synthetic dimerizer of FGF receptor-1. Lab. Investig. 87, 828–835. doi:10.1038/labinvest.3700597

Ohkubo, T., Ozawa, M., 1999. p120(ctn) binds to the membrane-proximal region of the E-cadherin cytoplasmic domain and is involved in modulation of adhesion activity. J. Biol. Chem. 274, 21409–21415. doi:10.1074/jbc.274.30.21409

Ong, L.L., Kim, N., Mima, T., Cohen-Gould, L., Mikawa, T., 1998. Trabecular myocytes of the embryonic heart require N-cadherin for migratory unit identity. Dev. Biol. 193, 1–9. doi:10.1006/dbio.1997.8775

Ouyang, M., Lu, S., Kim, T., Chen, C.-E., Seong, J., Leckband, D.E., Wang, F., Reynolds, A.B., Schwartz, M.A., Wang, Y., 2013. N-cadherin regulates spatially polarized signals through distinct p120ctn and β-catenin-dependent signalling pathways. Nat. Commun. 4, 1589. doi:10.1038/ncomms2560

Paluch, E., Sykes, C., Prost, J., Bornens, M., 2006. Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol. 16, 5–10.

Parish, a, Schwaederle, M., Daniels, G., Piccioni, D., Fanta, P., Schwab, R., Shimabukuro, K., Parker, B. a, Helsten, T., Kurzrock, R., 2015. Fibroblast Growth Factor Family Aberrations in Cancers: Clinical and Molecular Characteristics. Cell Cycle 00–00.

Partanen, J., Schwartz, L., Rossant, J., 1998. Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for Fgfr1 in anteroposterior patterning of mouse embryos. Genes Dev. 12, 2332–2344.

Patel, S.D., Ciatto, C., Chen, C.P., Bahna, F., Rajebhosale, M., Arkus, N., Schieren, I., Jessell, T.M., Honig, B., Price, S.R., Shapiro, L., 2006. Type II Cadherin Ectodomain Structures: Implications for Classical Cadherin Specificity. Cell 124, 1255–1268. doi:10.1016/j.cell.2005.12.046

Pathak, A., Kumar, S., 2011. Biophysical regulation of tumor cell invasion: moving beyond matrix stiffness. Integr. Biol. 3, 267. doi:10.1039/c0ib00095g

Peglion, F., Llense, F., Etienne-Manneville, S., 2014. Adherens junction treadmilling during collective migration. Nat. Cell Biol. 16, 639–51.

Peluso, J.J., Pappalardo, A., Trolice, M.P., 1996. N-cadherin-mediated cell contact inhibits granulosa cell apoptosis in a progesterone-independent manner. Endocrinology 137, 1196–203.

Peterson, S.J., Krasnow, M.A., 2015. Subcellular trafficking of FGF controls tracheal invasion of Drosophila flight muscle. Cell 160, 313–23. doi:10.1016/j.cell.2014.11.043

Piedra, J., Miravet, S., Castaño, J., Pálmer, H.G., Heisterkamp, N., García de Herreros, A., Duñach, M., 2003. p120 Catenin-associated Fer and Fyn tyrosine kinases regulate beta-catenin Tyr-142 phosphorylation and beta-catenin-alpha-catenin Interaction. Mol. Cell. Biol. 23, 2287–97.

Pirotte, S., Lamour, V., Lambert, V., Alvarez Gonzalez, M.-L., Ormenese, S., Noël, A., Mottet, D., Castronovo, V., Bellahcène, A., 2011. Dentin matrix protein 1 induces membrane expression of VE-cadherin on endothelial cells and inhibits VEGF-induced angiogenesis by blocking VEGFR-2 phosphorylation. Blood 117, 2515–26.

Plestant, C., Strale, P.-O.P.-O., Seddiki, R., Nguyen, E., Ladoux, B., Mege, R.-M., Mège, R.-M., 2014a. Adhesive interactions of N-cadherin limit the recruitment of microtubules to cell-cell contacts through organization of actomyosin. J. Cell Sci. 127, 1660–71. doi:10.1242/jcs.131284

Plestant, C., Strale, P.-O.P.-O., Seddiki, R., Nguyen, E., Ladoux, B., Mege, R.-M., Mège, R.-M., Mege, R.-M., Mège, R.-M., 2014b. Adhesive interactions of N-cadherin limit the recruitment of microtubules to cell-cell contacts through organization of actomyosin. J. Cell Sci. 127, 1660–71.

doi:10.1242/jcs.131284

Pokutta, S., Weis, W.I., 2000. Structure of the dimerization and beta-catenin-binding region of alpha-catenin. Mol. Cell 5, 533–43.

Poling, J., Szibor, M., Schimanski, S., Ingelmann, M.-E., Rees, W., Gajawada, P., Kochfar, Z., Lorchner, H., Salwig, I., Shin, J.-Y., Wiebe, K., Kubin, T., Warnecke, H., Braun, T., 2011. Induction of Smooth Muscle Cell Migration During Arteriogenesis Is Mediated by Rap2. Arterioscler. Thromb. Vasc. Biol. 31, 2297–2305. doi:10.1161/ATVBAHA.111.232835

Powers, C.J., McLeskey, S.W., Wellstein, A., 2000. Fibroblast growth factors, their receptors and signaling. Endocr. Relat. Cancer 7, 165–197. doi:10.1677/erc.0.0070165

Presta, M., Dell'Era, P., Mitola, S., Moroni, E., Ronca, R., Rusnati, M., 2005. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 16, 159–78.

Qi, J., Chen, N., Wang, J., Siu, C., 2005. Transendothelial migration of melanoma cells involves N-cadherin-mediated adhesion and activation of the beta-catenin signaling pathway. Mol. Biol. Cell 16, 4386–97. doi:10.1091/mbc.E05

Qian, X., Anzovino, A., Kim, S., Suyama, K., Yao, J., Hulit, J., Agiostratidou, G., Chandiramani, N., McDaid, H.M., Nagi, C., Cohen, H.W., Phillips, G.R., Norton, L., Hazan, R.B., 2013. N-cadherin/FGFR promotes metastasis through epithelial-to-mesenchymal transition and stem/progenitor cell-like properties. Oncogene 33, 1–11.

Radice, G.L., 2013. N-Cadherin-Mediated Adhesion and Signaling from Development to Disease. pp. 263–289. doi:10.1016/B978-0-12-394311-8.00012-1

Radice, G.L., Rayburn, H., Matsunami, H., Knudsen, K.A., Takeichi, M., Hynes, R.O., 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev. Biol. 181, 64–78.

Rauch, B.H., Millette, E., Kenagy, R.D., Daum, G., Fischer, J.W., Clowes, A.W., 2005. Syndecan-4 Is Required for Thrombin-induced Migration and Proliferation in Human Vascular Smooth Muscle Cells. J. Biol. Chem. 280, 17507–17511. doi:10.1074/jbc.M410848200

Ravasio, A., Le, A.P., Saw, T.B., Tarle, V., Ong, H.T., Bertocchi, C., Mège, R.-M., Lim, C.T., Gov, N.S., Ladoux, B., 2015. Regulation of epithelial cell organization by tuning cell-substrate adhesion. Integr. Biol. (Camb). 7, 1228–41.

Redfield, A., Nieman, M.T., Knudsen, K.A., 1997. Cadherins Promote Skeletal Muscle Differentiation

in Three-dimensional Cultures. J. Cell Biol. 138, 1323–1331. doi:10.1083/jcb.138.6.1323

Reynolds, a B., Daniel, J., McCrea, P.D., Wheelock, M.J., Wu, J., Zhang, Z., 1994. Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes. Mol. Cell. Biol. 14, 8333–8342. doi:10.1128/MCB.14.12.8333

Reynolds, A.B., Carnahan, R.H., 2004. Regulation of cadherin stability and turnover by p120ctn: Implications in disease and cancer. Semin. Cell Dev. Biol. 15, 657–663.

Reynolds, A.B., Roczniak-Ferguson, A., 2004. Emerging roles for p120-catenin in cell adhesion and cancer. Oncogene 23, 7947–7956.

Rieger-Christ, K.M., Lee, P., Zagha, R., Kosakowski, M., Moinzadeh, A., Stoffel, J., Ben-Ze'ev, A., Libertino, J. a, Summerhayes, I.C., 2004. Novel expression of N-cadherin elicits in vitro bladder cell invasion via the Akt signaling pathway. Oncogene 23, 4745–4753.

Rimm, D.L., Koslov, E.R., Kebriaei, P., Cianci, C.D., Morrow, J.S., 1995. Alpha(1)(E)-Catenin Is An Actin-Binding And Actin-Bundling Protein Mediating The Attachment Of F-Actin To The Membrane Adhesion Complex. Proc. Natl. Acad. Sci. U. S. A. 92, 8813–8817. doi:10.1073/pnas.92.19.8813

Rørth, P., 2007. Collective guidance of collective cell migration. Trends Cell Biol. 17, 575–579. doi:10.1016/j.tcb.2007.09.007

Roura, S., Miravet, S., Piedra, J.J., García de Herreros, A., Duñach, M., Garcia de Herreros, A., Dunach, M., Garc??a De Herreros, A., Du??achl, M., 1999. Regulation of E-cadherin/catenin association by tyrosine phosphorylation. J. Biol. Chem. 274, 36734–36740. doi:10.1074/jbc.274.51.36734

Rousso, D.L., Pearson, C.A., Gaber, Z.B., Miquelajauregui, A., Li, S., Portera-Cailliau, C., Morrisey, E.E., Novitch, B.G., 2012. Foxp-mediated suppression of N-cadherin regulates neuroepithelial character and progenitor maintenance in the CNS. Neuron 74, 314–30. doi:10.1016/j.neuron.2012.02.024

Rubio, M.E., Curcio, C., Chauvet, N., Brusés, J.L., 2005. Assembly of the N-cadherin complex during synapse formation involves uncoupling of p120-catenin and association with presenilin 1. Mol. Cell. Neurosci. 30, 118–130. doi:10.1016/j.mcn.2005.06.005

Saffell, J.L., Williams, E.J., Mason, I.J., Walsh, F.S., Doherty, P., 1997. Expression of a dominant negative FGF receptor inhibits axonal growth and FGF receptor phosphorylation stimulated by

CAMs [published erratum appears in Neuron 1998 Mar;20(3):619]. Neuron 18, 231–242.

Salomon, D.S., Brandt, R., Ciardiello, F., Normanno, N., 1995. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit. Rev. Oncol. Hematol. 19, 183–232.

Sarabipour, S., Hristova, K., 2016. Mechanism of FGF receptor dimerization and activation. Nat. Commun. 7, 10262.

Sasaki, T., Hiroki, K., Yamashita, Y., 2013. The role of epidermal growth factor receptor in cancer metastasis and microenvironment. Biomed Res. Int. 2013, 546318. doi:10.1155/2013/546318

Sato, A., Scholl, A.M., Kuhn, E.B., Stadt, H.A., Decker, J.R., Pegram, K., Hutson, M.R., Kirby, M.L., 2011. FGF8 signaling is chemotactic for cardiac neural crest cells. Dev. Biol. 354, 18–30. doi:10.1016/j.ydbio.2011.03.010

Scarpa, E., Szabó, A., Bibonne, A., Theveneau, E., Parsons, M., Mayor, R., 2015. Cadherin Switch during EMT in Neural Crest Cells Leads to Contact Inhibition of Locomotion via Repolarization of Forces. Dev. Cell 34, 421–434. doi:10.1016/j.devcel.2015.06.012

Schlessinger, J., 2000. Cell Signaling by Receptor Tyrosine Kinases. Cell 103, 211–225.

Shan, W., Yagita, Y., Wang, Z., Koch, A., Svenningsen, A.F., Gruzglin, E., Pedraza, L., Colman, D.R., 2004. The Minimal Essential Unit for Cadherin-mediated Intercellular Adhesion Comprises Extracellular Domains 1 and 2. J. Biol. Chem. 279, 55914–55923. doi:10.1074/jbc.M407827200

Shan, W.S., Tanaka, H., Phillips, G.R., Arndt, K., Yoshida, M., Colman, D.R., Shapiro, L., 2000. Functional cis-heterodimers of N- and R-cadherins. J. Cell Biol. 148, 579–90.

Sharma, M., Henderson, B.R., 2007. IQ-domain GTPase-activating Protein 1 Regulates beta-Catenin at Membrane Ruffles and Its Role in Macropinocytosis of N-cadherin and Adenomatous Polyposis Coli. J. Biol. Chem. 282, 8545–8556. doi:10.1074/jbc.M610272200

Shewan, A.M., 2005. Myosin 2 Is a Key Rho Kinase Target Necessary for the Local Concentration of E-Cadherin at Cell-Cell Contacts. Mol. Biol. Cell 16, 4531–4542. doi:10.1091/mbc.E05-04-0330

Shih, W., Yamada, S., 2012. N-cadherin-mediated cell-cell adhesion promotes cell migration in a three-dimensional matrix. J. Cell Sci. 125, 3661–70.

Shikanai, M., Nakajima, K., Kawauchi, T., 2011. N-Cadherin regulates radial glial fiber-dependent migration of cortical locomoting neurons. Commun. Integr. Biol. 4, 326–330.

Shore, E.M., Nelson, W.J., 1991. Biosynthesis of the Cell Adhesion Molecule Uvomorulin (E-Cadherin) in MAdin-Darby Canine Kidney Epithelial Cells. J. Biol. Chem. 266, 19672–19680.

Steiling, H., Werner, S., 2003. Fibroblast growth factors: key players in epithelial morphogenesis, repair and cytoprotection. Curr. Opin. Biotechnol. 14, 533–7.

Stepniak, E., Radice, G.L., Vasioukhin, V., 2009. Adhesive and signaling functions of cadherins and catenins in vertebrate development. Cold Spring Harb. Perspect. Biol. 1, a002949. doi:10.1101/cshperspect.a002949

Strale, P.-O., Duchesne, L., Peyret, G., Montel, L., Nguyen, T., Png, E., Tampe, R., Troyanovsky, S., Henon, S., Ladoux, B., Mege, R.-M., 2015. The formation of ordered nanoclusters controls cadherin anchoring to actin and cell-cell contact fluidity. J. Cell Biol. 210, 333–346. doi:10.1083/jcb.201410111

Suyama, K., Shapiro, I., Guttman, M., Hazan, R.B., 2002. A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2, 301–14.

Taeger, J., Moser, C., Hellerbrand, C., Mycielska, M.E., Glockzin, G., Schlitt, H.J., Geissler, E.K., Stoeltzing, O., Lang, S. a., 2011. Targeting FGFR/PDGFR/VEGFR Impairs Tumor Growth, Angiogenesis, and Metastasis by Effects on Tumor Cells, Endothelial Cells, and Pericytes in Pancreatic Cancer. Mol. Cancer Ther. 10, 2157–2167.

Tai, C.-Y., Mysore, S.P., Chiu, C., Schuman, E.M., 2007. Activity-Regulated N-Cadherin Endocytosis. Neuron 54, 771–785. doi:10.1016/j.neuron.2007.05.013

Takahashi, K., Matafonov, A., Sumarriva, K., Ito, H., Lauhan, C., Zemel, D., Tsuboi, N., Chen, J., Reynolds, A., Takahashi, T., 2014. CD148 tyrosine phosphatase promotes cadherin cell adhesion. PLoS One 9.

Takehara, T., Teramura, T., Onodera, Y., Frampton, J., Fukuda, K., 2015. Cdh2 stabilizes FGFR1 and contributes to primed-state pluripotency in mouse epiblast stem cells. Sci. Rep. 5, 14722.

Taniguchi, H., Kawauchi, D., Nishida, K., Development, F.M., Taniguchi, H., Kawauchi, D., Nishida, K., Murakami, F., 2006a. Classic cadherins regulate tangential migration of precerebellar neurons in the caudal hindbrain. Development 133, 1923–1931. doi:10.1242/dev.02414

Taniguchi, H., Kawauchi, D., Nishida, K., Murakami, F., 2006b. Classic cadherins regulate tangential migration of precerebellar neurons in the caudal hindbrain. Development 133, 1923–31. doi:10.1242/dev.02354

Taulet, N., Comunale, F., Favard, C., Charrasse, S., Bodin, S., Gauthier-Rouviere, C., 2009. N-cadherin/p120 Catenin Association at Cell-Cell Contacts Occurs in Cholesterol-rich Membrane Domains and Is Required for RhoA Activation and Myogenesis. J. Biol. Chem. 284, 23137–23145. doi:10.1074/jbc.M109.017665

Theisen, C.S., Wahl, J.K., Johnson, K.R., Wheelock, M.J., 2007. NHERF links the N-cadherin/catenin complex to the platelet-derived growth factor receptor to modulate the actin cytoskeleton and regulate cell motility. Mol. Biol. Cell 18, 1220–32. doi:10.1091/mbc.E06-10-0960

Théveneau, E., Duband, J.-L., Altabef, M., 2007. Ets-1 confers cranial features on neural crest delamination. PLoS One 2, e1142. doi:10.1371/journal.pone.0001142

Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moepps, B., Parsons, M., Mayor, R., 2010. Collective Chemotaxis Requires Contact-Dependent Cell Polarity. Dev. Cell 19, 39–53. doi:10.1016/j.devcel.2010.06.012

Thiery, J.P., 2002. Epithelial–mesenchymal transitions in tumour progression. Nat. Rev. Cancer 2, 442–454. doi:10.1038/nrc822

Thiery, J.P., Engl, W., Viasnoff, V., Dufour, S., 2012. Biochemical and biophysical origins of cadherin selectivity and adhesion strength. Curr. Opin. Cell Biol. 24, 614–619. doi:10.1016/j.ceb.2012.06.007

Thoreson, M.A., Anastasiadis, P.Z., Daniel, J.M., Ireton, R.C., Wheelock, M.J., Johnson, K.R., Hummingbird, D.K., Reynolds, A.B., 2000. Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. J. Cell Biol. 148, 189–201. doi:10.1083/jcb.148.1.189

Thoumine, O., Lambert, M., Mège, R.-M., Choquet, D., 2006. Regulation of N-cadherin dynamics at neuronal contacts by ligand binding and cytoskeletal coupling. Mol. Biol. Cell 17, 862–75. doi:10.1091/mbc.E05-04-0335

Togashi, H., Abe, K., Mizoguchi, A., Takaoka, K., Chisaka, O., Takeichi, M., 2002. Cadherin Regulates Dendritic Spine Morphogenesis. Neuron 35, 77–89. doi:10.1016/S0896-6273(02)00748-1

Trepat, X., Fredberg, J.J., 2011. Plithotaxis and emergent dynamics in collective cellular migration. Trends Cell Biol. 21, 638–646. doi:10.1016/j.tcb.2011.06.006

Trepat, X., Wasserman, M.R., Angelini, T.E., Millet, E., Weitz, D.A., Butler, J.P., Fredberg, J.J., 2009. Physical forces during collective cell migration. Nat. Phys. 5, 426–430.

doi:10.1038/nphys1269

Trokovic, N., Trokovic, R., Partanen, J., 2005. Fibroblast growth factor signalling and regional specification of the pharyngeal ectoderm. Int. J. Dev. Biol. 49, 797–805. doi:10.1387/ijdb.051976nt

Trokovic, R., Trokovic, N., Hernesniemi, S., Pirvola, U., Vogt Weisenhorn, D.M., Rossant, J., McMahon, A.P., Wurst, W., Partanen, J., 2003. FGFR1 is independently required in both developing mid- and hindbrain for sustained response to isthmic signals. EMBO J. 22, 1811–1823.

Trolice, M.P., Pappalardo, A., Peluso, J.J., 1997. Basic fibroblast growth factor and N-cadherin maintain rat granulosa cell and ovarian surface epithelial cell viability by stimulating the tyrosine phosphorylation of the fibroblast growth factor receptors. Endocrinology 138, 107–13. doi:10.1210/endo.138.1.4836

Twiss, F., de Rooij, J., 2013. Cadherin mechanotransduction in tissue remodeling. Cell. Mol. Life Sci. 70, 4101–4116. doi:10.1007/s00018-013-1329-x

Utton, M.A., Eickholt, B., Howell, F. V., Wallis, J., Doherty, P., 2001. Soluble N-cadherin stimulates fibroblast growth factor receptor dependent neurite outgrowth and N-cadherin and the fibroblast growth factor receptor co-cluster in cells. J. Neurochem. 76, 1421–1430. doi:10.1046/j.1471-4159.2001.00140.x

Utton, M.A., Eickholt, B., Howell, F. V, Wallis, J., Doherty, P., 2001. Soluble N-cadherin stimulates fibroblast growth factor receptor dependent neurite outgrowth and N-cadherin and the fibroblast growth factor receptor co-cluster in cells. J. Neurochem. 76, 1421–30.

Vaezi, A., Bauer, C., Vasioukhin, V., Fuchs, E., 2002. Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architercture in the early steps of assembling a stratified epithelium. Dev. Cell 3, 367–381. doi:10.1016/S1534-5807(02)00259-9

Van Roy, F., Berx, G., 2008. The cell-cell adhesion molecule E-cadherin. Cell. Mol. Life Sci. 65, 3756–3788. doi:10.1007/s00018-008-8281-1

Vargas, P., Terriac, E., Lennon-Duménil, A.-M., Piel, M., 2014. Study of cell migration in microfabricated channels. J. Vis. Exp. e51099.

Vedula, S.R.K., Leong, M.C., Lai, T.L., Hersen, P., Kabla, A.J., Lim, C.T., Ladoux, B., 2012. Emerging modes of collective cell migration induced by geometrical constraints. Proc. Natl.

Acad. Sci. U. S. A. 109, 12974–9.

Vedula, S.R.K., Ravasio, A., Anon, E., Chen, T., Peyret, G., Ashraf, M., Ladoux, B., 2014. Microfabricated environments to study collective cell behaviors. Methods Cell Biol. 120, 235–52.

Vitorino, P., Meyer, T., 2008. Modular control of endothelial sheet migration. Genes Dev. 22, 3268–81. doi:10.1101/gad.1725808

Ware, K.E., Hinz, T.K., Kleczko, E., Singleton, K.R., Marek, L.A., Helfrich, B.A., Cummings, C.T., Graham, D.K., Astling, D., Tan, A.-C., Heasley, L.E., 2013. A mechanism of resistance to gefitinib mediated by cellular reprogramming and the acquisition of an FGF2-FGFR1 autocrine growth loop. Oncogenesis 2, e39.

Weis, S., Cui, J., Barnes, L., Cheresh, D., 2004. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. J. Cell Biol. 167, 223–229.

Werner, S., Duan, D.S., de Vries, C., Peters, K.G., Johnson, D.E., Williams, L.T., 1992. Differential splicing in the extracellular region of fibroblast growth factor receptor 1 generates receptor variants with different ligand-binding specificities. Mol. Cell. Biol. 12, 82–88. doi:10.1128/MCB.12.1.82

Werner, S., Smola, H., Liao, X., Longaker, M.T., Krieg, T., Hofschneider, P.H., Williams, L.T., 1994. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science 266, 819–22.

Wesche, J., Haglund, K., Haugsten, E.M., 2011. Fibroblast growth factors and their receptors in cancer. Biochem. J. 437, 199–213. doi:10.1042/BJ20101603

Wheelock, M.J., Soler, A.P., Knudsen, K.A., 2001. Cadherin junctions in mammary tumors. J. Mammary Gland Biol. Neoplasia 6, 275–85.

Williams, E.J., Furness, J., Walsh, F.S., Doherty, P., 1994. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. Neuron 13, 583–94.

Williams, E.J., Williams, G., Howell, F. V, Skaper, S.D., Walsh, F.S., Doherty, P., 2001. Identification of an N-cadherin motif that can interact with the fibroblast growth factor receptor and is required for axonal growth. J. Biol. Chem. 276, 43879–86.

Xiao, K., 2003. Cellular levels of p120 catenin function as a set point for cadherin expression levels in microvascular endothelial cells. J. Cell Biol. 163, 535–545.

Xu, L., Meng, F., Ni, M., Lee, Y., Li, G., 2013. N-cadherin regulates osteogenesis and migration of bone marrow-derived mesenchymal stem cells. Mol. Biol. Rep. 40, 2533–9.

Xu, X., Li, W.E.I., Huang, G.Y., Meyer, R., Chen, T., Luo, Y., Thomas, M.P., Radice, G.L., Lo, C.W., 2001. Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap junctions. J. Cell Biol. 154, 217–230. doi:10.1083/jcb.200105047

Yamada, S., Nelson, W.J., 2007. Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. J. Cell Biol. 178, 517–527. doi:10.1083/jcb.200701058

Yamaguchi, T.P., Harpal, K., Henkemeyer, M., Rossant, J., 1994. fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. Genes Dev. 8, 3032–3044.

Yoshida, K., Soldati, T., 2006. Dissection of amoeboid movement into two mechanically distinct modes. J. Cell Sci. 119, 3833–3844. doi:10.1242/jcs.03152

Zaromytidou, A.-I., 2014. Mechanotransduction in collective cell migration. Nat. Cell Biol. 16, 628–628. doi:10.1038/ncb3008

Zhan, X., Plourde, C., Hu, X., Friesel, R., Maciag, T., 1994. Association of fibroblast growth factor receptor-1 with c-Src correlates with association between c-Src and cortactin. J. Biol. Chem. 269, 20221–4.

Zhang, X., Ibrahimi, O.A., Olsen, S.K., Umemori, H., Mohammadi, M., Ornitz, D.M., 2006. Receptor specificity of the fibroblast growth factor family: The complete mammalian FGF family. J. Biol. Chem. 281, 15694–15700. doi:10.1074/jbc.M601252200

Zhang, Y., Sivasankar, S., Nelson, W.J., Chu, S., 2009. Resolving cadherin interactions and binding cooperativity at the single-molecule level. Proc. Natl. Acad. Sci. U. S. A. 106, 109–14. doi:10.1073/pnas.0811350106

Zou, L., Cao, S., Kang, N., Huebert, R.C., Shah, V.H., 2012. Fibronectin Induces Endothelial Cell Migration through 1 Integrin and Src-dependent Phosphorylation of Fibroblast Growth Factor Receptor-1 at Tyrosines 653/654 and 766. J. Biol. Chem. 287, 7190–7202. doi:10.1074/jbc.M111.304972

7 Annexes

N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in thecontrol of developmental and cancer cell migration

The formation of ordered nanoclusters controls cadherin anchoring to actin and cell–cell contact fluidity

European Journal of Cell Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Research paper

N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations

Thao Nguyen, René Marc Mège*

Institut Jacques Monod, CNRS, Université Paris Diderot, Paris, France

ARTICLE INFO

Article history:
Received 24 March 2016
Received in revised form 13 May 2016
Accepted 24 May 2016

Keywords:
Cadherin
FGFR
Cell migration
Cell invasion
Cell signalling
Cancer

ABSTRACT

Cell migrations are diverse. They constitutemajor morphogenetic driving forces during embryogenesis, but they contribute also to the loss of tissue homeostasis and cancer growth. Capabilities of cells to migrate as single cells or as collectives are controlled by internal and external signalling, leading to the reorganisation of their cytoskeleton as well as by the rebalancing of cell-matrix and cell-cell adhesions. Among the genes altered in numerous cancers, cadherins and growth factor receptors are of particular interest for cell migration regulation. In particular, cadherins such as N-cadherin and a class of growth factor receptors, namely FGFRs cooperate to regulate embryonic and cancer cell behaviours. In this review, we discuss on reciprocal crosstalk between N-cadherin and FGFRs during cell migration. Finally, we aim at clarifying the synergy between N-cadherin and FGFR signalling that ensure cellular reorganization during cell movements, mainly during cancer cell migration and metastasis but also during developmental processes.

1. Introduction

1.1. Cell adhesion and migration

Cell migration is a complex and central process of tissue organisation during embryogenesis and wound healing. Dysregulation of cell migration is associated with numerous diseases such as congenital malformations, neurological disorders and cancers. As a simple view, there are two main modes of cell migration, depending on cell types and cellular environment. Some cells migrate individually such as of immune system cells or fibroblasts (Friedl et al., 2001; Le Clainche and Carlier, 2008). Other cells while migrating remain associated to their neighbours inside cell cohorts or sheets, and therefore move collectively (Arboleda-Estudillo et al., 2010; Dumortier et al., 2012; Montell, 2001). Interestingly, cancer cells can migrate individually, collectively or switch from one mode to the other (Clark and Vignjevic, 2015; Friedl et al., 2012).

Cadherins, one of the core transmembrane components of *Adherens Junctions* (AJs) play an essential role during these processes. For instance, E-cadherin is critical for border cell migration in *Drosophila* egg chamber (Niewiadomska et al., 1999) or for directional epithelial sheet migration during wound healing in mouse cornea (Danjo and Gipson, 1998). Another cadherin, N-cadherin is a major regulator of neuronal progenitors migration (Kadowaki et al., 2007; Lien et al., 2006). Moreover, the switch from E-cadherin to N-cadherin expression during Epithelial to Mesenchymal Transition (EMT) confers to epithelia-originating cancer cells drastic changes in their migratory behaviour (Li et al., 2001; Nakashima et al., 2003; Rieger-Christ et al., 2004; Taeger et al., 2011; Kolijn et al., 2015).

FGFRs (Fibroblast Growth Factor Receptors) belong to a family of single pass transmembrane Receptors Tyrosine Kinases (RTK). Activation of FGFRs, elicited by the binding of their ligands FGFs, trigger numerous intracellular signalling pathways orchestrating key cellular events including cell migration (Lemmon and Schlessinger, 2011). For example, FGFR 1 and 2 have been reported as key regulators for keratinocyte migration in wounded mouse skin (Meyer et al., 2012). During *Drosophila* tracheal morphogenesis, interaction of FGFR signalling with the regulation of the actin network is regulating cell migration (Okenve-Ramos and Llimargas, 2014). Remarkably, a functional link between FGFR signalling and cadherin-mediated adhesion has been reported for the regulation

Abbreviations: AJ, *adherens* junction; CAFs, cancer-associated fibroblasts; CNS, central nervous system; DN, dominant negative; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial to mesenchymal transition; FA, focal adhesion; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FRS2a, FGFR substrate 2a; GC, granulosa cell; GF, growth factor; KD, kinase death; mEpiSC, mouse epiblast stem cell; ROSE, ovarian surface epithelial cell; RTK, receptors tyrosine kinase; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

* Corresponding author.
E-mail addresses: rene-marc.mege@ijm.fr, rene.marc.mege@gmail.com (R.M. Mège).

http://dx.doi.org/10.1016/j.ejcb.2016.05.002
0171-9335/

of neural or tumour cell migration (Suyama et al., 2002; Williams et al., 2001). The synergistic action of these two transmembrane proteins would thus provide an unexpected additional level of regulation of cell migration.

1.2. Individual and collective cell migration modes

Numerous culture systems have been developed to study the migration of individual cells *in vitro* and the most used is the well-known Boyden chamber (Boyden, 1962). Recent progresses in micro-fabrication techniques brought out the possibility to produce one-cell-size adhesive areas with different protein surfaces (Maiuri et al., 2012), providing powerful tools to control and study the mode of migration of single cells.

Most of the cells migrate individually through a cyclic process involving adhesion/de-adhesion and acto-myosin polymerisation/contractility phases (Blanchoin et al., 2014; Krause and Gautreau, 2014). The successive steps are: the induction of cell polarity, the protrusion of the leading edge by actin polymerisation, the attachment of protrusive membrane to new adhesion sites or Focal Adhesions (FAs) on the Extra Cellular Matrix (ECM), and the contraction of acto-myosin to promote the sliding and detachment of the cell rear. Attachment/detachment of the cell from the ECM is one of the most important checkpoints in this process of so-called lamellipodial migration.

However, this mode of migration is not universal as revealed, by *in vivo* and *in vitro* studies of cell migration performed by immune surveillance cells (or dendritic cells) exiting the blood flow to patrol in tissue upon inflammation or cancer cell migration (Friedl et al., 2001). The development of micro-channel assays to study cell migration in confined environment of well-defined geometry (Heuzé et al., 2011; Vargas et al., 2014) allowed to demonstrate that dendritic cells perform single cell migration by a totally different mode, the so-called amoeboid migration (Friedl and Wolf, 2010; Paluch et al., 2006). Amoeboid migration refers to cells of round morphology that do not form adhesions with the substratum and thus lack FAs and stress fibres. Amoeboid cells insteat migrate by forming membrane blebs at their front, materialized by a disruption of the plasma-membrane actomyosin cortex link, then by the reconstitution of the actomyosin network within the protruding bled (Smith et al., 2007; Yoshida and Soldati, 2006). In a confined environment amoeboid cells migrate by pushing laterally on their environment thanks to a retrograde intracellular actomyosin flow (Paluch et al., 2006).

Epithelial, glial and some cancer cells migrate collectively. The simplest approach to study collective migration *in vitro* is the 2D migration of cell sheets on a cell-free surface in wound scratch assays (Cory, 2011) or after the release of a constraint barrier on an ECM-coated surface (Van Horssen and ten Hagen, 2011; Vedula et al., 2014). In this case, cells at the front, as well as some cells inside the group, migrate through lamellipodial mode; however, they remain attached to their neighbours allowing them to improve their migration efficiency and to acquire emerging collective properties (Ladoux et al., 2016). As a result, AJs and their ligands, cadherins, play a primordial role in this mode of cell migration (Trepat et al., 2009; Vedula et al., 2012). The maintenance of intercellular adhesion allows cells to migrate on the substrate by a tug of war mechanism (Ladoux et al., 2016). Embryonic cells, especially during gastrulation (Aman and Piotrowski, 2010), as well as differentiated epithelial cells during wound healing (Arciero et al., 2011; Trepat et al., 2009; Vedula et al., 2012) follow this mode of migration.

1.3. Migration of cancer cells

Tumor cells engage the two modes of migration in order to reach and invade distant tissues (Friedl and Wolf, 2003). Local invasion is the result of protruding cohorts of cells that still maintain the contact with the primary tumour. This invasive mode is observed in epithelial cancers such as oral squamous cell carcinoma, mammary carcinoma and colon carcinoma (Friedl et al., 2012). On the contrary, isolated or/and clusters of cancer cells can completely detach from the primary tumour to invade distant tissues as seen in melanoma in lung cancer, breast cancer and melanoma (Friedl et al., 2012). Finally, cells can detach from each other to escape and migrate individually. In this case, either cells migrate as mesenchymal cells with the formation of a lamellipodium adhering and pulling on the ECM at the leading edge and an uropod, or cells adopt and amoeboid morphology-like and squeeze to glide through the matrix (Friedl and Wolf, 2010; Friedl et al., 2012). This type of migration occurs in lymphoma and small cell lung cancer, leukemia or mesenchymal type like fibrosarcoma and glioblastoma tumours. Apart from intrinsic properties, the ability of cancer cells to migrate enormously depends on adhesion to the surrounding tumour microenvironment, and especially to cancer-associated fibroblasts (CAFs) which can increase their migratory and invasive characters, as reported for colorectal cancer cells (Lorusso and Rüegg, 2008).

To summarize, cancer cells can migrate individually or collectively, depending of their origin. In the latter case, reinforcing adhesion with their neighbours may foster their collective migration by increasing the efficiency of the tug of war mechanism (Trepat et al., 2009). Therefore, under these different conditions, cell-cell adhesion could contribute to promote or inhibit migration, and cadherins appear thus as potential key regulators of cancer cell migration and metastasis.

2. Cadherins, tissue organization and cell migrations

Cadherins constitute the most important family of cell-cell adhesion molecules (Takeichi, 1990). We will consider here a subtype of these molecules, the so-called classical cadherin subfamily: E (Epithelial)-, N (Neuronal)-, P (Placental)- and R (Retinal)-cadherins and the closely related VE (Vascular/Endothelial)-cadherin. These integral plasma membrane proteins are the intercellular ligands of AJ. They are equally involved in biochemical and mechanical signals transduction at AJ (Ladoux et al., 2015; Mège et al., 2006). Cadherin functions require the association of their cytoplasmic domain to catenins (α, β and p120), which connect them to actin filaments maintained under tension by myosin II motors. Besides this role in the mechanical linkage of AJ to actin, catenins associate to various structural and signalling proteins involved in the regulation of cadherin functions and/or in signalling cascades downstream of cell-cell contact formation (Hoffman and Yap, 2015; Padmanabhan et al., 2015).

2.1. Role of N-cadherin in cell migration during nervous system development

N-cadherin is mainly expressed in neural and mesenchymal tissues. It controls various migration processes responsible for tissue organization in different systems and organs: brain, spinal cord and nerves, lens, muscle and blood vessels. N-cadherin is ubiquitously expressed in the nervous system at the initiation of neurulation (Duband et al., 1987; Radice et al., 1997). It is required for proper cohesion and polarisation of neuroepithelial cells, and for migration of neuronal progenitors from their first location in the neuroepithelium to their final destination as mature neurons or glial cells.

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

Altering N-cadherin expression disturbs cell sorting and morphogenesis of neuronal cell layers.

For example, in *zebrafish* bearing mutation in N-cadherin gene, neuroectodermal cell adhesion, positioning and migration are altered. As a result neural tube formation is impaired (Lele et al., 2002). In chick embryos, expression of a Dominant Negative (DN) form of N-cadherin or ectopic expression of the N-cadherin induces defects in segregation and emigration of neural crest cells out of the neural tube. The latter is distorted, overgrown and folded (Coles et al., 2007; Nakagawa and Takeichi, 1998). Abnormal neural tube closure was also observed after N-cadherin RNA injection in *Xenopus* embryos and ectopic expression in the ectoderm (Detrick et al., 1990). N-cadherin is required for the maintenance of the cohesion, polarization and controlled proliferation of neuroepithelial cells, as revealed by neuroepithelium defects described in N-cadherin and α-catenin mutant mice (Kadowaki et al., 2007; Lien et al., 2006). At later stages of chick development, N-cadherin regulates selective adhesion and sorting between both neuronal cell bodies and neuronal extensions, allowing the formation of specific brain nuclei and nerve tracks (Redies and Takeichi, 1996).

During brain development, the mammalian neocortex builds in different layers resulting from the radial migration along radial glial cells of neuronal progenitor pools, from ventricular walls to their final locations in the cortical layers. In mouse, N-cadherin is necessary for the radial migration of these precursors (Jossin and Cooper, 2011; Franco et al., 2011; Kadowaki et al., 2007). N-cadherin ensures mild cohesion between neurons and radial glial cells ensuring neuron to adhere and de-adhere on glial fibres. During this process, N-cadherin undergoes endocytosis to maintain optimal surface levels and allowing the effective migration of neurons (Jossin and Cooper, 2011). In rats, the altered export of N-cadherin at the plasma membrane of neural stem cells causes abnormalities in neurogenesis including abnormal neuroblast migration, and leads to hydrocephaly (Guerra et al., 2015). In addition, N-cadherin is required for proper long distance migration and maintained polarization of tangentially migrating interneuron precursors (Luccardini et al., 2013).

In vitro, N-cadherin behaves as a suppressor of oligodendrocyte migration on astrocyte monolayer (Schnädelbach et al., 2000). Moreover, N-cadherin has been long recognized as a substrate for neurite outgrowth (Bard et al., 2008; Boscher and Mège, 2008; Matsunaga et al., 1988; Williams et al., 1994). Two pathways have been involved in N-cadherin-induced neurite outgrowth: the mechanical coupling of adhesion sites with the acto-myosin treadmilling (Bard et al., 2008; Giannone et al., 2009) and the activation of an FGFR-dependent biochemical signalling cascades (Boscher and Mège, 2008; Williams et al., 2001, 1994; Utton et al., 2001).

2.2. Role of N-cadherin in cell adhesion and migration in others tissues

N-cadherin is involved in the migration of neural crest derivatives during the development of the enteric nervous system (Broders-Bondon et al., 2012). In eye development, E-cadherin and N-cadherin expression levels control normal cell sorting leading to the separation of invaginating lens vesicles (Pontoriero et al., 2009). Double Knock-Out (KO) of E and N-cadherin genes induces abnormal migration and infiltration of iris tissue into the anterior chamber. During angiogenesis, N-cadherin is primordial to form contact between pericytes and endothelial cells and consequently to stabilize forming vessels through FGF/FGFR activity (Gerhardt et al., 2000). However, N-cadherin modulates also endothelial cell-cell interactions through the regulation of VE-cadherin expression at the plasma membrane and consequently its conditional KO in endothelial cells is very similar to VE-cadherin KO (Luo and Radice, 2005).

2.3. E-Cadherin, VE-cadherin and cell migration

E-cadherin has been mostly studied for its roles in controlling epithelia cohesion and differentiation (Gumbiner, 2005), but it is also involved in epithelial cell migration during development. During oocyte development in *Drosophila*, E-cadherin in border cells forms homologous junctions with E-cadherin in neighbouring border cells and heterologous junctions with E-cadherin of nurse cells, forming cohesive clusters of cells and promoting migration, respectively (Niewiadomska et al., 1999). Montell et al., showed that E-cadherin supports collective cell migration by regulating adhesion and force generation between the group of migrating border cells and adjacent resident tissue cells including nurse cells (Cai et al., 2014). During the development of *Drosophila* posterior midgut, E-cadherin ensures adhesion and controls the migration of mesenchymal cells over epithelial cells (Campbell and Casanova, 2015). Active recycling of E-cadherin in both cell types enables this process, and high levels of expression of E-cadherin slow down the migration of mesenchymal and epithelial cells. These observations suggest that the level of cadherin-mediated adhesion needs to be adjusted to ensure a correct balance between adhesion and efficient migration.

While E and N-cadherins are expressed in numerous cells types, VE-cadherin is solely expressed in endothelial cells. It is not only required for the maintenance of endothelium integrity but also necessary for the migration and the assembly of endothelial cells during angiogenesis (Giannotta et al., 2013; Lamalice et al., 2007; Navarro et al., 1998). Endothelial cell migration, vessel sprouting, as well as transmigration of leukocytes and cancer cells across vessels, require a transient reduction of endo-endothelial and endo-stromal cell-cell adhesion which may rely on VE-cadherin prevalence at the surface of endothelial cells (Giannotta et al., 2013; van Buul et al., 2002). VE-cadherin plasma membrane levels are controlled by endocytosis processes depending on tyrosine phosphorylation (Hatanaka et al., 2011), and on p120 (Xiao et al., 2005, 2003). These regulations likely modulate cell-cell adhesion strength, actin reorganization and *in fine* the stabilization of endothelial AJs *versus* the acquisition of a motile phenotype (Giannotta et al., 2013).

Taken together, these observations show that cadherin-mediated adhesion controls cell migration during development. Importantly, cadherin expression levels or activity should be strictly regulated. There is likely an optimal level of adhesion to sustain efficient migration, and this is primarily achieved through the regulation of protein trafficking/recycling at the plasma membrane. Abnormal expression or intracellular localizations of cadherins may thus induce abnormal migration and therefore the development of pathologies.

3. FGF, FGFR and cell migrations in tissue organisation during embryogenesis

Fibroblast Growth Factors (FGFs) are morphogens that control patterning of the embryo. FGFs can bind to the ECM that serves as a reservoir of FGFs, thus promoting long range FGF signalling (Duchesne et al., 2012). FGF gradients control the establishment of the dorso-ventral body axis, the extension of the antero-posterior body axis (Balasubramanian and Zhang, 2015) and many other morphogenetic processes. FGFR family constitutes one of the largest families of RTKs in vertebrates (Schlessinger, 2000). RTKs and their ligands known as Growth Factors (GFs) are wide regulators of multiple cell behaviours, including cell migration. FGFRs are transmembrane proteins with 3 IgG-like domains in their ectodomain

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

and a tyrosine kinase domain in their intracellular part. FGFR family includes 4 receptors (numerated from 1 to 4) that bind 18 flavours of FGF (1 to 18) (Ornitz and Itoh, 2015; Powers et al., 2000).

3.1. Mechanisms of FGFR activation

The fixation of FGF allows the homo-dimerization of the FGFR allowing the cross phosphorylation of each receptor subunit and their activation (Ornitz and Itoh, 2015; Powers et al., 2000). Subsequently, activated receptors bind adaptor proteins and phosphorylate cytoplasmic substrates (Powers et al., 2000; Zhang et al., 2006), eliciting various downstream signalling pathways that control cell proliferation, migration and survival. The classical view postulates that RTKs including FGFRs are monomers in the absence of ligand, but dimerized upon ligand binding. However, the existence of unliganded FGFR dimers was recently revealed (Sarabipour and Hristova, 2016).

The specificities in the cellular answer upon FGFs binding to FGFRs are unclear (Ornitz and Itoh, 2015; Zhang et al., 2006). Considering the same receptor, fixation of different FGFs trigger different downstream responses. Responses mediated by different receptors in response to the binding of the same ligand are also distinct. These specificities in cell responses could be related to the tendencies of FGFRs to dimerize in the absence of ligand (Sarabipour and Hristova, 2016). FGFR1 is predominantly monomeric, whereas unliganded FGFR 2 and 3 form homodimers that become phosphorylated. However, FGFs binding triggers structural changes in the FGFR dimers which increase FGFR phosphorylation, but are different for FGF1 and FGF2, establishing the existence of multiple active states (Sarabipour and Hristova, 2016).

Phosphorylation of FGFRs activates signalling cascades involved in their internalization. It has been reported that the binding of FGF1 to FGFR1 and FGFR3 leads to the ubiquitination of the receptors decreasing the protein levels by targeting it to degradation (Haugsten et al., 2005). Thus, FGFs-FGFRs association states contribute also to FGFR trafficking, and consequently to FGFR crowding at the plasma membrane.

3.2. Failure in FGFR expression induces developmental defects

As expected, perturbations of FGFR signalling are deleterious at numerous developmental steps. Impairment of FGFR functions causes failure of gastrulation in numerous species. Gastrulation defects were reported in FGFR1 mutant mice and in *Zebrafish* or *Xenopus* embryos injected with mRNA coding for a DN form of FGFR1 (Amaya et al., 1993; Griffin et al., 1995). In mouse, the expression of Kinase Dead (KD) FGFR1 disrupts the migration of epiblastic cells toward the anterior pole and leads to the accumulation of these cells at the posterior streak (Deng et al., 1994; Yamaguchi et al., 1994). Moreover, in FGFR1$^{-/-}$ mice mesodermal cells are unable to migrate away from the primitive streak (Ciruna et al., 1997). It has been proposed that FGFR1 regulates the migration of mesodermal cells by differentially regulating intercellular adhesion properties of progenitor population in the primitive streak (Ciruna and Rossant, 2001; Ciruna et al., 1997; Partanen et al., 1998; Yamaguchi et al., 1994). Many evidences for FGFR implication in the regulation of cell adhesion and/or cell migration have been reported in *Drosophila*. A mutation in FGFR2 results in the failure of mesodermal cells to migrate away from the midline during gastrulation (Gisselbrecht et al., 1996). Notably, FGFRs are essential for migration of tracheal cells (Klambt et al., 1992). Live imaging revealed that FGFR signalling leads to dynamic cytoskeletal reorganization controlling the migration of these cells (Chu et al., 2013; Lebreton and Casanova, 2016, 2014; Peterson and Krasnow, 2015).

In mice, specific deletion of FGFR1 in the Central Nervous System (CNS) revealed its necessary contribution in mid- and hindbrain development. It controls cell-cell contact formation at the midbrain-hindbrain junction, which is necessary for maintaining the isthmic organizer and normal tissue patterning (Trokovic et al., 2003). FGFRs play also a fundamental role at mid and late stage of neocortical development. Indeed, FGFRs activities confer a migratory capacity to nascent neuronal progeny to organize the laminar patterning of the cortex (Hasegawa, 2004). Altering FGFR signalling in the developing mouse olfactory bulb inhibits motility of neuroblasts that migrate from the subventricular zone to the rostral side, thus providing direct evidence for FGFR function (Zhou et al., 2015). In optical system of the moth *Manduca sexta*, blockade of FGFR kinase activity leads to absence of glia cell migration and aberrant outgrowth of olfactory receptor neurons (Gibson et al., 2012).

FGFRs are also involved in adult tissue homeostasis, as revealed by studies on skin wound healing. Indeed, a transgenic mouse expressing a DN form of FGFR2 in keratinocytes displays severe delay in epidermis repair after injury (Werner et al., 1994). Conditional FGFR1 and FGFR2 null mutant mice in keratinocytes show aberrant re-epithelialization due to an impaired migration of keratinocytes in the mounded epidermis (Meyer et al., 2012).

In conclusion, these data indicate that FGFRs modulate cell migration. As a matter of fact, these receptors could regulate cell adhesion and thus modify the capabilities of cells to migrate. As a consequence, aberrant regulation of FGF/FGFR signalling could provoke pathological migration involved in morphogenetic malformations, as well as cancer development.

4. Abnormal expression/function of N-cadherin and FGFRs associated to cancer

There are numerous evidences pointing out the dysfunction of N-cadherin or/and FGFR in quantity, in quality as well as in localization in cancer cells. This worsens the tumour malignancy characterized by enhanced migratory and invasive properties.

4.1. N-Cadherin signalling increases invasiveness of cancer cells

N-cadherin is acknowledged as a marker of invasive, malignant state of tumours. In some epithelial cancer types, cells acquire motility and invasiveness by upregulating N-cadherin to the detriment of E-cadherin (Li et al., 2001). This switch of cadherin subtype expression is observed in breast (Rieger-Christ et al., 2004), prostate (Kolijn et al., 2015) and lung cancer cells (Nakashima et al., 2003; Taeger et al., 2011). Transfection of exogenous N-cadherin into breast cancer cells increases cell migration, invasion and metastasis (Hazan et al., 1997). Besides, N-cadherin and cadherin-11 are co-localized at sites of adhesion formed between cancer cells and the stroma (McAndrews et al., 2015). Blocking of stromal N-cadherin function reverses this attachment, suggesting that N-cadherin is involved in cancer cell engraftment to stroma in invasive tumour (McAndrews et al., 2015). Silencing of N-cadherin expression, blocking of Src activity, or overexpressing of T860F mutant N-cadherin in melanoma cells prevent their trans-endothelial migration (Qi et al., 2005; Qi et al., 2006). It has been proposed that heterotypic N-cadherin adhesion may induce Src activation leading to N-cadherin phosphorylation on Tyr 860 and subsequent dissociation of β-catenin, allowing the transmigration of melanoma cells. Cancer cells migration may thus be favoured by an upregulation of N-cadherin expression.

The catenin p120 is a substrate of Src family protein kinases. p120 binds to the cadherin juxtamembrane domain and promotes cadherin-catenins complex exocytosis (Chen et al., 2003), and regulates the complex stability at the plasma membrane (Davis et al., 2003; Xiao et al., 2003) by lowering its susceptibility to undergo endocytosis and proteasomal degradation (Davis et al., 2003). To

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

be noted, p120 was reported to contribute to oligodendrocyte collective cell migration by regulating N-cadherin recycling and AJ dynamics (Peglion et al., 2014). In addition, Ozawa and Ohkubo reported that Src-dependent phosphorylation of p120 reduces its association to E-cadherin and consequently cell aggregation (Ozawa and Ohkubo, 2001). p120 is frequently altered and/or lost in tumours of the colon, bladder, stomach, breast, prostate, lung, and pancreas. Moreover, in some cases, p120 loss appears to be an early event in tumour progression, possibly preceding the loss of E-cadherin (Thoreson and Reynolds, 2002). Thus, changes in expression and trafficking of N-cadherin, regulated by p120, Src and other signalling proteins, by modifying its availability at the plasma membrane, may contribute to cancer cell adhesion, transmigration and invasion.

4.2. FGFR activation increases cancer aggressiveness

FGF/FGFR signalling has been associated to cancer development as well. FGFRs promote proliferation and migration of numerous cancer cell types (Coleman et al., 2014; Künstlinger et al., 2015; Parish et al., 2015). Genes coding for FGFRs undergo a large panel of mutations and rearrangements in cancers (Parish et al., 2015). Mutational activation of FGFR2 was reported in endometrial cancer cells, and drug treatment against FGFR2 kinase activity decreases the migration and invasion of these cells (Kim et al., 2015). Interestingly, drug-mediated inhibition of FGFRs delocalizes FGFR1 and FGF2 from the plasma membrane to the cytoplasm and the nucleus. Moreover, this drug-induced membranous delocalization reduces invasion of pancreatic cancer cells (Coleman et al., 2014), as well as invasion of breast cancer cells (Chioni and Grose, 2012). Along the same line, nuclear accumulation of FGFR1 correlates with the ability of head and neck squamous carcinoma cells to escape from the primary tumour site, to invade stroma and migrate to distant sites (Nguyen et al., 2013). Interestingly, blockade of FGFR1 activity reduces cell growth and invasion (Nguyen et al., 2013). An increase in cell contact-dependent migration and invasion of colorectal cancer cells, triggered by Src-dependent FGF2/FGFR1 signalling pathway, has been reported (Knuchel et al., 2015). Finally, targeting FGFR activity by specific inhibitors blocks downstream signalling pathways, impairs proliferation and induces apoptosis of breast tumour cells (Dey et al., 2010). Cell migration and invasion has also been reported to be enhanced by FGF10/FGFR2 signalling in pancreatic cancers (Nomura et al., 2008). To resume, enhanced activity of FGFRs is generally associated to cancer cells aggressiveness.

Thus, the two kinds of receptor (N-cadherin and FGFRs) are involved in the control of cell migration during normal development, and their deregulation is correlated with cancer progression. However, the crosstalk between N-cadherin and FGFR, as well as the combined effects of their deregulations, are barely understood (Table 1), as developed below.

5. Synergies between FGFR and N-cadherin in developmental processes

5.1. Examples of N-cadherin/FGFR cross-talk

Importantly, a synergistic action of N-cadherin and FGFRs was demonstrated in the regulation of the pluripotency of epiblast stem cell (Takehara et al., 2015), of ovarian cell survival (Trolice et al., 1997) and of osteogenesis (Debiais et al., 2001). N-cadherin stabilizes FGFRs to maintain pluripotency of mouse epiblast stem cells (mEpiSC) upon FGF2 stimulation. Indeed, the overexpression of N-cadherin prevents the downregulation of FGFRs at the plasma membrane after FGF2 addition in mEpiSC cells (Takehara et al., 2015). Moreover, downstream effectors of FGFR signalling, such as ERK and Akt, are significantly activated in cells overexpressing N-cadherin. At the opposite, N-cadherin silencing accelerates FGFR degradation, suggesting that N-cadherin inhibits FGFR protein degradation (Takehara et al., 2015).

In addition, FGFR and N-cadherin are both involved in the survival of granulosa cells (GCs) and ovarian surface epithelial cells (ROSEs) in the rat gonad generative cycle (Peluso et al., 1996; Trolice et al., 1997). GC and ROSE cell death is regulated by growth factors including FGF, but depends also on cell surface N-cadherin (Trolice et al., 1997). Neutralization of N-cadherin by antibodies reduces the phosphorylation level of FGFR by 50% in GCs and ROSEs, even in the absence of FGF. Authors speculate that both homophilic N-cadherin binding and FGF/FGFR binding enhance FGFR Tyr phosphorylation, and that subsequent activation of FGFR triggers downstream signalling to prevent apoptosis of aggregated cells (Peluso et al., 1996; Trolice et al., 1997). Trolice's group results further suggest that N-cadherin regulates FGFR phosphorylation by interfering with the capacity of FGF to bind.

Finally, the expression of a constitutively active form of FGFR increases the expression of N-cadherin reinforcing cell-cell adhesion in human osteogenic cells (Debiais et al., 2001). Moreover, an increase of N-cadherin expression at cell-cell contacts occurs upon FGF2 treatment in a Src-dependent manner. Thus, activated FGFR reinforces N-cadherin osteoblastic cell-cell junctions. Interestingly, E-cadherin level in these cells was not affected by FGF2 treatment, showing the specific effect of FGF/FGFR complex activity on N-cadherin functions. From above studies, we appreciate the mutual crosstalk of FGFR and N-cadherin, involving the stabilization at the plasma membrane of these two proteins and leading to the reinforcement cell-cell contacts and FGFR signalling pathways.

5.2. N-Cadherin/FGFR complex promotes axonal outgrowth

The Doherty's lab pioneered the study of the functional interaction between FGFR and N-cadherin during neurite outgrowth *in vitro* (Williams et al., 1994). Indeed, recombinant N-cadherin induces neurite outgrowth through the activation of signalling cascade (lipase/CAM kinase, MAP Kinases and PI3 kinase pathways) known to act downstream of FGFRs (Doherty et al., 2000; Williams et al., 1994). Moreover, the expression of DN FGFR inhibits axonal growth, as well as FGFR phosphorylation stimulated by N-cadherin (Brittis et al., 1996). Similarly, it has been reported a crosstalk between FGFR and immobilized N-cadherin or cadherin-11 to promote neurite outgrowth (Boscher and Mège, 2008). Indeed, biomimetic surfaces grafted with recombinant N-cadherin or cadherin-11 stimulates neurite outgrowth in the absence of FGF, and this response is severely reduced by FGFR inhibitor treatment. These observations suggested that FGFR activation is primordial for neuritogenesis induced by N-cadherin-mediated adhesion. Beside, FGFRs and N-cadherin are co-clustered at the surface of neuronal cells (Utton et al., 2001). These results suggest that FGFRs and N-cadherin interact at the cell membrane, triggering FGFR signalling and axonal outgrowth. We can speculate that FGFRs recruited at the cell membrane by N-cadherin provokes their activation.

6. Synergies between FGFRs and N-cadherin in cancer invasion and metastasis

6.1. N-Cadherin/FGFR crosstalk exacerbates cancer cell dissemination

In lung cancer cells, FGFR expression is positively correlated with ZEB expression, a transcription factor promoting N-cadherin expression and inducing migratory, invasive cell prop-

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

			stem cells	FGFR by N-cadherin at cell membrane	maintenance		(2015)
Ovary development	N-cadherin	FGFRs	rat granulosa cells and ovary superficial epithelia	co-immunoprecipitation in cell extract	cell viability	Akt sustained FGFR phosphorylation	Peluso et al. (1996), Trolice et al. (1997)
Osteogenesis	N-cadherin	FGFRs	human calvaria osteoblastic cells		osteoblastic cell adhesion osteogenesis	PKC	Debiais et al. (2001)
Neuronaldifferentiation	N-cadherin	FGFRs/FGFR1	mouse cerebellar neurons ventral spinal cord	co-clustering, N-cadherin-dependent activation of FGFR	neurite outgrowth	Src PLCγ	Saffell et al. (1997), Utton et al. (2001), Boscher and Mège (2008)
Retina development	N-cadherin	FGFRs/FGFR 1	*zebrafish*, *xenopus* RGC (Retina Ganglion Cells)	N-cadherin-dependent activation of FGFR	RGC extension and guidance	DAG/CAMK PI3K DAG/CAMK	Lom et al., (1998); Masai et al. (2003)
Breast cancer	N-cadherin	FGFRs	poliomavirus middle T-antigen (PvVmT)-induced mince mammary cancer cells	N-cadherin-dependent activation of FGFR	increase in invasion and pulmonary metastasis	MAPK/ERK	Hulit et al. (2007)
		FGFR1	human breast cancer cells	co-clustering, N-cadherin −dependent- increase of FGFR membranous stability N-cadherin prevents FGFR endocytosis induced by FGF2	increased invasion and metastasis	Src independent signalling MAPK/ERK increase in MMP9 mRNA expression	Suyama et al. (2002)
		FGFRs	mouse breast cancer cells	N-cadherin −dependent increase of FGFR 1-4 expression and phosphorylation N-cadherin-dependent increase of FGFR1 and 2 mRNA expression	increase in tumour invasion, proliferation and metastasis	ERK Akt	Qian et al. (2013)
Pancreatic cancer	N-cadherin	FGFR4	mouse β-cells	clustering in complex with NCAM	increase in tumour adhesion to ECM produced by vessel	PLCy	Cavallaro et al. (2001)
		FGFR2	mouse pancreatic ductal cell	clustering in complex with NCAM	increase in tumour cell migration, invasion and tumour architecture disruption	Pp60 Src MAPK/Akt	Deramaudt et al. (2006)
		FGFR2	mouse pancreatic ductal adenocarcinoma (PDA)	N-cadherin haploinsufficiency enhances effective FGFR2 signalling	increase in tumour cell survival, growth, migration and invasion	ERK	Su et al. (2012)
		FGFR	xenografed human cancer cells	FGFR increases total proteic expression of N-cadherin	increase in cancer cells motility	ERK	Taeger et al. (2011)
Pituitary cancer	N-cadherin	FGFR4	xenografted GH4 human pituitary cancer cells	N-cadherin, FGFR and NCAM co-clustering. FGFR kinase activity increases	increase in tumour growth, invasive, metastasis		Ezzat et al. (2006)

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

Table 1 (*Continued*)

process	cadherin	RTK	cell	interrelation	cellular response	molecular pathway	references
Colon cancer	VE-cadherin	VEGFR	VEGF-expressing murin colon tumour cancer	VEGF uncouples VE-cadherin junctions creating endothelial gaps	increase in tumour metastasis to lung and liver	Src	Weis et al. (2004)
Lung cancer	E-cadherin	EGFR	human lung cancer cells	EGFR antibody blocking relocalizes the E-cadherin/catenin complex at cell membrane	EGFR blockade inhibits cancer cell motility		Al Moustafa et al. (2002)
Neo angiogenesis	E-cadherin	FGFR	human melanoma/endothelia cells	VE/FGFR1: endo/melanoma adhesion	melanoma cell transmigration		Sandig et al. (1997)
	VE-cadherin	VEGFR2	human endothelial cells	co-clustering at cell-cell junctions	attenuated intercellular adhesion, increased angiogenesis and vascular permeability	DMP1	Esser et al., 1998
				VEGFR 2 sustains VE-cadherin phosphorylation		Src	
	VE-cadherin	VEGFR2, 3	human endothelial cells	co-clustering	increase in endothelial cells viability	Bcl2/Akt	Carmeliet et al. (1999)
	VE-cadherin	VEGFR 2	human endothelial cells	E-cadherin binds to VEGFR2, preventing its endocytosis and promoting its inactivation at the cell surface	increased contact inhibition of cell growth, limitation of endothelial cell proliferation	PLCy	Lampugnani et al. (2006)
	VE-cadherin	VEGFR2, 3	human endothelial cells	co-clustering facilitates ligand-independent transactivation of VEGFR 2,3	enhanced endothelial response to flow shear stress	MAPK	Coon et al. (2015)

erties (Gemmill et al., 2011; Ware et al., 2013). Direct evidence of a FGFR and N-cadherin complex formation came from biochemical analysis in breast cancer cells (Suyama et al., 2002). Moreover, N-cadherin prevents FGFR from undergoing ligand-induced internalization, which results in its stabilisation at the plasma membrane and ultimately in sustained FGFR signalling (Suyama et al., 2002). N-cadherin/FGFR signalling enhances ERK phosphorylation, leading to invasive phenotype development of tumor cells (Hulit et al., 2007). Moreover, N-cadherin co-precipitates with FGFR in human pancreatic β-cells (Cavallaro et al., 2001). In the case of mice xenografted with human pancreatic cancer cells, inhibition of FGFR leads to decreased expression of N-cadherin and to the reduction of cancer cell motility. As a consequence, FGFR inhibition in pancreatic cancer cells reduces invasive properties and improves sensitivity to gemcitabine treatment (Taeger et al., 2011).

Several molecular aberrations have been implicated in the pathogenesis of pituitary tumours, among which the production of an alternative cytoplasmic isoform of FGFR4 which lacks most of the extracellular domain (Ezzat et al., 2006). Truncated FGFR4 expressing tumours show reduced membrane levels of N-cadherin. The pivotal role of N-cadherin as a mediator of cell growth was demonstrated by experiment using small interfering RNA. N-cadherin silencing promotes invasive pituitary cell growth in xenografted mice (Ezzat et al., 2006). Interestingly, selective inhibition of FGFRs with PD173074 restores membrane distribution of N-cadherin, and significantly reduces the tumour growth and invasion, emphasizing the critical partnership of N-cadherin and FGFRs in promoting invasive functions in pituitary cancer development.

6.2. N-Cadherin/FGFR and neoangiogenesis

N-cadherin and FGFRs can also induce tumour angiogenesis (Nakashima et al., 2003; Presta et al., 2005). Knock down or drug inhibition of FGFRs perturb angiogenesis during tumour development (Murakami et al., 2008). Endothelial cells express both N- and VE-cadherins, and deletion of either of these cadherins leads to severe vascular defects in mice (Carmeliet et al., 1999; Giampietro et al., 2012). However, endothelial cells overexpressing N-cadherin (N cells) express higher levels of FGF2 than endothelial cells overexpressing VE-cadherin (VE cells) (Giampietro et al., 2012). In N cells, the phosphorylation of FGFR substrate 2a (FRS2a) and the MAPK pathway activation are increased in comparison with VE cells. Consistently, inhibition of FGF2/FGFR1 signalling strongly reduces migration of N cells.

As reported above, N-cadherin can interact with FGFRs in tumour cells, thus limiting its internalization and subsequently increasing its signalling activity (Hulit et al., 2007; Qian et al., 2013; Suyama et al., 2002). Surprisingly, VE-cadherin, but not N-cadherin, co-immunoprecipitates with FGFR1 in endothelial cell extracts, and authors suggested that VE-cadherin may antagonize FGFR activation. As a matter of fact, the higher level of FGFR signalling in N cells may parallel a higher migratory phenotype required for the sprouting and the elongation of newly vessels. In contrast, VE-cadherin, once engaged at cell-to-cell junctions, would promote vascular stability by limiting cell motility and growth, in accordance with observations made in transgenic mice. An inhibitory action of VE-cadherin on FGFR signalling may contribute to this process (Giampietro et al., 2012).

Another study reported that the attenuation of N-cadherin function in endothelial cells by antagonistic peptides results in the impairment of cadherin-mediated endothelial interaction and causes apoptosis (Erez et al., 2004). This effect appears to be mediated by FGFR signalling, since N-cadherin inhibitory peptides reduces FRS2 phosphorylation, and exogenous addition of FGF2 completely rescues the phenotype (Erez et al., 2004). Taken together, it is reasonable to claim that N-cadherin and FGFRs establish a reciprocal crosstalk to drive tumour angiogenesis.

6.3. Cadherins, FGFR and transmigration

The upregulation of N-cadherin to the detriment of E-cadherin observed in some invasive cancer cells could be correlated with the fact that the endothelium abundantly expresses N-cadherin. Thus, the E- to N-cadherin switch may facilitate the adhesion of cancer cells on endothelial cells required for transendothelium migration, as reported for melanoma cells (Qi et al., 2005). Interestingly, although FGFR1 was reported to co-immunoprecipitate with N-cadherin in some breast cancer cells (Hazan et al., 1997) and to contribute with N-cadherin to mammary cancer cell metastasis (Qian et al., 2013). In contrast, FGFR1co-immunoprecipitates with VE-cadherin but not with N-cadherin in melanoma cancer cells undergoing transendothelial migration (Qi et al., 2005). Thus, a more complex communication between FGFR and subtype-cadherins would exist during cancer cell migration and transmigration.

7. Molecular bases of RTK/cadherin crosstalks

7.1. Possible regulation of N-cadherin availability at the cell surface

One way to tightly control N-cadherin activity and thus cell-cell adhesion is to regulate its abundance at the cell surface. N-cadherin recycling properties may allow the direct regulation of N-cadherin amount at the plasma membrane. The catenin p120 is acknowledged to stabilize N-cadherin at the cell-cell contacts by regulating its biogenesis (Davis et al., 2003; Reynolds and Roczniak-Ferguson, 2004) and/or preventing its internalization (Chen et al., 2003). Interestingly, p120 lacking cancer cells show more malignant and invasive proprieties (Davis et al., 2003; Qi et al., 2006; Reynolds and Carnahan, 2004; Takahashi et al., 2014). Besides, phosphorylation of p120 has pro-tumorigenic activity in renal and breast cancer (Kourtidis et al., 2015). Interestingly, phosphorylation of p120 by Src has been reported to prevent its interaction with N-cadherin cytoplasmic tail (Kourtidis et al., 2015).

On the other hand, FGFR activate phosphastases such as Shp2-a (Ryan et al., 1998) or/and CD148 (Takahashi et al., 2014) which are able to inactivate Src. Inactivation of Src promotes p120 binding to N-cadherin, leading to the stabilization of N-cadherin at cell-cell contacts. Besides, β-arrestin could be involved in N-cadherin recycling. Indeed, it has been shown to be involved in VE-cadherin internalization in endothelial cells (Gavard and Gutkind, 2006). Of interest, β-arrestin binds to Src and possibly associates in a complex with p120 and β-catenin (Penela et al., 2001). We can thus speculate on an endocytosis-regulated co-stabilization of N-cadherin and FGFR, involving Src protein kinases and the catenin p120.

7.2. Cross-talk between other classic cadherins and RTKs in cancer

There are additional evidences concerning the specific crosstalk between RTKs and cadherins in the control of cell migration, metastasis and neoangiogenesis (Chiasson-MacKenzie et al., 2015; Fedor-Chaiken et al., 2003; Lugo-Martínez et al., 2009; Mateus et al., 2007; Sasaki et al., 2013), or between VE-cadherin and VEGFR (Coon et al., 2015; Esser et al., 1998; Lampugnani et al., 2006; Pirotte et al., 2011).

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

7.3. E-Cadherin and EGFR crosstalk

Like FGFR, EGFR is upregulated in numerous cancer cell types (Salomon et al., 1995). Many data argue for a cross-talk between the E-cadherin and EGFR pathways in the regulation of cancer cells growth (Chiasson-MacKenzie et al., 2015; Fedor-Chaiken et al., 2003; Lugo-Martínez et al., 2009; Mateus et al., 2007; Sasaki et al., 2013). It has also been reported that E-cadherin binds to EGFR *via* its extracellular domain leading to a reduced EGFR activation (Al Moustafa et al., 2002). Moreover, activated receptor induces the endocytosis of E-cadherin, down-regulating E-cadherin levels at the cell surface and in AJs. E-cadherin mutations in gastric cancers, preventing its binding to EGFR, lead to an increased activation of the EGFR, and results in increased cell migration (Mateus et al., 2007). Other studies suggested that E-cadherin may stabilize EGFR at cell-cell contacts through modulation of the contractility of the cortical actomyosin network by involving Merlin/NF2 (Chiasson-MacKenzie et al., 2015). Importantly, EGFR does not associate with and is not activated by N-cadherin in epithelial cells (Fedor-Chaiken et al., 2003), further supporting the hypothesis that there are specific functional interactions between cadherin subtypes and RTK members.

(Muhamed et al., 2016) reported this year an E-cadherin-based force transduction pathway triggering global changes in cell mechanics affecting also cell-ECM interactions. Interestingly, this pathway involves EGFR and a PI3 K pathway. Altogether, these reports indicate that E-cadherin-mediated cell adhesion, EGFR related pathways and cell mechanics are linked. As cell mechanics has strong incidence on cell-cell and cell-ECM adhesions, this feedback loop may be of primary importance of the regulation of cell migratory behaviour.

7.4. VE-Cadherin and VEGFR

In the same line, VE-cadherin was reported to bind to VEGFR2 and VEGFR3 at the plasma membrane of endothelial cells (Coon et al., 2015; Esser et al., 1998). The recruitment of VEGFR2 by VE-cadherin activates the receptor, leading to the weakening of cell-cell contacts. As a consequence, the vessels barrier is more permeable and cell sprouting and migration are favoured (Esser et al., 1998). Dejana group studied the mechanism by which VE-cadherin regulates VEGFR2 signalling. When VE-cadherin is absent or not engaged at cell-cell contacts, VEGFR2 is endocytosed more rapidly and remains in endosomal compartments for a longer time (Lampugnani et al., 2006). At the opposite, pharmacological inhibition of VEGFR2 stabilizes endothelial barrier junctions maintained mainly by VE-cadherin accumulated at AJs. Src protein is involved in the phosphorylation of VEGFR induced by VE-cadherine (Pirotte et al., 2011) and Src inhibition produces the same result. These inhibitions lead to the reduction of extravasion and lung cancer metastasis (Weis et al., 2004).

8. Conclusion

In this review, we propose a reciprocal crosstalk between cadherins and RTKs during cell migration process through a dynamic control of cell-cell contact strength. Cluster of cancer cells migration is favoured by tight adhesions to cellular neighbours. N-cadherin and FGFR mutually modify their recruitment at the plasma membrane to regulate cell-cell contact strengths. The decreased of N-cadherin and/or FGFR recruitment at cell-cell contacts would weaken cell-cell junctions. On the contrary, increased N-cadherin recruitment strengthens cell adhesion. Thus FGFRs and N-cadherin prevalence at the cell surface, which are altered in various cancer types, could have strong impact on the migration mode of neoplastic cells. Moreover, these deregulations may differentially contribute to the distinct steps of cancer cell migration including transmigration. We could gain on the understanding of the underlying mechanisms by studying more deeply the contribution of the two receptor types to developmental processes. However, more mechanistic studies of the interactions between FGFRs and cadherins at the plasma membrane and of the crosstalk between downstream signalling cascades are now required.

Acknowledgements

This work was supported by grants from CNRS, ARC foundation, Human Frontier Science Program (HFSP grant RPG0040/2012), and Agence Nationale de la Recherche (ANR 2010 Blan1515). TN was supported by a HFSP grant RPG0040/2012 funded graduate student fellowship. We thank Delphine Delacour for fruitful discussions and valuable input on manuscript. We would like also to thank all present and past member of the Cell Adhesion & Mechanics lab at the Institute Jacques Monod for constant support and exchange.

References

Al Moustafa, A.-E., Yen, L., Benlimame, N., Alaoui-Jamali, M.A., 2002. Regulation of E-cadherin/catenin complex patterns by epidermal growth factor receptor modulation in human lung cancer cells. Lung Cancer 37, 49–56.
Aman, A., Piotrowski, T., 2010. Cell migration during morphogenesis. Dev. Biol. 341, 20–33.
Amaya, E., Stein, P.A., Musci, T.J., Kirschner, M.W., 1993. FGF signalling in the early specification of mesoderm in Xenopus. Development 118, 477–487.
Arboleda-Estudillo, Y., Krieg, M., Stühmer, J., Licata, N.a., Muller, D.J., Heisenberg, C.P., 2010. Movement directionality in collective migration of germ layer progenitors. Curr. Biol. 20, 161–169.
Arciero, J.C., Mi, Q., Branca, M.F., Hackam, D.J., Swigon, D., 2011. Continuum model of collective cell migration in wound healing and colony expansion. Biophys. J. 100, 535–543.
Balasubramanian, R., Zhang, X., 2015. Mechanisms of FGF gradient formation during embryogenesis. Semin. Cell Dev. Biol., http://dx.doi.org/10.1016/j.semcdb.2015.10.004.
Bard, L., Boscher, C., Lambert, M., Mege, R.-M., Choquet, D., Thoumine, O., 2008. A molecular clutch between the actin flow and N-cadherin adhesions drives growth cone migration. J. Neurosci. 28, 5879–5890.
Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., Plastino, J., 2014. Actin dynamics architecture, and mechanics in cell motility. Physiol. Rev. 94, 235–263.
Boscher, C., Mège, R.M., 2008. Cadherin-11 interacts with the FGF receptor and induces neurite outgrowth through associated downstream signalling. Cell. Signal. 20, 1061–1072.
Boyden, S., 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J. Exp. Med. 115, 453–466.
Brittis, P.A., Silver, J., Walsh, F.S., Doherty, P., 1996. Fibroblast growth factor receptor function is required for the orderly projection of ganglion cell axons in the developing mammalian retina. Mol. Cell. Neurosci. 8, 120–128.
Broders-Bondon, F., Paul-Gilloteaux, P., Carlier, C., Radice, G.L., Dufour, S., 2012. N-Cadherin and β1-integrins cooperate during the development of the enteric nervous system. Dev. Biol. 364, 178–191.
Cai, D., Chen, S.-C., Prasad, M., He, L., Wang, X., Choesmel-Cadamuro, V., Sawyer, J.K., Danuser, G., Montell, D.J., 2014. Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. Cell 157, 1146–1159.
Campbell, K., Casanova, J., 2015. A role for E-cadherin in ensuring cohesive migration of a heterogeneous population of non-epithelial cells. Nat. Commun. 6, 7998.
Carmeliet, P., Lampugnani, M.G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oosthuyse, B., Dewerchin, M., Zanetti, A., Angellilo, A., Mattot, V., Nuyens, D., Lutgens, E., Clotman, F., De Ruiter, M.C., Groot, A.G., De Poelmann, R., Lupu, F., Herbert, J.M., Collen, D., Dejana, E., 1999. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell 98, 147–157.
Cavallaro, U., Niedermeyer, J., Fuxa, M., Christofori, G., 2001. N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. Nat. Cell Biol. 3, 650–657.
Chen, X., Kojima, S.I., Borisy, G.G., Green, K.J., 2003. P120 catenin associates with kinesin and facilitates the transport of cadherin-catenin complexes to intercellular junctions. J. Cell Biol. 163, 547–557.
Chiasson-MacKenzie, C., Morris, Z.S., Baca, Q., Morris, B., Coker, J.K., Mirchev, R., Jensen, A.E., Carey, T., Stott, S.L., Golan, D.E., McClatchey, A.I., 2015. NF2/merlin mediates contact-dependent inhibition of EGFR mobility and internalization via cortical actomyosin. J. Cell Biol. 211, 391–405.
Chioni, A.M., Grose, R., 2012. FGFR1 cleavage and nuclear translocation regulates breast cancer cell behavior. J. Cell Biol. 197, 801–817.

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

Chu, W.-C., Lee, Y.-M., Henry Sun, Y., 2013. FGF/FGFR signal induces trachea extension in the drosophila visual system. PLoS One 8, e73878.
Ciruna, B., Rossant, J., 2001. Mesoderm cell fate specification and morphogenetic movement at the primitive streak. Dev. Cell 1, 37–49.
Ciruna, B.G., Schwartz, L., Harpal, K., Yamaguchi, T.P., Rossant, J., 1997. Chimeric analysis of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenetic movement through the primitive streak. Development 124, 2829–2841.
Clark, A.G., Vignjevic, D.M., 2015. Modes of cancer cell invasion and the role of the microenvironment. Curr. Opin. Cell Biol. 36, 13–22.
Coleman, S.J., Chioni, A., Ghallab, M., Anderson, R.K., Nicholas, R., 2014. Nuclear translocation of FGFR 1 and FGF2 in pancreatic stellate cells facilitates pancreatic cancer cell invasion. EMBO Mol. Med. 6, 467–482.
Coles, E.G., Taneyhill, L.A., Bronner-Fraser, M., 2007. A critical role for Cadherin6B in regulating avian neural crest emigration. Dev. Biol. 312, 533–544.
Coon, B.G., Baeyens, N., Han, J., Budatha, M., Ross, T.D., Fang, J.S., Yun, S., Thomas, J.L., Schwartz, M.A., 2015. Intramembrane binding of VE-cadherin to VEGFR2 and VEGFR3 assembles the endothelial mechanosensory complex. J. Cell Biol. 208, 975.
Cory, G., 2011. Scratch-wound assay. Methods Mol. Biol. 769, 25–30.
Danjo, Y., Gipson, I.K., 1998. Actin purse string filaments are anchored by E-cadherin-mediated adherens junctions at the leading edge of the epithelial wound, providing coordinated cell movement. J. Cell Sci. 111, 3323–3332.
Davis, M.A., Ireton, R.C., Reynolds, A.B., 2003. A core function for p120-catenin in cadherin turnover. J. Cell Biol. 163, 525–534.
Debiais, F., Lemonnier, J., Hay, E., Delannoy, P., Caverzasio, J., Marie, P.J., 2001. Fibroblast growth factor-2 (FGF-2) increases N-cadherin expression through protein kinase C and Src-kinase pathways in human calvaria osteoblasts. J. Cell. Biochem. 81, 68–81.
Deng, C., Shen, M.M., Daugherty, C., Ornitz, D.M., Leder, P., 1994. Murine FGFR-1 is required for early postimplantation growth and axial organization. Genes Dev. 8, 3045–3057.
Deramaudt, T.B., Takaoka, M., Upadhyay, R., Bowser, M.J., Porter, J., Lee, A., Rhoades, B., Johnstone, C.N., Weissleder, R., Hingorani, S.R., Mahmood, U., Rustgi, A.K., 2006. N-cadherin and keratinocyte growth factor receptor mediate the functional interplay between Ki-RASG12V and p53V143A in promoting pancreatic cell migration, invasion, and tissue architecture disruption. Mol. Cell. Biol. 26 (11), 4185–4200, PubMed PMID: 16705170; PubMed Central PMCID: PMC1489079.
Detrick, R.J., Dickey, D., Kintner, C.R., 1990. The effects of N-cadherin misexpression on morphogenesis in Xenopus embryos. Neuron 4, 493–506.
Dey, J.H., Bianchi, F., Voshol, J., Bonenfant, D., Oakeley, E.J., Hynes, N.E., 2010. Targeting fibroblast growth factor receptors blocks PI3K/AKT signaling induces apoptosis, and impairs mammary tumor outgrowth and metastasis. Cancer Res. 70, 4151–4162.
Doherty, P., Williams, G., Williams, E.J., 2000. CAMs and axonal growth: a critical evaluation of the role of calcium and the MAPK cascade. Mol. Cell. Neurosci. 16, 283–295.
Duband, J.L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G.M., Thiery, J.P., 1987. Adhesion molecules during somitogenesis in the avian embryo. J. Cell Biol. 104, 1361–1374.
Duchesne, L., Octeau, V., Bearon, R.N., Beckett, A., Prior, I.A., Lounis, B., Fernig, D.G., 2012. Transport of fibroblast growth factor 2 in the pericellular matrix is controlled by the spatial distribution of its binding sites in heparan sulfate. PLoS Biol. 10, 16.
Dumortier, J.G., Martin, S., Meyer, D., Rosa, F.M., David, N.B., 2012. Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. Proc. Natl. Acad. Sci. U. S. A. 109, 16945–16950.
Erez, N., Zamir, E., Gour, B.J., Blaschuk, O.W., Geiger, B., 2004. Induction of apoptosis in cultured endothelial cells by a cadherin antagonist peptide: involvement of fibroblast growth factor receptor-mediated signalling. Exp. Cell Res. 294, 366–378.
Esser, S., Lampugnani, M.G., Corada, M., Dejana, E., Risau, W., 1998. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. J. Cell Sci. 111, 1853–1865.
Ezzat, S., Zheng, L., Winer, D., Asa, S.L., 2006. Targeting N-cadherin through fibroblast growth factor receptor-4: distinct pathogenetic and therapeutic implications. Mol. Endocrinol. 20, 2965–2975.
Fedor-Chaiken, M., Hein, P.W., Stewart, J.C., Brackenbury, R., Kinch, M.S., 2003. E-cadherin binding modulates EGF receptor activation. Cell Commun. Adhes. 10, 105–118.
Franco, S.J., Martinez-Garay, I., Gil-Sanz, C., Harkins-Perry, S.R., Müller, U., 2011. Reelin regulates cadherin function via Dab1/Rap1 to control neuronal migration and lamination in the neocortex. Neuron 69, 482–497.
Friedl, P., Borgmann, S., Bröcker, E.B., 2001. Amoeboid leukocyte crawling through extracellular matrix: lessons from the Dictyostelium paradigm of cell movement. J. Leukoc. Biol. 70, 491–509.
Friedl, P., Locker, J., Sahai, E., Segall, J.E., 2012. Classifying collective cancer cell invasion. Nat. Cell Biol. 14, 777–783.
Friedl, P., Wolf, K., 2003. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat. Rev. Cancer 3, 362–374.
Friedl, P., Wolf, K., 2010. Plasticity of cell migration: a multiscale tuning model. J. Cell Biol. 188, 11–19.
Gavard, J., Gutkind, J.S., 2006. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat. Cell Biol. 8, 1223–1234.
Gemmill, R.M., Roche, J., Potiron, V.A., Nasarre, P., Mitas, M., Coldren, C.D., Helfrich, B.A., Garrett-Mayer, E., Bunn, P.A., Drabkin, H.A., 2011. ZEB1-responsive genes in non-small cell lung cancer. Cancer Lett. 300, 66–78.
Gerhardt, H., Wolburg, H., Redies, C., 2000. N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken. Dev. Dyn. 218, 472–479.
Giampietro, C., Taddei a Corada, M., Sarra-Ferraris, G.M., Alcalay, M., Cavallaro, U., Orsenigo, F., Lampugnani, M.G., Dejana, E., 2012. Overlapping and divergent signalling pathways of N- and VE-cadherin in endothelial cells. Blood 119, 2159–2170.
Giannone, G., Mège, R.M., Thoumine, O., 2009. Multi-level molecular clutches in motile cell processes. Trends Cell. Biol. 19, 475–486.
Giannotta, M., Trani, M., Dejana, E., 2013. VE-Cadherin and endothelial adherens junctions: active guardians of vascular integrity. Dev. Cell 26, 441–454.
Gibson, N.J., Tolbert, L.P., Oland, L.A., 2012. Activation of glial FGFRs is essential in glial migration, proliferation, and survival and in glia-neuron signaling during olfactory system development. PLoS One 7, e33828.
Gisselbrecht, S., Skeath, J.B., Doe, C.Q., Michelson, A.M., 1996. heartless encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the Drosophila embryo. Genes Dev. 10, 3003–3017.
Griffin, K., Patient, R., Holder, N., 1995. Analysis of FGF function in normal and no tail zebrafish embryos reveals separate mechanisms for formation of the trunk and the tail. Development 121, 2983–2994.
Guerra, M.M., Henzi, R., Ortloff, A., Lichtin, N., Vio, K., Jimenez, A.J., Dominguez-Pinos, M.D., Gonzalez, C., Jara, M.C., Hinostroza, F., Rodriguez, S., Jara, M., Ortega, E., Guerra, F., Sival, D.A., den Dunnen, W.F., Perez-Figares, J.M., McAllister, J.P., Johanson, C.E., Rodriguez, E.M., 2015. Cell junction pathology of neural stem cells is associated with ventricular zone disruption, hydrocephalus, and abnormal neurogenesis. J. Neuropathol. Exp. Neurol. 74, 653–671.
Gumbiner, B.M., 2005. Regulation of cadherin-mediated adhesion in morphogenesis. Nat. Rev. Mol. Cell Biol. 6, 622–634.
Hasegawa, H., 2004. Laminar patterning in the developing neocortex by temporally coordinated fibroblast growth factor signaling. J. Neurosci. 24, 8711–8719.
Hatanaka, K., Simons, M., Murakami, M., 2011. Phosphorylation of VE-cadherin controls endothelial phenotypes via p120-catenin coupling and Rac1 activation. Am. J. Physiol. Heart Circ. Physiol. 300, H162–H172.
Haugsten, E.M., Sørensen, V., Brech, A., Olsnes, S., Wesche, J., 2005. Different intracellular trafficking of FGF1 endocytosed by the four homologous FGF receptors. J. Cell Sci. 118, 3869–3881.
Hazan, R.B., Kang, L., Whooley, B.P., Borgen, P.I., 1997. N-Cadherin promotes adhesion between invasive breast cancer cells and the stroma. Cell Adhes. Commun. 4, 399–411.
Heuzé, M.L., Collin, O., Terriac, E., Lennon-Duménil, A.-M., Piel, M., 2011. Cell migration in confinement: a micro-channel-based assay. Methods Mol. Biol 769, 415–434.
Hoffman, B.D., Yap, A.S., 2015. Towards a dynamic understanding of cadherin-based mechanobiology. Trends Cell Biol. 25, 803–814.
Hulit, J., Suyama, K., Chung, S., Keren, R., Agiostratidou, G., Shan, W., Dong, X., Williams, T.M., Lisanti, M.P., Knudsen, K., Hazan, R.B., 2007. N-Cadherin signaling potentiates mammary tumor metastasis via enhanced extracellular signal-regulated kinase activation. Cancer Res. 67, 3106–3116.
Jossin, Y., Cooper, J.A., 2011. Reelin: Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. 14, 697–703.
Kadowaki, M., Nakamura, S., Machon, O., Krauss, S., Radice, G.L., Takeichi, M., 2007. N-Cadherin mediates cortical organization in the mouse brain. Dev. Biol. 304, 22–33.
Kim, D.-H., Kwak, Y., Kim, N.D., Sim, T., 2015. Antitumor effects and molecular mechanisms of ponatinib on endometrial cancer cells harboring activating FGFR2 mutations. Cancer Biol. 17, 65–78.
Klambt, C., Glazer, L., Shilo, B.Z., 1992. Breathless, a Drosophila FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. Genes Dev. 6, 1668–1678.
Knuchel, S., Anderle, P., Werfelli, P., Diamantis, E., Rüegg, C., 2015. Fibroblast surface-associated FGF-2 promotes contact-dependent colorectal cancer cell migration and invasion through FGFR-SRC signaling and integrin αvβ5-mediated adhesion. Oncotarget 6, 14300–14317.
Kolijn, K., Verhoef, E.I., van Leenders, G.J.L.H., 2015. Morphological and immunohistochemical identification of epithelial-to-mesenchymal transition in clinical prostate cancer. Oncotarget 6, 24488–24498.
Kourtidis, A., Yanagisawa, M., Huveldt, D., Copland, J.a., Anastasiadis, P.Z., 2015. Pro-tumorigenic phosphorylation of p120 catenin in renal and breast cancer. PLoS One 10, e0129964.
Krause, M., Gautreau, A., 2014. Steering cell migration: lamellipodium dynamics and the regulation of directional persistence. Nat. Rev. Mol. Cell Biol. 15, 577–590.
Künstlinger, H., Fassunke, J., Schildhaus, H.-U., Brors, B., Heydt, C., Angelika Ihle, M., Mechtersheimer, G., Wardelmann, E., Büttner, R., Merkelbach-Bruse, S., 2015. FGFR2 is overexpressed in myxoid liposarcoma and inhibition of FGFR signaling impairs tumor growth in vitro. Oncotarget 6, 20215–20230.
Ladoux, B., Mège, R.-M., Trepat, X., 2016. Front-rear polarization by mechanical cues: from single cells to tissues. Trends Cell Biol., http://dx.doi.org/10.1016/j.tcb.2016.02.002.
Ladoux, B., Nelson, W.J., Yan, J., Mège, R.M., 2015. The mechanotransduction machinery at work at adherens junctions. Integr. Biol. (Camb.) 7, 1109–1119.

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

Lamalice, L., Le Boeuf, F., Huot, J., 2007. Endothelial cell migration during angiogenesis. Circ. Res. 100, 782–794.
Lampugnani, M.G., Orsenigo, F., Gagliani, M.C., Tacchetti, C., Dejana, E., 2006. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. J. Cell Biol. 174, 593–604.
Lele, Z., Folchert, A., Concha, M., Rauch, G.J., Geisler, R., Rosa, F., Wilson, S.W., Hammerschmidt, M., Bally-Cuif, L., 2002. Parachute/n-cadherin is required for morphogenesis and maintained integrity of the zebrafish neural tube. Development 129 (14), 3281–3294, PubMed PMID: 12091300.
Le Clainche, C., Carlier, M.-F., 2008. Regulation of actin assembly associated with protrusion and adhesion in cell migration. Physiol. Rev. 88, 489–513.
Lebreton, G., Casanova, J., 2016. Ligand-binding and constitutive FGF receptors in single Drosophila tracheal cells: implications for the role of FGF in collective migration. Dev. Dyn. 245, 372–378.
Lebreton, G., Casanova, J., 2014. Specification of leading and trailing cell features during collective migration in the Drosophila trachea. J. Cell Sci. 127, 465–474.
Lemmon, M., Schlessinger, J.a., 2011. NIH public access. Biochemistry 141, 1117–1134.
Li, G., Satyamoorthy, K., Herlyn, M., 2001. N-Cadherin-mediated intercellular interactions promote survival and migration of melanoma cells. Cancer Res. 61, 3819–3825.
Lien, W.-H., Klezovitch, O., Fernandez, T.E., Delrow, J., Vasioukhin, V., 2006. alphaE-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. Science 311, 1609–1612.
Lom, B., Höpker, V., McFarlane, S., Bixby, J.L., Holt, C.E., 1998. Fibroblast growth factor receptor signaling in Xenopus retinal axon extension. J. Neurobiol. 37 (4), 633–641, PubMed PMID: 9858264.
Lorusso, G., Rüegg, C., 2008. The tumor microenvironment and its contribution to tumor evolution toward metastasis. Histochem. Cell Biol. 130, 1091–1103.
Luccardini, C., Hennekinne, L., Viou, L., Yanagida, M., Murakami, F., Kessaris, N., Ma, X., Adelstein, R.S., Mège, R.-M., Métin, C., 2013. N-Cadherin sustains motility and polarity of future cortical interneurons during tangential migration. J. Neurosci. 33, 18149–18160.
Lugo-Martínez, V.-H., Petit, C.S., Fouquet, S., Le Beyec, J., Chambaz, J., Pinçon-Raymond, M., Cardot, P., Thenet, S., 2009. Epidermal growth factor receptor is involved in enterocyte anoikis through the dismantling of E-cadherin-mediated junctions. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G235–G244.
Luo, Y., Radice, G.L., 2005. N-Cadherin acts upstream of VE-cadherin in controlling vascular morphogenesis. J. Cell Biol. 169, 29–34.
Maiuri, P., Terriac, E., Paul-Gilloteaux, P., Vignaud, T., McNally, K., Onuffer, J., Thorn, K., Nguyen, P.A., Georgoulia, N., Soong, D., Jayo, A., Beil, N., Beneke, J., Lim, J.C.H., Sim, C.P.-Y., Chu, Y.-S., participants, W.C.R., Jiménez-Dalmaroni, A., Joanny, J.-F., Thiery, J.-P., Erfle, H., Parsons, M., Mitchison, T.J., Lim, W.A., Lennon-Duménil, A.-M., Piel, M., Théry, M., 2012. The first world cell race. Curr. Biol. 22, R673–5.
Masai, I., Lele, Z., Yamaguchi, M., Komori, A., Nakata, A., Nishiwaki, Y., Wada, H., Tanaka, H., Nojima, Y., Hammerschmidt, M., Wilson, S.W., Okamoto, H., 2003. N-cadherin mediates retinal lamination, maintenance of forebrain compartments and patterning of retinal neurites. Development 130 (11), 2479–2494, PubMed PMID: 12702661.
Mateus, A.R., Seruca, R., Machado, J.C., Keller, G., Oliveira, M.J., Suriano, G., Luber, B., 2007. EGFR regulates RhoA-GTP dependent cell motility in E-cadherin mutant cells. Hum. Mol. Genet. 16, 1639–1647.
Matsunaga, M., Hatta, K., Takeichi, M., 1988. Role of N-cadherin cell adhesion molecules in the histogenesis of neural retina. Neuron 1, 289–295.
McAndrews, K.M., Yi, J., McGrail, D.J., Dawson, M.R., 2015. Enhanced adhesion of stromal cells to invasive cancer cells regulated by cadherin 11. ACS Chem. Biol. 10, 1932–1938.
Mège, R.-M., Gavard, J., Lambert, M., 2006. Regulation of cell-cell junctions by the cytoskeleton. Curr. Opin. Cell Biol. 18, 541–548.
Meyer, M., Müller, A.-K., Yang, J., Moik, D., Ponzio, G., Ornitz, D.M., Grose, R., Werner, S., 2012. FGF receptors 1 and 2 are key regulators of keratinocyte migration in vitro and in wounded skin. J. Cell Sci. 125, 5690–5701.
Montell, D.J., 2001. Command and control: regulatory pathways controlling invasive behavior of the border cells. Mech. Dev. 105, 19–25.
Muhamed, I., Wu, J., Sehgal, P., Kong, X., Tajik, A., Wang, N., Leckband, D.E., 2016. E-Cadherin-mediated force transduction signals regulate global cell mechanics. J. Cell Sci. 129, 1843–1854.
Murakami, M., Nguyen, L.T., Zhang, Z.W., Moodie, K.L., Carmeliet, P., Stan, R.V., Simons, M., 2008. The FGF system has a key role in regulating vascular integrity. J. Clin. Invest. 118, 3355–3366.
Nakagawa, S., Takeichi, M., 1998. Neural crest emigration from the neural tube depends on regulated cadherin expression. Development 125, 2963–2971.
Nakashima, T., Huang, C., Liu, D., Kameyama, K., Masuya, D., Kobayashi, S., Kinoshita, M., Yokomise, H., 2003. Neural-cadherin expression associated with angiogenesis in non-small-cell lung cancer patients. Br. J. Cancer 88, 1727–1733.
Navarro, P., Ruco, L., Dejana, E., 1998. Differential localization of VE- and N-cadherins in human endothelial cells: VE-cadherin competes with N-cadherin for junctional localization. J. Cell Biol. 140, 1475–1484.
Nguyen, P.T., Tsunematsu, T., Yanagisawa, S., Kudo, Y., Miyauchi, M., Kamata, N., Takata, T., 2013. The FGFR1 inhibitor PD173074 induces mesenchymal-epithelial transition through the transcription factor AP-1. Br. J. Cancer 109, 2248–2258.
Niewiadomska, P., Godt, D., Tepass, U., 1999. DE-Cadherin is required for intercellular motility during Drosophila oogenesis. J. Cell Biol. 144, 533–547.
Nomura, S., Yoshitomi, H., Takano, S., Shida, T., Kobayashi, S., Ohtsuka, M., Kimura, F., Shimizu, H., Yoshidome, H., Kato, A., Miyazaki, M., 2008. FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer. Br. J. Cancer 99, 305–313.
Okenve-Ramos, P., Llimargas, M., 2014. Fascin links Btl/FGFR signalling to the actin cytoskeleton during Drosophila tracheal morphogenesis. Development 141, 929–939.
Ornitz, D.M., Itoh, N., 2015. The fibroblast growth factor signaling pathway. Wiley Interdiscip. Rev. Dev. Biol. 4, 215–266.
Ozawa, M., Ohkubo, T., 2001. Tyrosine phosphorylation of p120(ctn) in v-Src transfected L cells depends on its association with E-cadherin and reduces adhesion activity. J. Cell Sci. 114, 503–512.
Padmanabhan, A., Rao, M.V., Wu, Y., Zaidel-Bar, R., 2015. Jack of all trades: functional modularity in the adherens junction. Curr. Opin. Cell Biol. 36, 32–40.
Paluch, E., Sykes, C., Prost, J., Bornens, M., 2006. Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol. 16, 5–10.
Parish, A., Schwaederle, M., Daniels, G., Piccioni, D., Fanta, P., Schwab, R., Shimabukuro, K., Parker, B.a., Helsten, T., Kurzrock, R., 2015. Fibroblast growth factor family aberrations in cancers: clinical and molecular characteristics. Cell Cycle 14, 5–10.
Partanen, J., Schwartz, L., Rossant, J., 1998. Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for Fgfr1 in anteroposterior patterning of mouse embryos. Genes Dev. 12, 2332–2344.
Peglion, F., Llense, F., Etienne-Manneville, S., 2014. Adherens junction treadmilling during collective migration. Nat. Cell Biol. 16, 639–651.
Peluso, J.J., Pappalardo, A., Trolice, M.P., 1996. N-Cadherin-mediated cell contact inhibits granulosa cell apoptosis in a progesterone-independent manner. Endocrinology 137, 1196–1203.
Penela, P., Elorza, A., Sarnago, S., Mayor, F., 2001. Beta-arrestin- and c-Src-dependent degradation of G-protein-coupled receptor kinase 2. EMBO J. 20, 5129–5138.
Peterson, S.J., Krasnow, M.A., 2015. Subcellular trafficking of FGF controls tracheal invasion of Drosophila flight muscle. Cell 160, 313–323.
Pirotte, S., Lamour, V., Lambert, V., Alvarez Gonzalez, M.-L., Ormenese, S., Noël, A., Mottet, D., Castronovo, V., Bellahcène, A., 2011. Dentin matrix protein 1 induces membrane expression of VE-cadherin on endothelial cells and inhibits VEGF-induced angiogenesis by blocking VEGFR-2 phosphorylation. Blood 117, 2515–2526.
Pontoriero, G.F., Smith, A.N., Miller, L.-A.D., Radice, G.L., West-Mays, J.A., Lang, R.A., 2009. Co-operative roles for E-cadherin and N-cadherin during lens vesicle separation and lens epithelial cell survival. Dev. Biol. 326, 403–417.
Powers, C.J., McLeskey, S.W., Wellstein, A., 2000. Fibroblast growth factors, their receptors and signaling. Endocr. Relat. Cancer 7, 165–197.
Presta, M., Dell'Era, P., Mitola, S., Moroni, E., Ronca, R., Rusnati, M., 2005. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 16, 159–178.
Qi, J., Chen, N., Wang, J., Siu, C.-H., 2005. Transendothelial migration of melanoma cells involves N-cadherin-mediated adhesion and activation of the beta-catenin signaling pathway. Mol. Biol. Cell 16, 4386–4397.
Qi, J., Wang, J., Romanyuk, O., Siu, C.-H., 2006. Involvement of Src family kinases in N-cadherin phosphorylation and beta-catenin dissociation during transendothelial migration of melanoma cells. Mol. Biol. Cell 17, 1261–1272.
Qian, X., Anzovino, A., Kim, S., Suyama, K., Yao, J., Hulit, J., Agiostratidou, G., Chandiramani, N., McDaid, H.M., Nagi, C., Cohen, H.W., Phillips, G.R., Norton, L., Hazan, R.B., 2013. N-Cadherin/FGFR promotes metastasis through epithelial-to-mesenchymal transition and stem/progenitor cell-like properties. Oncogene 33, 1–11.
Radice, G.L., Rayburn, H., Matsunami, H., Knudsen, K.A., Takeichi, M., Hynes, R.O., 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev. Biol. 181, 64–78.
Redies, C., Takeichi, M., 1996. Cadherins in the developing central nervous system: an adhesive code for segmental and functional subdivisions. Dev. Biol. 180, 413–423.
Reynolds, A.B., Carnahan, R.H., 2004. Regulation of cadherin stability and turnover by p120ctn: Implications in disease and cancer. Semin. Cell Dev. Biol. 15, 657–663.
Reynolds, A.B., Roczniak-Ferguson, A., 2004. Emerging roles for p120-catenin in cell adhesion and cancer. Oncogene 23, 7947–7956.
Rieger-Christ, K.M., Lee, P., Zagha, R., Kosakowski, M., Moinzadeh, A., Stoffel, J., Ben-Ze'ev, A., Libertino, J.a., Summerhayes, I.C., 2004. Novel expression of N-cadherin elicits in vitro bladder cell invasion via the Akt signaling pathway. Oncogene 23, 4745–4753.
Ryan, P.J., Paterno, G.D., Gillespie, L.L., 1998. Identification of phosphorylated proteins associated with the fibroblast growth factor receptor type I during early Xenopus development. Biochem. Biophys. Res. Commun. 244, 763–767.
Saffell, J.L., Williams, E.J., Mason, I.J., Walsh, F.S., Doherty, P., 1997. Expression of a dominant negative FGF receptor inhibits axonal growth and FGF receptor phosphorylation stimulated by CAMs. Neuron 18 (2), 231–242, PubMed PMID: 9052794.
Salomon, D.S., Brandt, R., Ciardiello, F., Normanno, N., 1995. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit. Rev. Oncol. Hematol. 19, 183–232.
Sandig, M., Voura, E.B., Kalnins, V.I., Siu, C.H., 1997. Role of cadherins in the transendothelial migration of melanoma cells in culture. Cell Motil. Cytoskeleton 38 (4), 351–364, PubMed PMID: 9415377.

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

Sarabipour, S., Hristova, K., 2016. Mechanism of FGF receptor dimerization and activation. Nat. Commun. 7, 10262.
Sasaki, T., Hiroki, K., Yamashita, Y., 2013. The role of epidermal growth factor receptor in cancer metastasis and microenvironment. Biomed Res. Int., 546318.
Schlessinger, J., 2000. Cell signaling by receptor tyrosine kinases. Cell 103, 211–225.
Schnädelbach, O., Blaschuk, O.W., Symonds, M., Gour, B.J., Doherty, P., Fawcett, J.W., 2000. N-Cadherin influences migration of oligodendrocytes on astrocyte monolayers. Mol. Cell. Neurosci. 15, 288–302.
Smith, L.A., Aranda-Espinoza, H., Haun, J.B., Dembo, M., Hammer, D.A., 2007. Neutrophil traction stresses are concentrated in the uropod during migration. Biophys. J. 92, L58–L60.
Su, Y., Li, J., Witkiewicz, A.K., Brennan, D., Neill, T., Talarico, J., Radice, G.L., 2012. N-cadherinhaploinsufficiency increases survival in a mouse model of pancreaticcancer. Oncogene 31 (41), 4484–4489, http://dx.doi.org/10.1038/onc.2011.574, Epub 2011 Dec 12. PubMed PMID: 22158044; PubMed Central PMCID: PMC3714178.
Suyama, K., Shapiro, I., Guttman, M., Hazan, R.B., 2002. A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2, 301–314.
Taeger, J., Moser, C., Hellerbrand, C., Mycielska, M.E., Glockzin, G., Schlitt, H.J., Geissler, E.K., Stoeltzing, O., Lang, S.a., 2011. Targeting FGFR/PDGFR/VEGFR impairs tumor growth, angiogenesis, and metastasis by effects on tumor cells, endothelial cells, and pericytes in pancreatic cancer. Mol. Cancer Ther. 10, 2157–2167.
Takahashi, K., Matafonov, A., Sumarriva, K., Ito, H., Lauhan, C., Zemel, D., Tsuboi, N., Chen, J., Reynolds, A., Takahashi, T., 2014. CD148 tyrosine phosphatase promotes cadherin cell adhesion. PLoS One 9, e112753.
Takehara, T., Teramura, T., Onodera, Y., Frampton, J., Fukuda, K., 2015. Cdh2 stabilizes FGFR1 and contributes to primed-state pluripotency in mouse epiblast stem cells. Sci. Rep. 5, 14722.
Takeichi, M., 1990. Cadherins: a molecular family important in selective cell-cell adhesion. Annu. Rev. Biochem. 59, 237–252.
Thoreson, M.A., Reynolds, A.B., 2002. Altered expression of the catenin p120 in human cancer: implications for tumor progression. Differentiation 70, 583–589.
Trepat, X., Wasserman, M.R., Angelini, T.E., Millet, E., Weitz, D.A., Butler, J.P., Fredberg, J.J., 2009. Physical forces during collective cell migration. Nat. Phys. 5, 426–430.
Trokovic, R., Trokovic, N., Hernesniemi, S., Pirvola, U., Vogt Weisenhorn, D.M., Rossant, J., McMahon, A.P., Wurst, W., Partanen, J., 2003. FGFR1 is independently required in both developing mid- and hindbrain for sustained response to isthmic signals. EMBO J. 22, 1811–1823.
Trolice, M.P., Pappalardo, A., Peluso, J.J., 1997. Basic fibroblast growth factor and N-cadherin maintain rat granulosa cell and ovarian surface epithelial cell viability by stimulating the tyrosine phosphorylation of the fibroblast growth factor receptors. Endocrinology 138, 107–113.
Utton, M.A., Eickholt, B., Howell, F.V., Wallis, J., Doherty, P., 2001. Soluble N-cadherin stimulates fibroblast growth factor receptor dependent neurite outgrowth and N-cadherin and the fibroblast growth factor receptor co-cluster in cells. J. Neurochem. 76, 1421–1430.
van Buul, J.D., Voermans, C., van den Berg, V., Anthony, E.C., Mul, F.P., van Wetering, S., van der Schoot, C.E., Hordijk, P.L., van Wetering, S., 2002. Migration of human hematopoietic progenitor cells across bone marrow endothelium is regulated by vascular endothelial cadherin. J. Immunol. 168, 588–596.
Van Horssen, R., ten Hagen, T.L.M., 2011. Crossing barriers: the new dimension of 2D cell migration assays. J. Cell. Physiol. 226, 288–290.
Vargas, P., Terriac, E., Lennon-Duménil, A.-M., Piel, M., 2014. Study of cell migration in microfabricated channels. J. Vis. Exp., pe51099.
Vedula, S.R.K., Leong, M.C., Lai, T.L., Hersen, P., Kabla, A.J., Lim, C.T., Ladoux, B., 2012. Emerging modes of collective cell migration induced by geometrical constraints. Proc. Natl. Acad. Sci. U. S. A. 109, 12974–12979.
Vedula, S.R.K., Ravasio, A., Anon, E., Chen, T., Peyret, G., Ashraf, M., Ladoux, B., 2014. Microfabricated environments to study collective cell behaviors. Methods Cell Biol. 120, 235–252.
Ware, K.E., Hinz, T.K., Kleczko, E., Singleton, K.R., Marek, L.A., Helfrich, B.A., Cummings, C.T., Graham, D.K., Astling, D., Tan, A.-C., Heasley, L.E., 2013. A mechanism of resistance to gefitinib mediated by cellular reprogramming and the acquisition of an FGF2-FGFR1 autocrine growth loop. Oncogenesis 2, e39.
Weis, S., Cui, J., Barnes, L., Cheresh, D., 2004. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. J. Cell Biol. 167, 223–229.
Werner, S., Smola, H., Liao, X., Longaker, M.T., Krieg, T., Hofschneider, P.H., Williams, L.T., 1994. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science 266, 819–822.
Williams, E.J., Furness, J., Walsh, F.S., Doherty, P., 1994. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. Neuron 13, 583–594.
Williams, E.J., Williams, G., Howell, F.V., Skaper, S.D., Walsh, F.S., Doherty, P., 2001. Identification of an N-cadherin motif that can interact with the fibroblast growth factor receptor and is required for axonal growth. J. Biol. Chem. 276, 43879–43886.
Xiao, K., Allison, D.F., Buckley, K.M., Kottke, M.D., Vincent, P.A., Faundez, V., Kowalczyk, A.P., 2003. Cellular levels of p120 catenin function as a set point for cadherin expression levels in microvascular endothelial cells. J. Cell Biol. 163, 535–545.
Xiao, K., Garner, J., Buckley, K.M., Vincent, P.A., Chiasson, C.M., Dejana, E., Faundez, V., Kowalczyk, A.P., 2005. p120-Catenin regulates clathrin-dependent endocytosis of VE-cadherin. Mol. Biol. Cell 16, 5141–5151.
Yamaguchi, T.P., Harpal, K., Henkemeyer, M., Rossant, J., Hospital, M.S., Mg, C., 1994. fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. Genes Dev., 3032–3044.
Yoshida, K., Soldati, T., 2006. Dissection of amoeboid movement into two mechanically distinct modes. J. Cell Sci. 119, 3833–3844.
Zhang, X., Ibrahimi, O.A., Olsen, S.K., Umemori, H., Mohammadi, M., Ornitz, D.M., 2006. Receptor specificity of the fibroblast growth factor family: the complete mammalian FGF family. J. Biol. Chem. 281, 15694–15700.
Zhou, Y., Oudin, M.J., Gajendra, S., Sonego, M., Falenta, K., Williams, G., Lalli, G., Doherty, P., 2015. Regional effects of endocannabinoid: BDNF and FGF receptor signalling on neuroblast motility and guidance along the rostral migratory stream. Mol. Cell. Neurosci. 64, 32–43.

Please cite this article in press as: Nguyen, T., Mège, R.M., N-Cadherin and Fibroblast Growth Factor Receptors crosstalk in the control of developmental and cancer cell migrations. Eur. J. Cell Biol. (2016), http://dx.doi.org/10.1016/j.ejcb.2016.05.002

The formation of ordered nanoclusters controls cadherin anchoring to actin and cell–cell contact fluidity

Pierre-Olivier Strale,[1,3] Laurence Duchesne,[1,4] Grégoire Peyret,[1] Lorraine Montel,[2] Thao Nguyen,[1] Evelyn Png,[1,5] Robert Tampé,[6] Sergey Troyanovsky,[7] Sylvie Hénon,[2] Benoit Ladoux,[1,3] and René-Marc Mège[1]

[1]Institut Jacques Monod, Centre National de la Recherche Scientifique and [2]Laboratoire Matière et Systèmes Complexes, Centre National de la Recherche Scientifique, Université Paris Diderot, 75205 Paris, France
[3]Mechanobiology Institute, University of Singapore, Singapore 117411, Singapore
[4]Institut de Génétique et Développement de Rennes, Centre National de la Recherche Scientifique, Université de Rennes 1, 35042 Rennes, France
[5]Ocular Surface Research Group, Singapore Eye Research Institute, Singapore 169856, Singapore
[6]Institute of Biochemistry, Biocenter, Goethe-University Frankfurt, D-60438 Frankfurt, Germany
[7]Northwestern University, Chicago, Il 60208

Oligomerization of cadherins could provide the stability to ensure tissue cohesion. Cadherins mediate cell–cell adhesion by forming trans-interactions. They form cis-interactions whose role could be essential to stabilize intercellular junctions by shifting cadherin clusters from a fluid to an ordered phase. However, no evidence has been provided so far for cadherin oligomerization in cellulo and for its impact on cell–cell contact stability. Visualizing single cadherins within cell membrane at a nanometric resolution, we show that E-cadherins arrange in ordered clusters, providing the first demonstration of the existence of oligomeric cadherins at cell–cell contacts. Studying the consequences of the disruption of the cis-interface, we show that it is not essential for adherens junction formation. Its disruption, however, increased the mobility of junctional E-cadherin. This destabilization strongly affected E-cadherin anchoring to actin and cell–cell rearrangement during collective cell migration, indicating that the formation of oligomeric clusters controls the anchoring of cadherin to actin and cell–cell contact fluidity.

Introduction

Around 35% of proteins in cells are in an oligomeric state (Goodsell and Olson, 2000). Oligomerization provides several functional advantages such as a mechanism to resist degradation and, more importantly, to make higher order long-living subcellular structures such as cytoskeletal filaments and functional nanomachines. Tissue cohesion is ensured by cell adhesion molecules that establish short living intercellular protein–protein bonds at the single molecule level (Perret et al., 2004). Oligomerization could provide the necessary strength to support intercellular adhesion and resistance to mechanical stress. Cadherins are major cell adhesion molecules in animal cells (Hulpiau et al., 2013). Cadherins diffusing at the plasma membrane initiate cell–cell interactions by establishing homophilic intercellular bonds (Mège et al., 2006). These trans-interactions analyzed by atomic force microscopy or biomembrane force probe have been shown to be short living (Baumgartner et al., 2000; Perret et al., 2004), implying that some higher order processes must take place for cadherin-mediated adhesion to reach sufficient stability to sustain physiologically relevant resistance to mechanical stress. Nascent cell–cell contacts initiated by cadherin trans-interactions evolve in adhesion plaques by the growth of cadherin clusters gathering additional trans-interacting cadherin molecules by a diffusion trapping mode (Adams et al., 1998; Lambert et al., 2007). Upon anchorage to the underlying actin cytoskeleton, which may bring additional cooperativity in cadherin recruitment as well as stability (Lambert et al., 2002; Hong et al., 2013), these adhesion plaques eventually evolve in adherens junctions (AJs; Mège et al., 2006). However, whether cadherin clusters found in AJs are organized in oligomeric structures as connexins in gap junctions (Raviola and Gilula, 1975) or desmosomal cadherins in desmosomes (Al-Amoudi et al., 2007), or have no particular organization as contradictorily reported for desmosomal cadherins (He et al., 2003), remains an open question.

Structural data have brought important information on the organization of cadherins (Overduin et al., 1995; Shapiro et al.,

Correspondence to René-Marc Mège: rene-marc.mege@ijm.fr

Abbreviations used in this paper: AJ, adherens junction; MSD, mean square displacement; NP, nanoparticle; PDMS, polydimethylsiloxane; PIV, particle image velocimetry; TEM, transmission electron microscopy; wt, wild type.

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 210 No. 2 333–346
www.jcb.org/cgi/doi/10.1083/jcb.201410111

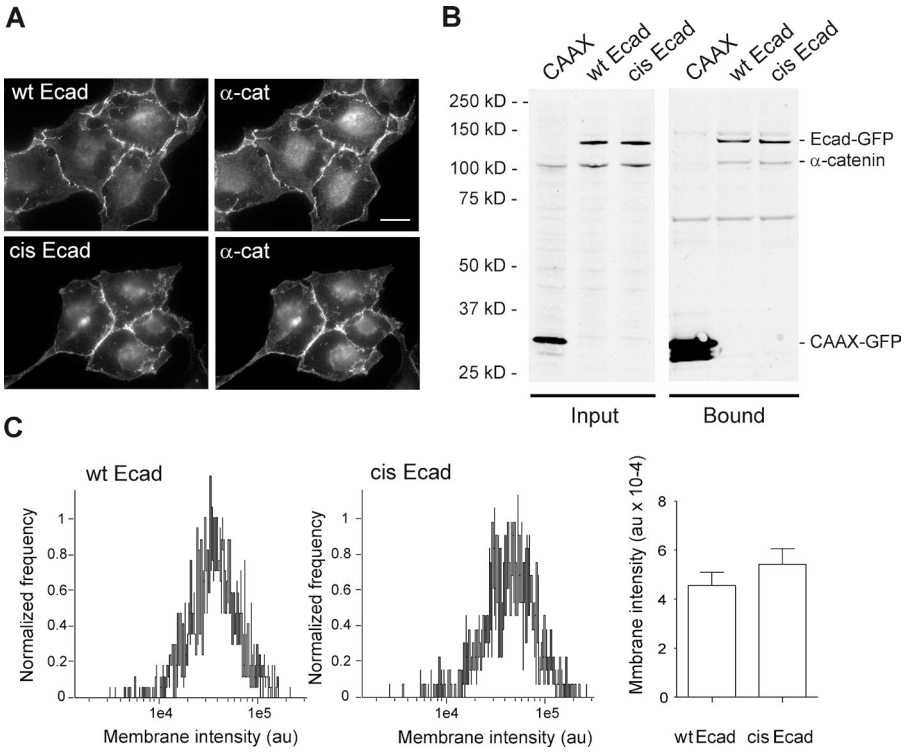


Figure 1. **wt Ecad and cis-Ecad expression restore Ecad-dependent cell–cell contacts in A431D cells.** (A) Fluorescence imaging of cells expressing wt Ecad-GFP (wt Ecad) or cis-Ecad-GFP (cis-Ecad) reveals indistinguishable coaccumulation of Ecad and α-catenin at cell–cell contacts. Bar, 20 µm. (B) GFP-tagged proteins were immunoprecipitated from transfected cell lysates and subjected to Western blotting to detect GFP and α-catenin (Bound). Western blot of the cellular extracts before immunoprecipitation is shown on the left (Input). CAAX-GFP expressing cells were used as a control. Both wt Ecad-GFP and cis-Ecad-GFP were expressed at the predicted molecular mass (140 kD) and at similar levels (Input). α-Catenin was coimmunoprecipitated at similar levels with wt Ecad-GFP and cis-Ecad-GFP. (C) Representative distributions of cell surface–associated fluorescent intensities (arbitrary units [au]) for wt and cis-Ecad-GFP–transfected cells, 24 h after transfection (1,500 and 1,300 objects analyzed, respectively). The histogram represents the mean of the median fluorescent intensities ± SEM obtained from three independent experiments.

1995; Boggon et al., 2002; Shapiro and Weis, 2009). The current hypothesis is that adhesion starts with trans-interaction of EC1 domains of cadherins from apposed cells. More recently, a cis-interface for E-cadherin (Ecad) has been identified in crystal lattices. It involves the nonsymmetrical interaction of the EC1 domain of one cadherin with the EC2 domain of a neighboring cadherin (Harrison et al., 2011). Site-directed mutagenesis in EC1 (V81D) and EC2 (L175D) domains abolishes the formation of a cis-interface in the crystal without affecting the formation of the trans-interface. V81D, L175D-mutated Ecad ectodomain failed to form ordered junction-like structures in a liposome system, whereas wild-type (wt) Ecad did. Further theoretical and simulation work predicted that Ecad organizes in linear or more complex nanometric arrays as a result of trans- and cis-interactions (Wu et al., 2011, 2013). However, although Ecad cluster size and distribution have been reported with unprecedented resolution in tissues thanks to super-resolution microscopy (Truong Quang et al., 2013; Wu et al., 2015), cadherins have never been imaged at a nanometric resolution and thus no direct proof of ordered organization of cadherin in clusters has been provided so far in cells. Harrison et al. (2011) data suggest that the cis-interface stabilizes junctional Ecad. However, these data have been obtained by expressing wt and cis-Ecad forms deleted from the cytoplasmic domain. Because anchorage of cadherin cytoplasmic domain to actin via catenins is a major factor of AJ formation and strengthening (Lambert et al., 2002; Cavey et al., 2008; Hong et al., 2013), one may ask questions on the influence of cadherin oligomerization on cadherin cytoplasmic tail anchoring to F-actin.

The purpose of this work is to provide evidence for the predicted formation of arrays of oligomeric Ecad in cellulo and to study the functional implication of Ecad oligomerization on the formation and maturation of cell–cell contacts. We expressed full-length wt and cis-Ecad (V81D, L175D double mutant) in Ecad-deficient A431D cells and used an electron microscopy approach to visualize single Ecad molecules at the cell membrane. We also studied the consequences of the disruption of the cis-interface on cell–cell contact formation and stability. Our data provide evidence for an ordered organization of Ecad in clusters, depending on the cis-interface. We show that the cis-interface was, however, not required for AJ formation. The effects of the V81D, L175D mutations on Ecad turnover, association to catenin, and anchoring to actin were analyzed by cell imaging, FRAP analysis, and manipulation of Ecad-coated beads with magnetic tweezers. Ecad cis-oligomerization perturbations had moderate effects on Ecad complex stability but dramatically impaired Ecad anchoring to the actin cytoskeleton, the overall stability of cell–cell contacts, and collective cell behavior.

Results

Effect of the disruption of the cis-interface on Ecad expression and cell–cell adhesion

To visualize Ecad oligomers at the cell membrane, we used A431D cells, as they do not express Ecad (Lewis et al., 1997). Both wt Ecad-GFP and cis-Ecad-GFP were accumulated at cell–cell contacts where they recruited α- (Fig. 1) and β-catenin (Fig. S1, A and B) as previously reported (Troyanovsky et al., 2015). Western blot analysis indicates that wt Ecad-GFP and cis-Ecad-GFP were expressed at similar levels. Similar levels of α- and β-catenin were coimmunoprecipitated with the wt and cis-mutant Ecad, indicating that the impairment of Ecad cis dimerization did not affect the association to catenin. Cell surface fluorescence imaging indicates that cis-Ecad-GFP was as efficiently accumulated at the plasma membrane as wt Ecad-GFP and even slightly more (Fig. 1 C).

To test whether the impairment of cis dimerization alters cadherin-mediated cell adhesion, we measured the ability of Ecad-Fc–coated beads to bind to transfected cells (Fig. S2, C

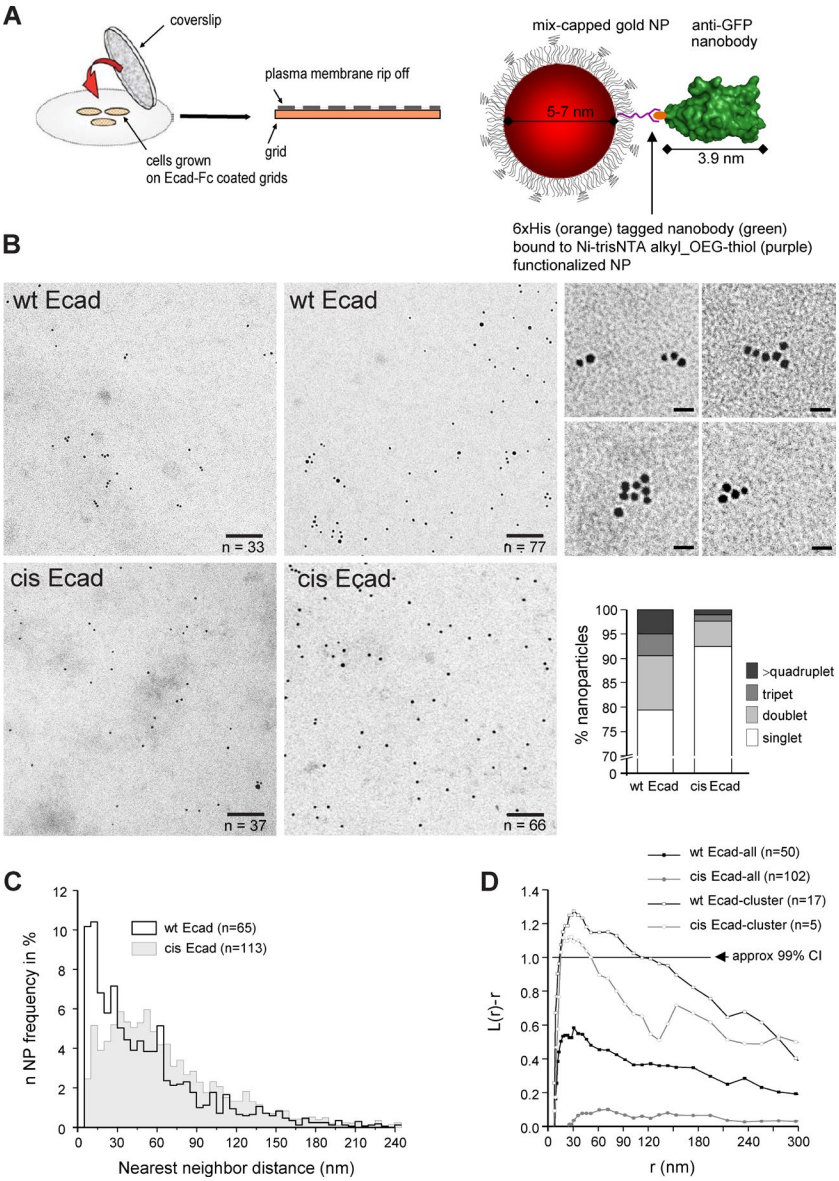


Figure 2. **wt Ecad-GFP molecules are organized in nanometric arrays at the cell membrane.** (A) Experimental approach. (left) Transfected cells were seeded at low density on Ecad-Fc–coated grids. After 2 h, the upper part of the cells were ripped off, leaving plasma membrane sheets corresponding to the bottom part of the cells (inner leaflet face up) on the electron microscopy grids (adapted from Hancock and Prior, 2005). Grids were then incubated with 5–7-nm-diameter mix-coated gold NPs (in red) conjugated to a single anti–GFP-NB (in green) and observed by TEM. As a control of binding specificity we observed only a few anti-GFP-NP on plasma membrane of nontransfected cells (not depicted). (B) TEM visualization of anti–GFP-NP on plasma membrane sheets of wt Ecad-GFP (wt Ecad) and cis-Ecad-GFP (cis Ecad) cells spread on Ecad-Fc. The four images on the left are representative images for wt Ecad and cis-Ecad, with around 35 and 70 NPs, left and right, respectively. The exact number of NPs in the picture is given at the bottom right. On the right is close-ups of NP aggregates found in the wt Ecad condition. Percentages of monomers, doublets, triplets, and higher oligomeric forms are given in the bottom right graph. χ^2 test shows significant difference between wt and cis-Ecad expressing cells regarding the proportion of singlets and oligomers ($P < 0.01$). Bars: (left) 100 nm; (right) 15 nm. (C) Distribution of the center to center distance between each NP and its nearest neighbor. *n*, number of images analyzed per condition; Kolmogorov-Smirnov non-parametric test reveals a significant difference between both distributions ($P = 0.006$). (D) NP clustering for wt Ecad (black) and cis-Ecad (gray) expressing cells was characterized by K-function analysis. Curves with solid symbols correspond to the mean $L(r) - r$ values calculated for all images. Curves with open symbols correspond to the mean $L(r) - r$ values calculated for individual images presenting significant clustering. Values of $L(r) - r$ above the 99% confidence interval (CI; black line) indicate significant clustering. Proportion of images having clustering of NP for wt and cis-Ecad is significantly different according to χ^2 test ($P < 0.01$).

and D). No significant difference was seen in the number of Ecad-Fc coated beads bound on cis and wt Ecad expressing cells. These observations indicate that the disruption of the cis-interface did not affect the ability of Ecad to mediate cell adhesion, extending previous observations showing that the disruption of the cis-interface did not impair cell aggregation in the context of cells expressing tailless Ecad (Harrison et al., 2011).

In cellulo visualization of Ecad cis-oligomers

In an attempt to visualize cadherin oligomeric organization in cellulo, we used an electron microscopy approach, allowing to stoichiometrically label GFP-tagged proteins on membrane patches (Fig. 2 A). Cells were grown on Ecad-Fc–coated electron microscope grids, and then ripped off from the surface, exposing the cytoplasmic face of the plasma membranes. Membrane rip-offs were then incubated with gold nanoparticles (NP) functionalized at a 1:1 stoichiometry with anti-GFP-NP. NPs were then observed by transmission electron microscopy (TEM), allowing the visualization of single GFP-tagged cadherins. Few areas with very high density of anti-GFP nanobodies were observed for wt Ecad (up to 2698 NP/μm^2) and were never observed for cis-Ecad (up to 279 NP/μm^2; Fig. S2, A and E). Because wt and cis-Ecad were expressed at similar levels and only a tiny area can be visualized by TEM, this difference could be the consequence of differences in distribution of wt and cis-Ecad or of a greater propensity of cis-Ecad membrane to be teared off when scratching the cell roof from the Ecad-coated grid.

To further compare the NP distribution between wt and cis-Ecad expressing cells only fields having a similar density of labeling (4–180 NP/μm^2) were considered for the analysis (Fig. 2 and Fig. S2, B and E). A fraction of NP-bound wt Ecad was distributed in doublets, triplets, or lines of a few particles as well as in small ordered clusters (Fig. 2 B and Fig. S2 C). Some aggregates of NP were seen for cis-Ecad but to a much lower extent. Analysis of the distance between each NP and its nearest neighbor revealed that 20.6% of the NPs were at <15-nm distance (Fig. 2 C). For this fraction, the mean center to center distance of neighboring particles was 10.1 ± 2.4 nm with a pick at 8 nm (Fig. S2 F); thus, in the range of the predicted distance between two adjacent cadherins interacting in cis (7.2 nm; Harrison et al., 2011). The proportion of oligomers and distance distribution were significantly different for cis-Ecad with only

7.6% of the NPs at <15 nm, a peak in the distance distribution between 30 to 55 nm, and very few NPs with a distance <10 nm (Fig. 2 C and Fig. S2 F). To determine whether the oligomeric forms observed reflect significant clustering, Ripley's K-function was used (Prior et al., 2003). 34% of the images acquired for wt Ecad cells present a significant clustering within a 15- to 120-nm range, with a maximum deviation out of the 99% confidence interval occurring at a radius of 30 nm (Fig. 2 D). In contrast, only 4.9% of the images acquired for cis-Ecad cells present a significant clustering within a 15- to 51-nm range, with a maximum deviation out of the 99% confidence interval occurring at a radius of 30 nm. cis-Ecad molecules were more randomly distributed, indicating that the cis-interface largely participates in the clustering of Ecad molecules. Altogether, these data provide supporting evidence that Ecads engaged in trans-interactions organize in ordered oligomers that require a proper cis-interface. In addition, the observed organization of particles within clusters, in particular in higher density images (Fig. S2 D), was highly reminiscent of the one predicted by theoretical models (Wu et al., 2011, 2013). This is, to our knowledge, the first time nanometric distribution of Ecad molecules can be visualized in a cellular context.

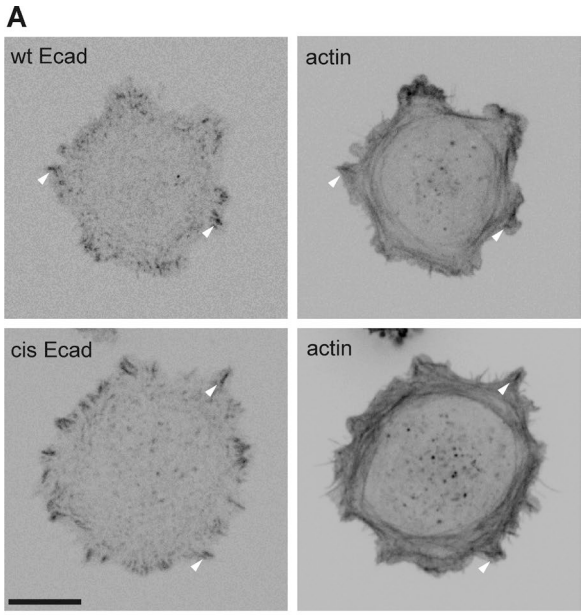
AJs form independently of Ecad cis-oligomerization

We then asked whether the disruption of this interface would affect the formation of AJs, which are believed to result from clustering of Ecads in restricted domains of cell–cell contacts (Mege et al., 1988; Harrison et al., 2011). We analyzed the propensity of transfected cells to organize Ecad in discrete cadherin adhesions when spread on Ecad-Fc, as a proxy of the ability of cells to form AJs (Gavard et al., 2004b). Both cis-Ecad and wt Ecad expressing cells spread on Ecad-Fc, and wt Ecad and cis-Ecad molecules were similarly recruited in radial cadherin adhesions, colocalizing with actin fibers (Fig. 3 A), suggesting that the formation of AJs may not involve the cis-interface.

To directly investigate the ability of cis-mutant proteins to induce the formation of AJs, we performed an ultrastructural examination of transfected cell monolayers by TEM. We searched for the presence of AJs, defined as zones of straight membrane apposition associated with the presence of dense material (Perez-Moreno et al., 2003). As reported by Lewis et al. (1997), A431D cells formed neither desmosomes nor AJs (unpublished data), allowing unambiguous interpretation of the observations on wt Ecad and cis-Ecad expressing A431D cells. The expression of wt Ecad drastically changed the cell–cell contact ultrastructure with the appearance of AJs with a mean intermembrane distance of 18.4 ± 0.7 nm (Fig. 3 B). Typically, cis-Ecad cells presented indistinguishable intercellular junctions with similar mean intermembrane spacing (18.0 ± 0.5 nm). Moreover, the mean lengths of these structures were similar. Altogether, TEM analysis demonstrates that the cis-interface is not required for the formation of AJs.

Ecad cis-oligomerization stabilizes junctional cadherins

We hypothesize that the cis-interface may, however, affect the dynamics of junctional molecules. To test whether mutations affecting the cis-interface had an effect on full-length Ecad dynamics, we performed FRAP experiments on wt and cis-Ecad expressing cells (Fig. 4, A and B). The disruption of the cis-interface induced a moderate but significant increase of



C

	AJ length	AJ width
wt Ecad	118.8 ± 7.6 µm	18.4 ± 0.8 µm
cis Ecad	116.0 ± 8.1 µm	18.0 ± 0.5 µm

Figure 3. **Ecad cis-oligomerization is not required for AJ formation.** (A) wt Ecad-GFP (wt Ecad) and cis-Ecad-GFP (cis Ecad) cells coexpressing LifeAct-Ruby were fixed after 2 h of spreading on Ecad-Fc–coated surfaces and imaged for Ecad-GFP and F-actin. Bar, 20 µm. The disruption of the cis-interface did not impact the ability of transfected cells to spread on Ecad-Fc and to recruit Ecad in cadherin adhesions (arrowheads). (B) Transmission electron micrographs showing the ultrastructure of intercellular contacts of wt Ecad-GFP (wt Ecad) and cis-Ecad-GFP (cis-Ecad) expressing cells. Bar, 100 nm. Insets show the junctional areas. (C) Table showing the mean length (± SEM) and the mean width (intermembrane distance; ± SEM) of AJs formed by wt Ecad (n = 36) and cis-Ecad-GFP expressing cells (n = 24). The disruption of the cis-interface does not prevent the formation of AJ.

the mobile fraction of Ecad. The $t_{1/2}$ value was not affected (Fig. 4 C), suggesting that the mobility of fast diffusing molecules was not altered. To have access to the dynamics of the cytoplasmic partners of Ecad, dual wavelength FRAP was performed on cells coexpressing α-catenin-mCherry. Impairment of cis-interactions induced similar trends in the dynamics of α-catenin (Fig. 4, B and C). The mobile fraction of α-catenin at cell–cell contacts significantly increased, whereas the $t_{1/2}$ was not affected. Altogether, these observations are in good agreement with a contribution of the cis-interface, through the formation of ordered oligomeric structures, in stabilizing junctional Ecad. The impairment of the Ecad cis-interface led

to a proportional increase of the α-catenin mobile fraction, suggesting that the increase in cadherin stability brought by ectodomain oligomerization has a direct intracellular impact through modifications of α-catenin dynamics. This may in turn affect cadherin anchoring to actin filaments. Such moderate changes in cadherin dynamics are expected to have limited impact on the steady-state recruitment of cadherin and catenin molecules and formation of AJs in agreement with photon end electron microscopy observations.

Ecad cis-oligomerization stiffens the mechanical coupling of cadherin adhesions to actin

The functional anchorage of cadherin–catenin complexes to F-actin has emerged as a major signaling pathway downstream of cadherins (Giannone et al., 2009; Takeichi, 2014), acting on the reinforcement of cell–cell contacts (le Duc et al., 2010; Yonemura et al., 2010; Thomas et al., 2013). To compare the functional anchorage of wt Ecad and cis-Ecad to actin we first studied actin dynamics in the lamellipodia of cells spread on Ecad-Fc–coated surfaces. Indeed, according to Mitchison and Kirschner (1988), a decreased actin treadmilling speed correlates with an increased friction between the cytoskeleton flowing underneath the plasma membrane and the membrane-anchored adhesion sites. Actin retrograde flow was visualized by live-cell imaging, thanks to the coexpression of LifeAct-Ruby (Fig. 5 A and Videos 1 and 2). The speed of the rearward flow of actin was increased by 30% in cis-Ecad compared with wt Ecad expressing cells, suggesting that cadherin oligomerization is involved in the coupling of the actin cytoskeleton to the plasma membrane. Thus, the increase in actin retrograde flow observed when the Ecad cis-interface was disrupted reflects a reduced association of Ecad clusters to the actin network. This increase in actin dynamics was correlated with an increase in cell protrusion (Fig. 5 B). Both the maximum amplitude and the frequency of lamellipodia edge back and forth movements were increased when the cis-interface was mutated (Fig. 5 C). These increases in actin rearward flow and protrusion activity in the lamellipodia of cells spread on Ecad-Fc are also suggestive of a decreased anchoring of cis-Ecad clusters to actin.

To directly test the mechanical coupling of wt and cis-Ecad to the underlying cytoskeleton, we probed the response to force of Ecad-coated magnetic beads bound to wt and cis-Ecad cells, using magnetic tweezers (Kollmannsberger and Fabry, 2007). We generated a magnetic field in the vicinity of beads applying forces ∼20 pN (Fig. 6 A). The semi-quantitative analysis of bead behavior as the magnetic power was turned on indicates that beads adhered less firmly to cis-Ecad expressing cells than to wt Ecad expressing cells (Fig. 6 B). Single beads bound to wt Ecad cells and cis-Ecad cells were then subjected to successive steps of force application (Fig. 6 C and Videos 3 and 4). No clear trend in the changes of the displacement amplitude in function of the number of cycles performed was noticed, suggesting that bead–cell mechanical coupling was not subject to force-dependent reinforcement as previously reported (Lambert et al., 2002). However, the amplitude of bead displacement was consistently higher for Ecad-Fc beads attached to cis-Ecad cells than for those bound to wt Ecad cells (Fig. 6 D). Altogether, these data indicate that Ecad cis-oligomerization increases the stiffness of Ecad complex coupling to internal structures.

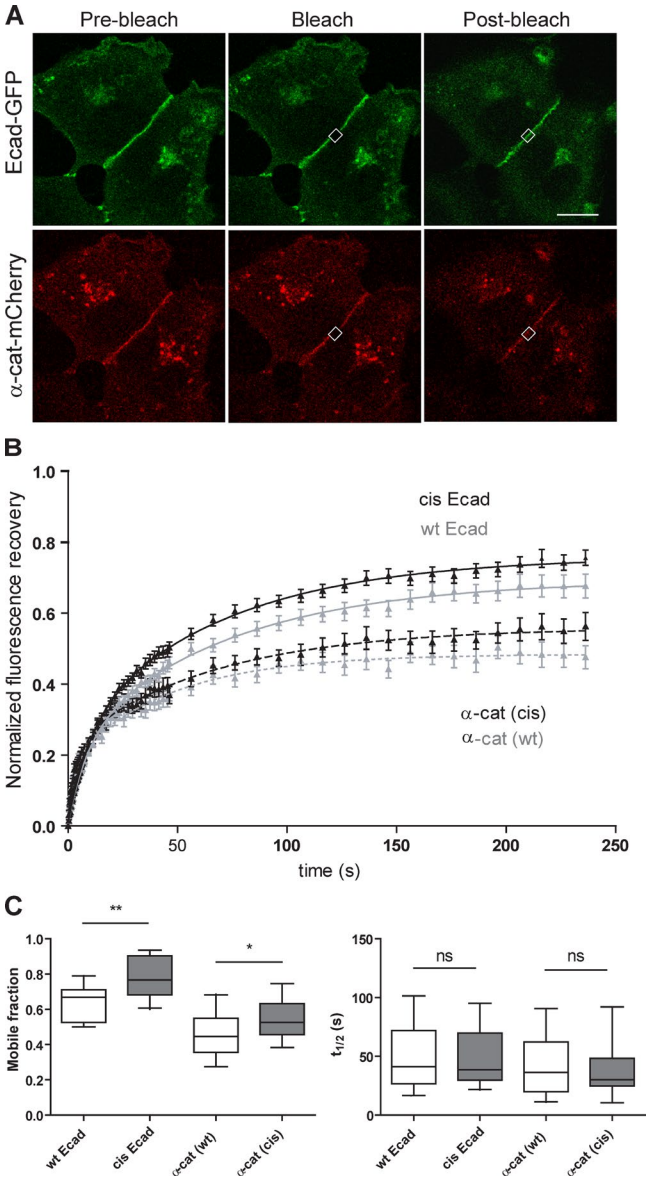


Figure 4. **Ecad cis-oligomerization stabilizes cadherin–catenin complexes at cell–cell contacts.** (A) Characteristic images of GFP and mCherry signal before (Pre-bleach), immediately after bleaching (Bleach), and 250 s after the bleach (Post-bleach) performed on wt Ecad-GFP and α-catenin-mCherry doubly transfected cells. White squares represent the bleached region. Bar, 20 µm. (B) Normalized fluorescence recovery curves versus time for wt Ecad-GFP, cis-Ecad-GFP, and α-catenin-mCherry in wt Ecad and cis-Ecad expressing cells ($n \geq 29$). (C) Box and whiskers plots (median + 10–90%) showing the mobile fraction (left) and the $t_{1/2}$ (right) extracted from a one-exponential decay fit of fluorescence recovery curves. The disruption of the cis-interface led to a moderate increase of the mobile fraction of Ecad and α-catenin molecules without apparent modification of the diffusion characteristic times. *, $P < 0.02$; **, $P < 0.05$; ns, not significant (paired Student's *t* test; $n \geq 23$).

We also took advantage of this experimental setup to extract information on the mobility of wt Ecad and cis-Ecad molecules in the cell membrane, which has been demonstrated to reflect anchoring of the molecules to the actin cytoskeleton (Lambert et al., 2002). Fig. 6 E shows successive x-y trajectories undergone by single beads bound to wt Ecad and cis-Ecad expressing cells in the absence of force. Beads bound on cis-Ecad expressing cells moved over larger areas than

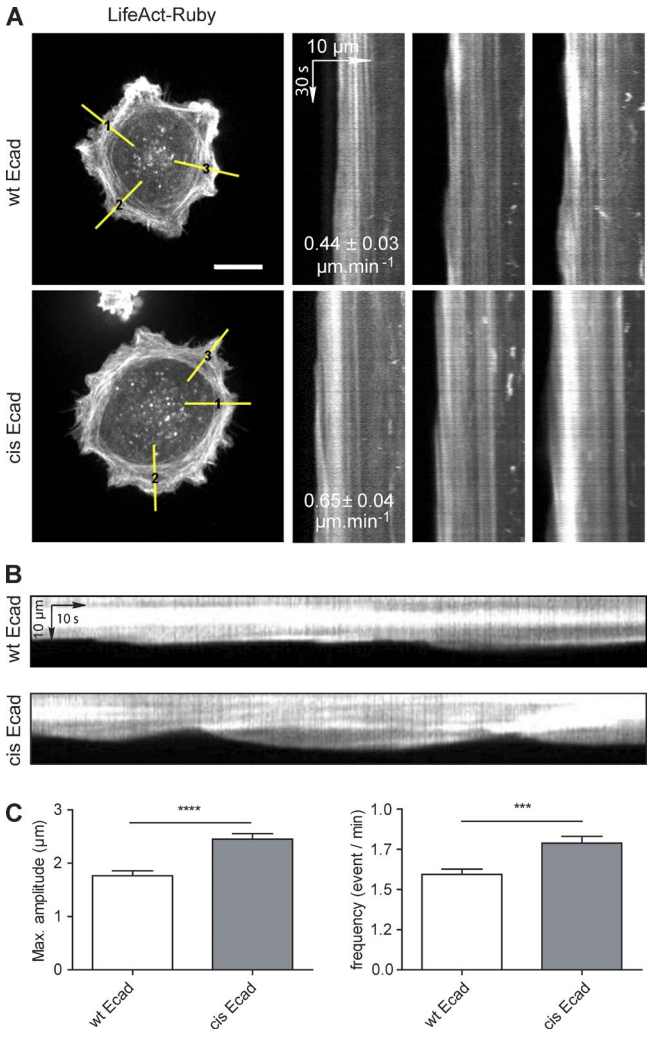


Figure 5. **Impairment of Ecad cis dimerization alters the actin dynamics of cells spread on Ecad-Fc.** Cells coexpressing wt Ecad-GFP or cis-Ecad-GFP and LifeAct-Ruby were seeded on Ecad-Fc substrates for 2 h and then subjected to spinning disk live-cell imaging for 3 min at a frequency of one image per 500 ms. (A) Still Images of LifeAct-Ruby distribution. Bar, 25 µm. The actin retrograde flow was quantified by kymograph analysis (yellow lines 1–3, 1 pixel width, perpendicular to the cell membrane in Ecad dense region). Superimposed on the kymographs are the means of actin retrograde flow speed for wt Ecad (n = 156 kymographs from 26 cells) and cis-Ecad (n = 192 kymographs from 32 cells) cells. The actin retrograde flow was significantly faster for cis-Ecad expressing cells than for cells expressing wt Ecad ($P \leq 0.0002$, Student's t test). (B) Similar kymographs of the LifeAct-Ruby signal extending on a longer time window revealed the cyclic protrusion of the edge of wt Ecad and cis-Ecad expressing cells. (C) Quantification of the maximum amplitude and frequency of membrane protrusions (mean values ± SEM; n = 100 kymographs from 26 cells for wt Ecad cells and n = 130 kymographs from 32 cells for cis-Ecad expressing cells). ****, $P \leq 0.0001$; ***, $P \leq 0.005$, Student's t test.

those bound on wt Ecad expressing cells, revealing a weaker anchorage to the underlying cytoskeleton. Extraction of mean square displacement (MSD) revealed a significant difference in the displacement of the probed Ecad forms, with cis-Ecad being more mobile than wt Ecad molecules (Fig. 6 F). These data demonstrate that disruption of the cis-interface increases Ecad mobility. They indicate that the formation of ordered clusters allowed by the cis-interface strongly regulates the anchoring of cadherins to the actin cytoskeleton, resulting in the stiffening of the cadherin–actin mechanical link.

Disruption of the cis-interface strongly impacts collective cell migration

Although wt Ecad and cis-Ecad cells did not display differences in the ability to form cell–cell contact at confluence, we postulated that the formation of structured clusters by its action on the strengthening of the Ecad–actin mechanical link may have an impact on the stability of cadherin adhesions, which may be revealed only when cell–cell adhesions are challenged, for example, during collective cell movement. Indeed, previous studies showed that changes in intercellular adhesion had a strong impact on collective cell migration behaviors (Petitjean et al., 2010; Tambe et al., 2011; Vedula et al., 2014). To unravel the impact of Ecad cis-interface disruption, we performed a cell layer expansion assay after release of confinement (Fig. S3 A). When confluence was reached the block was removed, freeing space for cell layer expansion. The migration front was followed over 24 h and analyzed for its progression and roughness (Fig. 7 A). Both wt and cis-Ecad cell monolayers expended linearly. However, this displacement was 1.6 times faster in the case of cis-Ecad cells. The roughness of the migration front increased during the first 12 h as front cells moved toward the freed area and then stabilized at a plateau. However, the roughness at the front of cis-Ecad expressing cell layers both increased more quickly and reached a higher value at plateau (Fig. 7 A). This was because of individual cis-Ecad expressing cells that tend to escape the monolayer. In addition, some cis-Ecad cells transiently detached from the monolayer, in such a way that has never been observed with wt Ecad expressing cells (Videos 5 and 6).

This analysis suggests that cis-Ecad expressing cells migrate faster as a result of reduced cell–cell cohesion releasing the constraints imposed by neighboring cells. However, faster progression of the migration front might also result from increased cell autonomous migratory behavior. We thus analyzed the migration of isolated wt Ecad and cis-Ecad cells (Fig. S4). Both cell types displayed comparable individual cell behavior, indicating that the faster migration of cis-Ecad cells was a result of collective cell behavior. Alternatively, during the extension of the monolayer, numerous divisions were taking place, suggesting that changes in the division rate might directly have an impact on monolayer front progression. To put aside this possibility, we verified that occurrence of mitosis in the extending monolayer was not different for wt Ecad and cis-Ecad cells (Fig. S5 A). In addition, 5-ethynyl-2-deoxyuridine incorporation showed that there was no difference in the proportion of cells in S phase in each population (Fig. S5 B). Thus the differences in the migratory behavior do not result from altered cell autonomous properties but is specific of collective behavior.

To further support this hypothesis we analyzed the trajectories of cells located either at the migration front or in the back (at least at the fourth row). For both cell types, individual cells at the front had very directional trajectory projecting for the majority in a 40–50° angle cone perpendicular to the migration front. In contrast, cells at the back had more randomly oriented trajectories (Fig. 7 B). However, cis-Ecad cells covered much larger distances both at the front and rear. cis-Ecad cells migrated almost two times faster than wt Ecad cells both at the front and rear (Fig. S3 B). To further describe the migration behavior of these cells, we extracted MSD values from individual cell trajectories (Fig. 7 C). The evolution of MSD as a function of time further showed that cells with the largest displacement

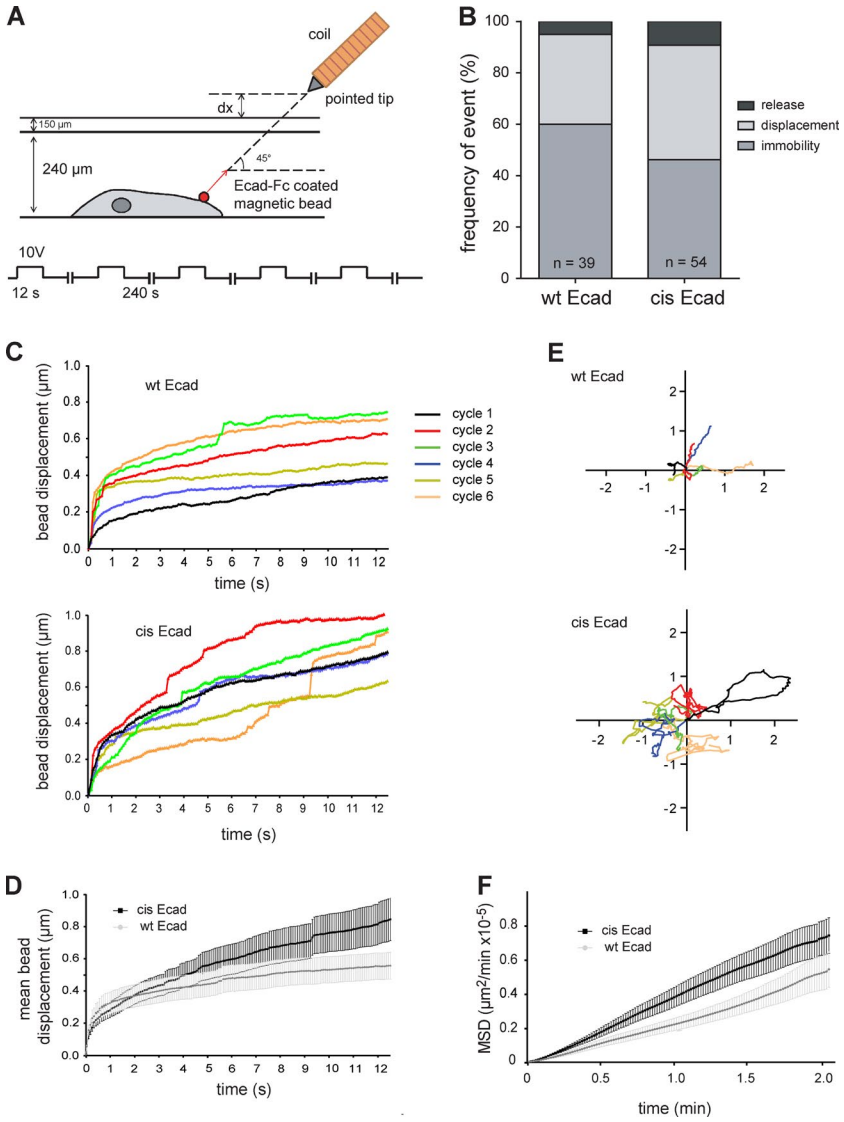


Figure 6. **Ecad oligomerization increases the anchoring of cadherin adhesions.** (A) Magnetic tweezers experimental setup. A pointed iron tip is wrapped with a copper coil under tension to generate a magnetic field in the vicinity of beads. 2.8 µm of Ecad-Fc–coated magnetic beads were preincubated 1 h on wt Ecad or cis-Ecad expressing A431D cells, and then the unbound beads were washed away. A 10-V magnetic field was applied during 10 s in the vicinity of a bound bead, and then the magnetic power was turn off for 240 s while acquiring phase-contrast images to follow bead displacement. Beads were tracked during and in between the application of forces. This sequence was repeated six times over each analyzed bead. (B) Distribution in three classes (release, displacement, and immobility) of the responses to the magnetic field of Ecad-Fc–coated beads bound to wt Ecad (39 cells) and cis-Ecad (54 cells) expressing cells. (C) Bead displacement under force: representative traces of the displacement from origin of single beads bound to wt Ecad cells and cis-Ecad cells, respectively, in response to six successive cycles of magnetic force application. (D) Curves showing the mean displacement from the origin in response to forces of beads bound to wt Ecad cells (77 displacements measured on 12 independent beads) and cis-Ecad cells (72 displacements measured on 12 independent beads). (E) Bead displacement under zero force: successive trajectories (125 s long) undergone by a single bead bound to wt Ecad and cis-Ecad expressing cells, respectively, during the six successive steps of force release. Data shown are representative of the behavior of 12 beads for each condition. (F) MSD in the absence of force calculated over the six cycles for n = 12 beads attached to wt Ecad (gray) and cis-Ecad (black) expressing cells.

were front cis-Ecad cells followed by rear cis-Ecad, and then front wt Ecad and rear wt Ecad cells. MSD curves were fitted using the equation MSD = $4Dt + v^2t^2$, where $4Dt$ represents the Brownian motion component and v^2t^2 represents the directed motion component (Fig. 7 D). Diffusion coefficent (D) value for cis-Ecad cells both at the front and rear were significantly higher than the ones of wt Ecad cells, revealing the increased exploratory behavior of the cis-mutant cells versus their wt counterparts. This can be interpreted as an increased fluidity of the cis-Ecad monolayers caused by a higher instability of cell–cell contacts and an increased ability of cells to exchange partners. The comparison of the second term of the equation (v: velocity of the directed movement) shows that front cells of both phenotypes display a more directed motion than the cells at the rear, which is expected because these cells face an empty space. However, the directed motion velocity of cis-Ecad cells is twice higher than the one of wt cells. Altogether these results suggest that the migration of wt Ecad cells is restrained by stable cell–cell contacts formed between neighboring cells within the monolayer. These results thus demonstrate that the stabilization of cell–cell contacts brought by the formation of the cis-oligomers is essential for coordinated cell behavior during collective cell migration.

Disruption of the cis-interface strongly reduces cell–cell coordination

To confirm that cell–cell coordination was affected by cis-interface impairment, we further analyzed the dynamic properties of expanding wt Ecad and cis-Ecad cell monolayers by particle image velocimetry (PIV; Fig. 8). PIV has been used as a powerful tool for quantitative analysis of tissue fluidity resulting from cell–cell rearrangements during collective cell migration (Vedula et al., 2012; Doxzen et al., 2013). PIV analysis confirmed that the mean instantaneous migration speed of cis-Ecad expressing cells was twice the one of wt Ecad cells (Fig. 8, A and B). From the velocity fields we calculated an order parameter as well as a correlation length. The order parameter reflects the degree of orientation of the velocity field in respect to a given direction, which is here defined as perpendicular to the initial migrating front (Fig. 8 C). Order parameter was maximum at the front but significantly lower for cis-Ecad than for wt Ecad cell layers. This order parameter decreased from the front toward the rear of the expanding monolayer. However, it was maintained at high values deeper in the monolayer for wt Ecad cells than for cis-Ecad cells. Finally, the correlation length, reflecting the mean distance at which velocity vectors are maintained in the same orientation, was also decreased

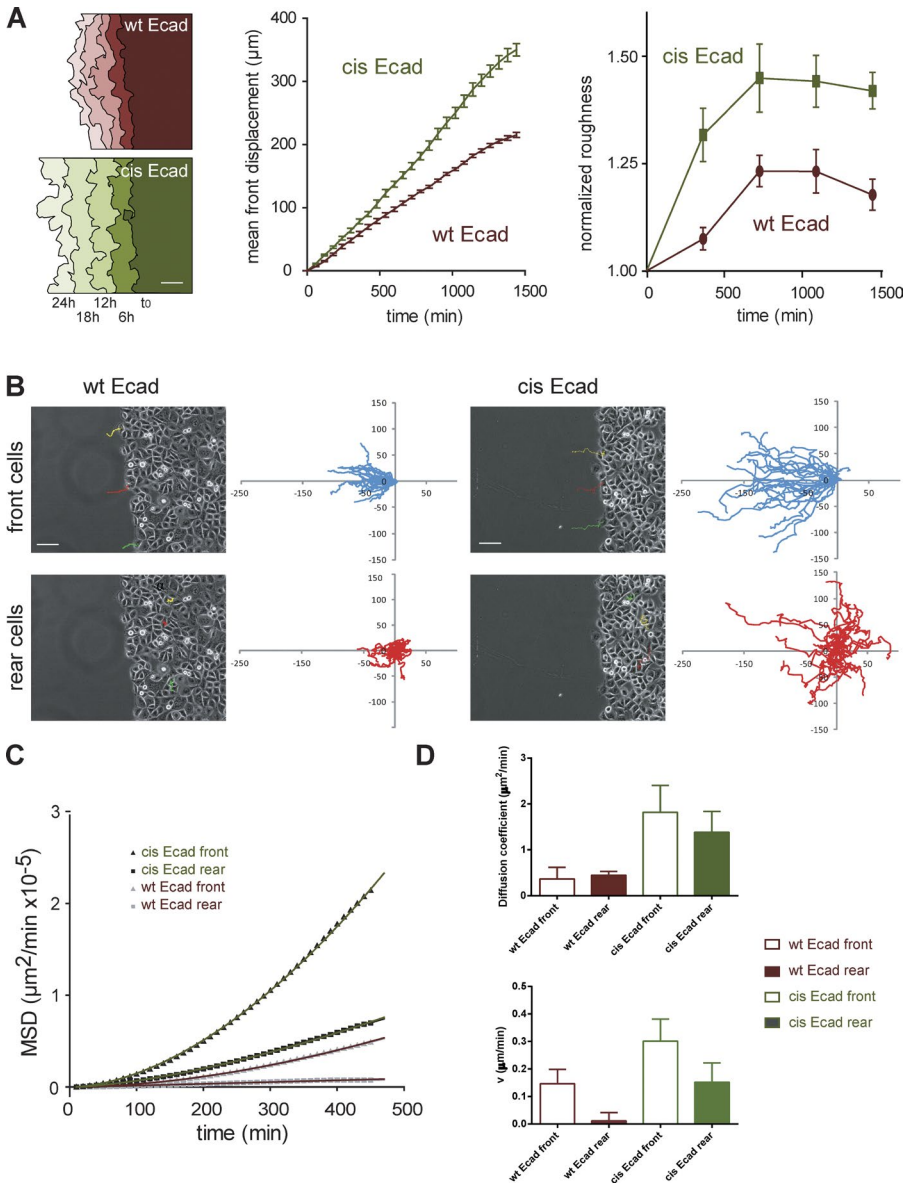


Figure 7. **The disruption of Ecad cis-oligomerization impairs collective cell migration.** Cells were phase-contrast imaged starting at the time the PDMS block was removed (t_0) and for 24 h. (A, left) Examples of the evolution of the migration front in function of time for wt Ecad and cis-Ecad expressing cells. Bar, 50 µm. Plots show the front migration displacement in function of time (middle) and the normalized front roughness in function of time (right). n = 12 and 17 for wt Ecad and cis-Ecad expressing cells, respectively. (B) Single cell tracking was performed over the first 8 h of the 24-h movies. Phase-contrast images of wt Ecad (left) and cis-Ecad (right) expressing monolayers taken at t_0 with superimposed 8-h trajectories of single cells at the front and rear (cells of the fifth row away from the edge). Bars, 50 µm. Plots of 8-h trajectories of front (blue curves) and rear (red curves) cells for wt Ecad and cis-Ecad expressing monolayers. Axes are scaled in micrometers; n = 48 and 36 trajectories for wt Ecad and cis-Ecad expressing cells, respectively. cis-Ecad cells migrate on significant larger distances than wt Ecad cells both at the front and rear. (C) MSD as a function of time for trajectories presented in B and fits by the equation ($MSD = 4Dt^2 + v^2t^2$). D: Histograms showing the D and v values (± SEM) extracted from the fits for wt Ecad and cis-Ecad front and rear cells.

when the cis-interface was impaired (Fig. 8 D). This parameter reflects the length over which cells are mechanically coupled, which is in the order of two to three cells for wt Ecad cells and only one cell for cis-Ecad cells. Altogether these observations show that cis-Ecad cell monolayers behave as a more fluid material, implying more rearrangement between individual cells. In agreement with single cell tracking and front displacement analysis, these observations demonstrate that when the cis-interface was impaired the apparent fluidity of the cell monolayer was increased as a direct consequence of decreased cell–cell cohesion, allowing more cell–cell rearrangements.

Discussion

It is proposed that oligomerization of cell adhesion molecules and their association to the underlying cytoskeleton provide to intercellular junctions the necessary strength to ensure tissue cohesion. To address this hypothesis, we determined the nanometric organization of Ecad in the plasma membrane and studied the consequences of the disruption of the cis-interface on single molecule organization up to the multicellular scale. We provide the first description of the nanometric distribution of Ecad in cellulo. Surprisingly, Ecad cis-interface is not required for AJ formation. However, its mutation strongly impaired the mechanical coupling of adhesion complexes to actin filaments affecting cell–cell contact strength. This reduced linkage to actin strongly affects cell movement coordination, leading to increased cell migration. Altogether, we show that Ecad oligomerization occurs in a cellular context and we provide direct evidence that it participates in mechanical anchoring of cadherin clusters to the cytoskeleton.

We used NP labeling to reveal an ordered nanometric organization of Ecad molecules at the cell membrane. The minimal distance between two Ecads was 8 nm. However, the mean distance (10.1 nm) between two adjacent Ecads is significantly higher. This may be explained by steric hindrance between functionalized NP, which are ∼9 nm in diameter plus the flexible GFP-bearing arm. This flexible long arm may, however, favor efficient labeling of adjacent molecules. The fact that the

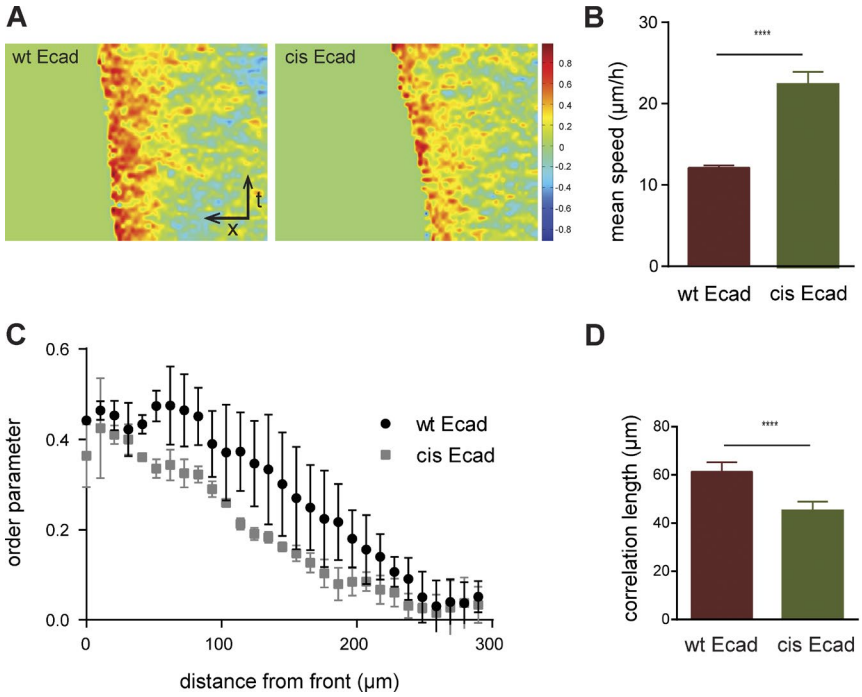


Figure 8. **PIV analysis of wt Ecad and cis-Ecad cell migration.** (A) Instantaneous velocities were extracted 250–300-µm deep from the migration front for each image and spatially averaged along the migrating axis in kymographs, giving heat maps of the order parameter (−1 means backward and +1 means forward movements). Mean instantaneous velocities (B), order parameters in the velocity field as a function of distance to the front (C), and mean correlation length (D) were extracted from the kymographs (± SEM). ****, $P \leq 0.0001$, Student's *t* test. wt Ecad cells displayed lower migration speed, migrate forward in a more directed fashion at the monolayer expansion front, and show better correlation in their movements.

cis-interface disruption significantly decreased the fraction of NP spaced <10 nm, as well as their clustering, strongly suggests that we indeed visualized predicted Ecad arrays stabilized by trans- and cis-interactions in the crystal (Harrison et al., 2011). We frequently observed a few (three to six) particles arranged in straight or broken lines as well as clusters of particles organized in two-dimensional arrays corresponding to the predicted organization of arrays predicted by modeling (Wu et al., 2011). The size of the nanoclusters is below the estimate of cluster size made by Truong Quang et al. (2013) using super-resolution imaging in *Drosophila melanogaster* embryos, although the comparison is difficult. Indeed, on the one hand, each Ecad molecule within a cluster might not be decorated with a NP and, on the other hand, super-resolutive imaging did not reach nanometric resolution, allowing to address whether Ecad molecules are packed in oligomeric nanoclusters. However, our data fit very well with the estimate of the number of molecules per cluster as well as of the surface of these clusters obtained by Wu et al. (2015) in mammalian cells.

The absence of the cis-interface did not prevent the formation of AJs in epithelial cells. This result recalls modeling data predicting the assembly of membrane-bound ligand–receptor complexes in microdomains (Weikl et al., 2002; Krobath et al., 2011; Bihr et al., 2012; Schmidt et al., 2012). In these models, an initial interaction brings locally the two membranes in close contact, increasing the probability of association of other freely diffusing ligands. This diffusion trap mechanism, similar to the one we proposed earlier for cadherin adhesions (Mège et al., 2006), leads to the buildup of densities of ligand–receptor complexes. Outside of these densities, the spacing of the two membranes superior to the length of the ligand–receptor complex prevents their growth from leading to the formation of discrete regularly spaced clusters. This remoteness has been attributed either to membrane thermal fluctuations or accumulations of membrane-bound glycoproteins. In these models, the increase in cooperatively brought by low energy cis-interactions (Wu et al., 2015) may be negligible. The absence of effect of cis-interface disruption on cadherin adhesion formation further supports this hypothesis. Indeed, cadherin adhesions formed on Ecad-Fc surfaces are found in areas of close contact between the plasma membrane and the substratum, whereas inter-cadherin adhesion areas are characterized by larger membrane-substratum spacing (Lambert et al., 2007). Although we cannot exclude that mutation of the cis-interface slightly destabilizes trans-interactions, the fact that cadherin adhesion and AJ formation are not affected does not advocate for this hypothesis. This is further supported by equilibrium analytical ultracentrifugation data showing that the K_D for Ecad ectodomain dimerization is not affected by this mutation (Harrison et al., 2011).

Oligomerization through cis-interactions slightly stabilizes cadherins at cell–cell contacts, in confirmation of previous FRAP experiments performed with tailless cadherins (Harrison et al., 2011). This increase in stability of Ecad was associated with an increase in stability of α-catenin at contact sites, indicating that Ecad ectodomain stability drives the dynamics of its associated partners. The formation of ordered clusters by stabilizing α-catenin may thus favor the association of the clusters to actin filaments. The disruption of the cis-interface significantly increases the retrograde flow of the actin networks in the lamellipodia of cells plated on Ecad-Fc. As reported previously (Plestant et al., 2014), this may result from a weaker coupling of the actin retrograde flow to the adhesion complexes. In addition, the amplitude and the frequency of membrane protrusion are increased in cis-Ecad expressing cells, which could also result from a weaker association to actin. To directly evaluate the anchoring of Ecad clusters to actin we analyzed the mobility of Ecad-Fc–coated beads bound to the surface of transfected cells because the restriction of cadherin mobility in the membrane has been associated with its anchoring to actin (Sako et al., 1998; Lambert et al., 2002). The binding of Ecad-Fc beads was not affected by the oligomeric status of Ecad. In contrast, the bead mobility was significantly higher when the cis-interface was mutated. The displacement of the beads under force was greater for cis-Ecad than for wt Ecad, indicating a weaker

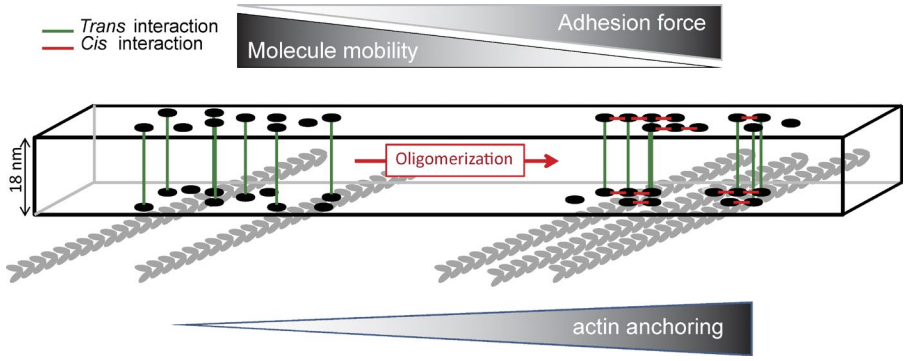


Figure 9. **Schematics of the cis-interface–dependent oligomerization of cadherin ectodomain and its effect on F-actin anchoring.** The slight stabilization of cell–cell contacts brought by cadherin oligomerization in oligomeric clusters has a strong influence on the anchoring of these clusters to the actomyosin network.

mechanical coupling of cellular Ecad to the underlying actin networks. Altogether these data indicate a contribution of oligomerization in the stiffening of the link between Ecad and actin. How can the stabilization of oligomeric clusters have such a strong influence on their anchoring to actin? The slight reduction in α-catenin accumulation may locally alter F-actin dynamics by regulating binding of nucleation and disassembly factors (Hansen et al., 2013). However, an obvious hypothesis is that the organization of Ecad molecules in clusters decreases the probability of rupturing their link to actin by a cooperative effect. Cadherin–catenin complexes within a nonorganized, fluid cluster would behave independently, preventing their cooperative binding to actin (Fig. 9).

The mechanical stabilization controlled by cadherin oligomerization had strong functional incidence on collective cell behavior. The disruption of the cis-interface increased by >70% of the speed of monolayer expansion. It was associated with a decrease of the order parameter. In other words, the monolayer composed of cis-Ecad cells behaves as a more fluid system. These changes in the fluidity of the monolayer can be directly attributed to a destabilization of the cell–cell contacts facilitating cell–cell contact reshaping and cell partner exchange. Thus, disruption of the cis-interface, albeit having a mild effect on junction formation, drastically alters cell cohesion. In conclusion, we show that cis-interactions stiffen Ecad molecule anchoring to the actin cytoskeleton, allowing cells to acquire more stable contacts and behave more collectively. Thus our results provide direct evidence that cadherin oligomerization indeed supplies the necessary strength to maintain tissue cohesion. Whether this pathway is modulated in vivo during collective cell migration, cell intercalation, wound healing, or cancer cell dissemination by factors such as cadherin glycosylation, cadherin and catenin phosphorylation, or other posttranslational modifications remains to be investigated.

Materials and methods

Cell culture and transient cell transfections

A431D Ecad-deficient epidermoid carcinoma cell line (Lewis et al., 1997; Hong et al., 2010) was grown in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 IU of penicillin, and 100 µg/ml streptomycin at 37°C in 5% CO_2. The plasmids encoding human wt Ecad-GFP (wt Ecad) and Ecad-V81D-L175D-GFP mutant (cis-Ecad) under the control of the CMV promotor, in the pRC-CMV vector, were derived from the corresponding Ecad constructs fused to Dendra (Harrison et al., 2011) by replacing exactly the Dendra coding sequence with the EGFP coding sequence in the C-terminal of the Ecad coding sequence. Cells were transfected with wt Ecad, cis-Ecad, CAAX-GFP, α-E-catenin-mCherry, and LifeAct-Ruby thanks to the Amaxa Cell Line Nucleofector Kit T (program X-001; Lonza), resulting in >80% transfection efficiency. Assays were performed 24–48 h after transfection. Cells were always used at $P < 20$.

Protein extraction and coimmunoprecipitation

Proteins were extracted from 5–10 × 10^6 transfected cells. Cells were rinsed in ice-cold PBS, detached with a cell scraper in cold PBS, and centrifuge at 1,000 rpm for 10 min at 4°C. Whole cell extracts were obtained by lysing cells into cold RIPA buffer (10 mM sodium phosphate buffer, pH 7.8, 60 mM NaCl, 1% Triton X-100, 10% glycerol, 25 mM β-glycerol-phosphate, 50 mM sodium fluoride, 2 mM sodium pyrophosphate, 1 mM orthovanadate, and protease inhibitor cocktail [Complete; Roche]). Lysates were agitated for 15 min at 4°C and cleared by centrifugation at 13,000 rpm for 10 min at 4°C, and protein concentration was estimated by micro-BCA assay (Thermo Fisher Scientific). GFP-tagged proteins were then coimmunoprecipitated using GFP-Trap according to the instructions of the manufacturer (ChromoTek). Protein samples (input and bound) were then subjected to SDS-PAGE and transferred on nitrocellulose membranes at 4°C. Membranes were then blocked with 5% nonfat milk and incubated with the corresponding primary antibody and then with IRDye-coupled secondary antibody (Rockland) against rabbit or mouse immunoglobulins, which were detected with Odyssey Imaging System (LI-COR Biosciences).

Immunocytochemistry

Cells were fixed for 12 min at room temperature using 4% paraformaldehyde in PBS, and then rinsed with PBS and permeabilized for 45 min in PBS supplemented with 1.5% BSA and 0.1% Triton X-100. Cells were then incubated for 1 h with mouse anti–α-catenin (BD) at 1:400 or rabbit anti–β-catenin (Sigma-Aldrich) at 1:400 dilution in PBS-BSA, rinsed, and incubated 1 h with anti–mouse or anti–rabbit Cy3–conjugated antibodies (Jackson ImmunoResearch Laboratories, Inc.) at 1:500 dilution. Preparations were mounted in Mowiol, 90% glycerol, and PBS. Images were taken with a microscope (DM6000; Leica) equipped with a 63× oil objective and Micromax charge coupled device (CCD) camera (Roper Scientific).

Cell membrane fluorescence analysis

Transfected cells were trypsinized, washed, fixed in 3.5% formaldehyde for 15 min, washed again, and imaged under flow using ImageStream X (Amnis) set with the 488-nm laser and 480–560 filter. Data were analyzed using the IDEAS software (Amnis). Regions corresponding to the cell membrane were extracted from bright field images. In brief, two masks were created by eroding and expanding the object by 4 pixels, respectively. The subtraction of the two masks corresponding to the cell membrane region was then applied on the fluorescence image to extract the cell membrane fluorescent intensity.

Data acquisition was performed for 300–1,200 cells for each condition and repeated three times.

Ecad-Fc and fibronectin coating procedure

Silanized glass coverslips or electron microscopy Formvar/carbon-coated gold grids (Oxford Instruments SAS) were coated with a human Ecad-human Fc chimera (R&D Systems) or fibronectin (EMD Millipore) as reported previously (Gavard et al., 2004a). In brief, 5 µg of anti–human IgG antibodies (Jackson ImmunoResearch Laboratories, Inc.) in 130 µl Ca^{2+} Mg^{2+} PBS were left to adsorb overnight at 4°C. The surfaces were washed three times with PBS, and then 10 µg of Ecad-Fc chimera proteins in PBS were allowed to bind for 2–3 h at room temperature. After three washes, coverslips were blocked for 45 min with PBS and 2.5% BSA.

Preparation of mix-capped gold NPs conjugated to GFP-NPs

Gold NPs used in this study were obtained from British Biocell International Ltd (5-nm diameter). PEGylated alkanethiol, HS-EC_{11}-EG_4 (HS-PEG), was purchased from ProChimia Surfaces and the CVVVT-ol peptidol (T-ol is for threoninol) is from Peptide and Protein Research. Mix-capped gold NPs (HS-PEG/CVVVT-ol, ratio 30:70) bearing only one Ni-*tris*NTA function per NP were prepared as described previously (Lata et al., 2005; Tinazli et al., 2005; Lévy et al., 2006; Duchesne et al., 2008). In brief, Mix-Matrix ligand solution at 2 mM final concentration was prepared by mixing 70 vol of CVVVT-ol at 2 mM with 30 vol of HS-PEG at 2 mM. A controlled molar ratio of 0.01% of HS-C_{16}-EG_3-*tris*NTA functional ligand (2-mM initial concentration) was then added to the Mix-Matrix solution. Capped NPs were prepared by adding 9 vol of colloidal gold solution to 1 vol of the previous ligand solution in a final buffer of PBS supplemented with 0.005% Tween-20 (PBST). Note that the ratio of the *tris*NTA functional ligand used (0.01% here) has been experimentally calculated to obtain Mix-Matrix–capped NPs bearing no more than one *tris*NTA group per NP (∼10% of NPs with one *tris*NTA function and ∼90% with none) and must be adjusted for each new batch of NPs and/or of ligands (matrix or functional). After an overnight incubation on a rotating wheel at room temperature, excess ligands were removed by G25 chromatography using water supplemented with 150 mM NaCl and 0.005% Tween-20 (HNT) as a mobile phase. Then, nickel loading was performed by adding $NiCl_2$ at 250 mM final concentration to the NP solution. After incubation for 1 h on a wheel at room temperature, excess $NiCl_2$ was removed by G25 chromatography using HNT as a mobile phase. Resulting Ni-*tris*NTA-NPs were purified by affinity chromatography (Histidine-resin). After elution with PBST supplemented with 200 mM imidazole, excess imidazole was removed by G25 chromatography using PBST as a mobile phase. When needed, capped NPs were concentrated by centrifugation at 60,000 *g* for 30 min. Resulting Ni-*tris*NTA-NPs were then coupled with polyhistidine-tagged camel anti–GFP-NPs (nanobody GFP-Trap; ChromoTek) as described previously (Duchesne et al., 2012) for polyhistidine-tagged FGF2 protein. In brief, nanobody GFP-Trap (6 µM final concentration) was mixed with purified Ni-*tris*NTA-NP (200 nM final concentration) in 10 µl PBST (vol/vol). The reaction was left 3 h at room temperature and PBST was added to a final volume of 200 µl. Centrifugation was performed for 90 min at 17,000 *g* at 4°C, and the supernatant, corresponding to free soluble anti-GFP-NP (uncoupled), was removed. The pellet was resuspended in 200 µl PBST and centrifuged again; a total of five cycles of centrifugation were performed. At the end, the pellet, which corresponds to the purified anti-GFP-NP conjugate (stoichiometry 1:1), was resuspended in PBS at a final concentration of 10 nM. The final concentration of conjugated NPs was calculated using ε_{520nm} of gold NPs given by the manufacturer.

Conjugated NP size

The actual measured diameter of the gold NPs was 6.9 ± 1.8 nm (n = 2,667 NPs from three independent images). They were surrounded by self-assembling monolayers of ∼2.5 nm (Harder et al., 1998; Duchesne et al., 2008), which give an inferred diameter of the functionalized NP of ∼9 nm. The length of the flexible Ni-*tris*NTA alkyl-OEG-thiol ligand (4.3 nm; Tinazli et al., 2005), of the nanobody itself, and of the GFP of both is in the range of 3 to 4 nm.

TEM experiments

For visualization of individual cadherin molecules, wt Ecad-GFP or cis-Ecad-GFP expressing cells were mechanically detached from the culture flask in the presence of PBS, 3.5 mM EDTA, and 2% BSA on ice and allowed to adhere for 2 h on Ecad-Fc– or fibronectin-coated electron microscopy grids, at 37°C in the absence of serum. After three washes with DMEM to remove the nonadherent cells, plasma membrane sheets on the electron microscopy grids were prepared ("rip-off" procedure) as described previously (Prior et al., 2003; Hancock and Prior, 2005) with some modifications. In brief, cells on grids were pressed onto a clean glass coverslip. The coverslip was turned over and 200 µl PBS was added quickly around the grids to separate them from the coverslip and to generate plasma membrane sheets on the grids (inner leaflet face up). Samples were then fixed with a solution of 0.1% glutaraldehyde (vol/vol) and 4% formaldehyde (wt/vol) for 10 min. The fixative was then quenched with three washes in 100 mM glycine. After three bathes in PBS, nonspecific sites were blocked for 10 min with PBS supplemented with 0.25% BSA. Grids were then incubated for 30 min with 3 nM anti-GFP-NP (or control uncoupled Ni-*tris*NTA-NP) in PBS and 0.25% BSA. After extensive washes with PBS and then deionized water, grids were treated with a mixture of 0.3% uranyl acetate (wt/vol) and 1.8% methylcellulose (wt/vol) for 10 min on ice. Grids were then individually picked up with homemade 5-mm-thick iron wire loops and left to dry overnight before storage. Preparations were digitally imaged using an 80-kV transmission electron microscope (CM100; Philips) equipped with an Orius CCD Camera (Gatan) or a 200-kV (Tecnai G2 T20 Sphera; FEI) transmission electron microscope equipped with a LaB6 electron source and a USC4000 CCD camera (Gatan). For the analysis, 3-µm^2 images acquired with the CM100 were cropped down to 0.55-µm^2 area to fit with the scale of the ones acquired with the Tecnai G2. Four and three different grids were visualized for wt and cis conditions, respectively. All 0.55-µm^2 images (742.7 × 742.7 nm) were processed using Fiji software (ImageJ, National Institutes of Health). In brief, digital pictures were converted to binary images and filtered to remove any residual noise, and then x,y coordinates of the NPs were calculated for each picture. To avoid bias that would be caused by a small number of pictures having a very high density of gold NP labeling, only images with 2 to 100 gold NPs per pictures (4–180 NP/µm^2) were used for further analysis. For oligomer counting, given a mean diameter for the functionalized NPs (∼9 nm) and the length of the flexible Ni-*Tris*NTA ligand (∼4.3 nm; see previous section), a maximal distance of 15 nm between NP centers was fixed to distinguish cadherin ectodomains in interaction from non-interacting monomers in cis. Calculation of the distance between each NP and its nearest neighbor (center to center) was performed using the NND (Nearest Neighbor Distance) imageJ plugin (from Y. Mao, Mississippi State University, Starville, MS). Cluster analysis was performed using the Univariate 725 macro (I. Prior, University of Liverpool, Liverpool, UK) as described in Hancock and Prior (2005). For such analysis, only fairly homogeneous images without vesicular profiles or large unlabeled areas were kept (n = 50 and 102 for wt and cis-Ecad expressing cells, respectively). In brief, K-function identifies systematic deviations of the NPs pattern from complete spatial randomness. The mean K-function is plotted as a linear transformation

$L(r) - r$. To interpret the statistical significance indicating clustering, a 99% confidence interval for $L(r) - r$ is generated using Monte Carlo simulations. Plots were generated for each individual image and $L(r) - r$ values were then standardized on the confidence interval for each image to allow comparison and averaging. Averaging was done for all images and specifically for images presenting a significant clustering between 0- to 300-nm radius. Values above 1 for the standardized $L(r) - r$ function indicate significant clustering (99% confidence interval) at the radius r and no deviation ($0 < L(r) - r < 1$) indicates a random pattern.

Graphs and statistical analyses were performed using OriginPro 8.6 software. Kolmogorov-Smirnov nonparametric test was used to compare distribution and χ^2 test was performed to compare proportions.

For analysis of intercellular junctions, transfected cells were deposited on a 12-well format cell culture insert at high density (pore size of 0.4 µm; BD). When the monolayer was confluent, cells were fixed with 3% glutaraldehyde in phosphate buffer for 1 h at room temperature. Samples were kept at 4°C in 0.1 M phosphate buffer, pH 7.4, until further treatment and embedding in epon resin was performed. Thin section chromatography was performed and samples were stained with uranyl acetate and observed using a transmission electron microscope (1011; JEOL) equipped with an Orius CCD camera.

FRAP

FRAP was measured at 37°C on cells coelectroporated with wt Ecad-GFP or cis-Ecad-GFP and αE-catenin-mCherry using a confocal microscope (TCS SP5; Leica) equipped with a 40× water immersion objective. After five prebleach scans (0.347 s), a rectangular region of interest (3.5 × 2.9 µm) was bleached and fluorescence recovery was acquired every 0.347 s (20 scans), then every 2 s (20 scans), and finally every 10 s (20 scans). The normalized recovery of fluorescence was expressed as a ratio of prebleach fluorescence rate after correction for photobleaching, as reported previously (Lambert et al., 2007). Fluorescence recovery in function of time were best fitted with a one-term exponential equation, allowing to extract a plateau value representing the fraction of diffusion-limited molecules (mobile fraction) and a recovery $t_{1/2}$ proportional to the apparent diffusion coefficient of diffusion-limited molecules (Thoumine et al., 2006). The mobile fraction and the $t_{1/2}$ were determined by fitting the normalized recovery curves using one-phase decay nonlinear regression function of the Prism 5.01 software (GraphPad Software).

Collective cell migration assay

A431D cells expressing wt Ecad or cis-Ecad were high density plated in 3.5-cm Petri dishes where a polydimethylsiloxane (PDMS) block was previously deposited to impose cells to grow on a restricted area of the dish. When cells reached confluence, the PDMS block was removed. Images were then acquired every 5 min during 24 h under a controlled temperature and CO_2 environment (5% CO_2 at 37°C; 10× objective; BioStation; Nikon). The surface occupied by the monolayer determined thanks to ImageJ was plotted as a function of time. Manual tracking of individual cells at the front or rear (at least four rows of cells away from the front) was performed with the MTrackJ plugin during the first 8 h. Individual trajectories were positioned on an orthonormal axis with the coordinates of the cell at $t_0 = (0, 0)$. The MSD was then extracted for each condition and plotted versus time. The direction persistence was calculated as the ratio of the cumulative distance over the Euclidian distance between the position of the cell at time 0 and its position at time t.

Actin dynamics and lamellipodial activity measurement

Cells coexpressing LifeAct-Ruby and wt Ecad-GFP or cis-Ecad-GFP were mechanically detached as described previously (Plestant et al., 2014) and plated in live cell imaging buffer (10 mM Hepes, pH 7.4, 128 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5.5 mM glucose, and 0.2% BSA) at low cell density ($<5 \times 10^4$ cells/cm^2) on Ecad-Fc–coated glass-bottom dishes for 2 h. Cells were then imaged every 500 ms for 3 min at 63× with a time-lapse confocal video microscope equipped with a Nipkov disk (spinning disk). Kymographs were made by generating time-lapse montages of a single line perpendicular to the cell edge for each frame of the video (ImageJ) along three lines normal to the free edge of each analyzed cell. Three flow rates were calculated for each kymograph (15 cells analyzed for each condition).

Preparation of the Ecad-coated beads

2.8 µm of magnetic protein A–coated beads (Dynabeads; Invitrogen) were coated with Ecad-hFc. In brief, 10 µl of the blurry solution was washed three times and resuspended in 200 µl of 0.1 M borate buffer, pH 8.0, before 2 × 30-s sonication. Then, 50 µl of goat anti–human IgG Fc fragment (2.4 mg/ml; Jackson ImmunoResearch Laboratories, Inc.) was added and left to incubate overnight on a wheel at room temperature. Beads are then washed three times and resuspended in 200 µl PBS (Life technologies) before 2 × 30-s sonication. Then 5 µl of recombinant Ecad-Fc were added and left to incubate for 3 h on a rotating wheel at room temperature. Finally, beads were washed and resuspended in 1 ml PBS supplemented with 1% BSA. 50 µl of this Ecad-coated bead solution was added to cells grown on a 22 × 22-mm glass coverslip placed on a 3.5-cm Petri dish for 1 h. After extensive washes to remove unbound beads, the medium was changed for phenol red–free DMEM supplemented with 20 mM Hepes, pH 7.4.

For bead binding assays, Ecad-hFc– or hFc-coated beads were deposited on nontransfected and wt Ecad– or cis-Ecad–transfected cells, left to adhere for 1 h, and gently washed before fixation. Images were taken with a DM6000 microscope equipped with a 10× objective and a micromax CCD camera. The number of bound beads per squared millimeter was then manually scored.

Magnetic tweezers assay

The forces were locally applied on bead-bearing cells with magnetic tweezers made of an electromagnet and the superparamagnetic microbeads mentioned in Preparation of the Ecad-coated beads. The electromagnet was 816 turns of 0.5-mm copper wire coil surrounding a soft iron core 5 mm in diameter with a 30° cone-shaped tip. It was mounted on a micromanipulator (InjectMan NI2; Eppendorf) at a 45° vertical angle, and the tip initially aligned at 700 µm from the center of the observation zone. The current was provided by a home-made voltage-controlled current and a function generator (TG1010; TT Instruments). This function generator was directly controlled from the computer through a control card (USB1208HS; Measurement Computing). The samples were mounted on a microscope (DMIRB; Leica) equipped with a CCD camera (Coolsnap HQ2; Roper Scientific) through a 100× oil objective. Both the camera and the current in the coil were controlled by the µManager software (version 1.4.8). 2.8 µm of Ecad-Fc–coated magnetic beads were preincubated for 1 h on wt or cis-Ecad cells, and then the unbound beads were washed away. Applications of current ranging from 0.5 to 1.2 A drew the beads toward the tip. The force exerted by the electromagnet was calibrated by measuring the velocity of a bead moving through a viscous fluid (PDMS). Six steps of forces were applied, each step consisted of the following: at $t = 0$, current in the coil was set to 1.2 A and camera received signal to start acquiring images in the burst mode (frequency of ∼13 frames/s) for 170 frames; the current was turned down and the camera was set to an acquiring rate of 2 frames/s for 114 s; the camera was then set to a 1-frame/s acquisition rate for an additional 125 s. Tracking of the bead position was done with Icy (Icy v1.4.3.5; Quantitative Image Analysis Unit, Institut Pasteur) using the Active Contour plugin.

PIV

PIV is an image correlation–based method usually used to obtain instantaneous velocity field measurements from the local displacements and heavily used in hydrodynamics. Images are divided into multiple interrogation subwindows. Each interrogation subwindow should contain sufficient numbers of tracers to enable comparison between the current time frame and the subsequent time frame. Cross-correlation techniques are then performed to compute the displacement vectors at each subwindow by finding their best match at the successive time frame. PIV analysis of monolayer movement (Petitjean et al., 2010) was performed as described previously (Vedula et al., 2012) using MatPIV v. 1.6.1 package and implemented in Matlab (MathWorks). The analysis was done with 32 × 32-pixel (19 × 19-µm) interrogation windows with an overlap of 50% using the same size of initial migrating front (300 µm). Order parameter and correlation length were calculated using the formula previously described (Doxzen et al., 2013; Vedula et al., 2014).

Statistical analysis and curve fitting and image processing

Statistical analysis and curves fitting were performed with Prism 5.0 software. Differences were considered significant for p-values ≤ 0.05. Image processing was done in ImageJ (or Matlab when indicated), and then with Photoshop and Illustrator (Adobe).

Online supplemental material

Fig. S1 shows the recruitment of β-catenin at cell–cell contacts and binding of Ecad-Fc beads independent of Ecad cis-oligomerization. Fig. S2 shows the nanometric organization of wt and cis-Ecad-GFP at the cell membrane of A431D-transfected cells. Fig. S3 shows the quantitative analysis of wt and cis-Ecad-GFP cell layer expansion. Fig. S4 shows the migration of single wt and cis-Ecad-GFP–transfected cells. Fig. S5 shows the division rate of wt and cis-Ecad-GFP–transfected cells. Videos 1 and 2 show actin retrograde flow in wt Ecad-GFP expressing cell spread on Ecad-Fc (Video 1) and in cis-Ecad-GFP expressing cell spread on Ecad-Fc (Video 2). Videos 3 and 4 show Ecad-Fc magnetic bead displacement under force in wt Ecad-GFP (Video 3) and in cis-Ecad-GFP expressing cells (Video 4). Videos 5 and 6 show collective cell migration of wt Ecad-GFP (Video 5) and of cis-Ecad-GFP expressing cells (Video 6). Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201410111/DC1.

Acknowledgments

We thank N. Boggetto for her help with flow imaging and D. Chrétien and A. Schmitt for help with electron microscopy. We thank Ian Prior for providing the Univariate 725 Excel macro.

This work was supported by grants from Centre National de la Recherche Scientifique, Fondation ARC (R.M. Mège), Human Frontier Science Program grant RPG0040/2012 (B. Ladoux and R.M. Mège), Agence Nationale de la Recherche (ANR 2010 Blan1515 [B. Ladoux and R.M. Mège] and Nanotechnology Program [B. Ladoux]), and European Research Council (ERC) under the European Union's Seventh Framework Program (FP7/2007-2013)/ERC grant agreement no. 617233 (B. Ladoux). Financial supports were from Mechanobiology Institute and Institut Universitaire de France to B. Ladoux. S. Troyanovsky's work was supported by grant AR44016 from the National Institutes of Health. G. Peyret, P.-O. Strale, and L. Duchesne were supported by fellowships from the University Paris-Diderot, Agence Nationale de la Recherche, and Fondation ARC, respectively. We acknowledge the Institut Jacques Monod ImagoSeine, Microscopy Rennes imaging center, and Institut Cochin Electron Microscopy facilities.

The authors declare no competing financial interests.

Submitted: 28 October 2014
Accepted: 10 June 2015

References

Adams, C.L., Y.T. Chen, S.J. Smith, and W.J. Nelson. 1998. Mechanisms of epithelial cell–cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin–green fluorescent protein. *J. Cell Biol.* 142:1105–1119. http://dx.doi.org/10.1083/jcb.142.4.1105

Al-Amoudi, A., D.C. Díez, M.J. Betts, and A.S. Frangakis. 2007. The molecular architecture of cadherins in native epidermal desmosomes. *Nature.* 450:832–837. http://dx.doi.org/10.1038/nature05994

Baumgartner, W., P. Hinterdorfer, W. Ness, A. Raab, D. Vestweber, H. Schindler, and D. Drenckhahn. 2000. Cadherin interaction probed by atomic force microscopy. *Proc. Natl. Acad. Sci. USA.* 97:4005–4010. http://dx.doi.org/10.1073/pnas.070052697

Bihr, T., U. Seifert, and A.-S. Smith. 2012. Nucleation of ligand-receptor domains in membrane adhesion. *Phys. Rev. Lett.* 109:258101. http://dx.doi.org/10.1103/PhysRevLett.109.258101

Boggon, T.J., J. Murray, S. Chappuis-Flament, E. Wong, B.M. Gumbiner, and L. Shapiro. 2002. C-cadherin ectodomain structure and implications for cell adhesion mechanisms. *Science.* 296:1308–1313. http://dx.doi.org/10.1126/science.1071559

Cavey, M., M. Rauzi, P.F. Lenne, and T. Lecuit. 2008. A two-tiered mechanism for stabilization and immobilization of E-cadherin. *Nature.* 453:751–756. http://dx.doi.org/10.1038/nature06953

Doxzen, K., S.R.K. Vedula, M.C. Leong, H. Hirata, N.S. Gov, A.J. Kabla, B. Ladoux, and C.T. Lim. 2013. Guidance of collective cell migration by substrate geometry. *Integr. Biol. (Camb.).* 5:1026–1035. http://dx.doi.org/10.1039/c3ib40054a

Duchesne, L., D. Gentili, M. Comes-Franchini, and D.G. Fernig. 2008. Robust ligand shells for biological applications of gold nanoparticles. *Langmuir.* 24:13572–13580. http://dx.doi.org/10.1021/la802876u

Duchesne, L., V. Octeau, R.N. Bearon, A. Beckett, I.A. Prior, B. Lounis, and D.G. Fernig. 2012. Transport of fibroblast growth factor 2 in the pericellular matrix is controlled by the spatial distribution of its binding sites in heparan sulfate. *PLoS Biol.* 10:e1001361. http://dx.doi.org/10.1371/journal.pbio.1001361

Gavard, J., M. Lambert, I. Grosheva, V. Marthiens, T. Irinopoulou, J.F. Riou, A. Bershadsky, and R.M. Mège. 2004a. Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. *J. Cell Sci.* 117:257–270. http://dx.doi.org/10.1242/jcs.00857

Gavard, J., V. Marthiens, C. Monnet, M. Lambert, and R.M. Mège. 2004b. N-cadherin activation substitutes for the cell contact control in cell cycle arrest and myogenic differentiation: involvement of p120 and β-catenin. *J. Biol. Chem.* 279:36795–36802. http://dx.doi.org/10.1074/jbc.M401705200

Giannone, G., R.M. Mège, and O. Thoumine. 2009. Multi-level molecular clutches in motile cell processes. *Trends Cell Biol.* 19:475–486. http://dx.doi.org/10.1016/j.tcb.2009.07.001

Goodsell, D.S., and A.J. Olson. 2000. Structural symmetry and protein function. *Annu. Rev. Biophys. Biomol. Struct.* 29:105–153. http://dx.doi.org/10.1146/annurev.biophys.29.1.105

Hancock, J.F., and I.A. Prior. 2005. Electron microscopic imaging of Ras signaling domains. *Methods.* 37:165–172. http://dx.doi.org/10.1016/j.ymeth.2005.05.018

Hansen, S.D., A.V. Kwiatkowski, C.Y. Ouyang, H. Liu, S. Pokutta, S.C. Watkins, N. Volkmann, D. Hanein, W.I. Weis, R.D. Mullins, and W.J. Nelson. 2013. αE-catenin actin-binding domain alters actin filament conformation and regulates binding of nucleation and disassembly factors. *Mol. Biol. Cell.* 24:3710–3720. http://dx.doi.org/10.1091/mbc.E13-07-0388

Harder, P., M. Grunze, and R. Dahint. 1998. Molecular conformation in oligo(ethylene glycol)-terminated self-assembled monolayers on gold and sylver surfaces determines their ability to resist protein adsorption. *J. Phys. Chem.* 102:426–436. http://dx.doi.org/10.1021/jp972635z

Harrison, O.J., X. Jin, S. Hong, F. Bahna, G. Ahlsen, J. Brasch, Y. Wu, J. Vendome, K. Felsovalyi, C.M. Hampton, et al. 2011. The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. *Structure.* 19:244–256. http://dx.doi.org/10.1016/j.str.2010.11.016

He, W., P. Cowin, and D.L. Stokes. 2003. Untangling desmosomal knots with electron tomography. *Science.* 302:109–113. http://dx.doi.org/10.1126/science.1086957

Hong, S., R.B. Troyanovsky, and S.M. Troyanovsky. 2010. Spontaneous assembly and active disassembly balance adherens junction homeostasis. *Proc. Natl. Acad. Sci. USA.* 107:3528–3533. http://dx.doi.org/10.1073/pnas.0911027107

Hong, S., R.B. Troyanovsky, and S.M. Troyanovsky. 2013. Binding to F-actin guides cadherin cluster assembly, stability, and movement. *J. Cell Biol.* 201:131–143.

Hulpiau, P., I.S. Gul, and F. van Roy. 2013. New insights into the evolution of metazoan cadherins and catenins. *Prog. Mol. Biol. Transl. Sci.* 116:71–94. http://dx.doi.org/10.1016/B978-0-12-394311-8.00004-2

Kollmannsberger, P., and B. Fabry. 2007. High-force magnetic tweezers with force feedback for biological applications. *Rev. Sci. Instrum.* 78:114301. http://dx.doi.org/10.1063/1.2804771

Krobath, H., B. Różycki, R. Lipowsky, and T.R. Weikl. 2011. Line tension and stability of domains in cell-adhesion zones mediated by long and short receptor-ligand complexes. *PLoS ONE.* 6:e23284. http://dx.doi.org/10.1371/journal.pone.0023284

Lambert, M., D. Choquet, and R.M. Mège. 2002. Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the actin cytoskeleton. *J. Cell Biol.* 157:469–479. http://dx.doi.org/10.1083/jcb.200107104

Lambert, M., O. Thoumine, J. Brevier, D. Choquet, D. Riveline, and R.M. Mège. 2007. Nucleation and growth of cadherin adhesions. *Exp. Cell Res.* 313:4025–4040. http://dx.doi.org/10.1016/j.yexcr.2007.07.035

Lata, S., A. Reichel, R. Brock, R. Tampé, and J. Piehler. 2005. High-affinity adaptors for switchable recognition of histidine-tagged proteins. *J. Am. Chem. Soc.* 127:10205–10215. http://dx.doi.org/10.1021/ja050690c

le Duc, Q., Q. Shi, I. Blonk, A. Sonnenberg, N. Wang, D. Leckband, and J. de Rooij. 2010. Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II–dependent manner. *J. Cell Biol.* 189:1107–1115. http://dx.doi.org/10.1083/jcb.201001149

Lévy, R., Z. Wang, L. Duchesne, R.C. Doty, A.I. Cooper, M. Brust, and D.G. Fernig. 2006. A generic approach to monofunctionalized protein-like gold nanoparticles based on immobilized metal ion affinity chromatography. *ChemBioChem.* 7:592–594. http://dx.doi.org/10.1002/cbic.200500457

Lewis, J.E., J.K. Wahl III, K.M. Sass, P.J. Jensen, K.R. Johnson, and M.J. Wheelock. 1997. Cross-talk between adherens junctions and desmosomes depends on plakoglobin. *J. Cell Biol.* 136:919–934. http://dx.doi.org/10.1083/jcb.136.4.919

Mege, R.M., F. Matsuzaki, W.J. Gallin, J.I. Goldberg, B.A. Cunningham, and G.M. Edelman. 1988. Construction of epithelioid sheets by transfection of mouse sarcoma cells with cDNAs for chicken cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 85:7274–7278. http://dx.doi.org/10.1073/pnas.85.19.7274

Mège, R.M., J. Gavard, and M. Lambert. 2006. Regulation of cell–cell junctions by the cytoskeleton. *Curr. Opin. Cell Biol.* 18:541–548. http://dx.doi.org/10.1016/j.ceb.2006.08.004

Mitchison, T., and M. Kirschner. 1988. Cytoskeletal dynamics and nerve growth. *Neuron.* 1:761–772. http://dx.doi.org/10.1016/0896-6273(88)90124-9

Overduin, M., T.S. Harvey, S. Bagby, K.I. Tong, P. Yau, M. Takeichi, and M. Ikura. 1995. Solution structure of the epithelial cadherin domain responsible for selective cell adhesion. *Science.* 267:386–389. http://dx.doi.org/10.1126/science.7824937

Perez-Moreno, M., C. Jamora, and E. Fuchs. 2003. Sticky business: orchestrating cellular signals at adherens junctions. *Cell.* 112:535–548. http://dx.doi.org/10.1016/S0092-8674(03)00108-9

Perret, E., A. Leung, H. Feracci, and E. Evans. 2004. Trans-bonded pairs of E-cadherin exhibit a remarkable hierarchy of mechanical strengths. *Proc. Natl. Acad. Sci. USA.* 101:16472–16477. http://dx.doi.org/10.1073/pnas.0402085101

Petitjean, L., M. Reffay, E. Grasland-Mongrain, M. Poujade, B. Ladoux, A. Buguin, and P. Silberzan. 2010. Velocity fields in a collectively migrating epithelium. *Biophys. J.* 98:1790–1800. http://dx.doi.org/10.1016/j.bpj.2010.01.030

Plestant, C., P.O. Strale, R. Seddiki, E. Nguyen, B. Ladoux, and R.M. Mège. 2014. Adhesive interactions of N-cadherin limit the recruitment of microtubules to cell-cell contacts through organization of actomyosin. *J. Cell Sci.* 127:1660–1671. http://dx.doi.org/10.1242/jcs.131284

Prior, I.A., C. Muncke, R.G. Parton, and J.F. Hancock. 2003. Direct visualization of Ras proteins in spatially distinct cell surface microdomains. *J. Cell Biol.* 160:165–170. http://dx.doi.org/10.1083/jcb.200209091

Raviola, E., and N.B. Gilula. 1975. Intramembrane organization of specialized contacts in the outer plexiform layer of the retina. A freeze-fracture study in monkeys and rabbits. *J. Cell Biol.* 65:192–222. http://dx.doi.org/10.1083/jcb.65.1.192

Sako, Y., A. Nagafuchi, S. Tsukita, M. Takeichi, and A. Kusumi. 1998. Cytoplasmic regulation of the movement of E-cadherin on the free cell surface as studied by optical tweezers and single particle tracking: corralling and tethering by the membrane skeleton. *J. Cell Biol.* 140:1227–1240. http://dx.doi.org/10.1083/jcb.140.5.1227

Schmidt, D., T. Bihr, U. Seifert, and A.-S. Smith. 2012. Coexistence of dilute and densely packed domains of ligand-receptor bonds in membrane adhesion. *Europhys. Lett.* 99:38003. http://dx.doi.org/10.1209/0295-5075/99/38003

Shapiro, L., and W.I. Weis. 2009. Structure and biochemistry of cadherins and catenins. *Cold Spring Harb. Perspect. Biol.* 1:a003053. http://dx.doi.org/10.1101/cshperspect.a003053

Shapiro, L., A.M. Fannon, P.D. Kwong, A. Thompson, M.S. Lehmann, G. Grübel, J.F. Legrand, J. Als-Nielsen, D.R. Colman, and W.A. Hendrickson. 1995. Structural basis of cell-cell adhesion by cadherins. *Nature.* 374:327–337. http://dx.doi.org/10.1038/374327a0

Takeichi, M. 2014. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. *Nat. Rev. Mol. Cell Biol.* 15:397–410. http://dx.doi.org/10.1038/nrm3802

Tambe, D.T., C.C. Hardin, T.E. Angelini, K. Rajendran, C.Y. Park, X. Serra-Picamal, E.H. Zhou, M.H. Zaman, J.P. Butler, D.A. Weitz, et al. 2011. Collective cell guidance by cooperative intercellular forces. *Nat. Mater.* 10:469–475. http://dx.doi.org/10.1038/nmat3025

Thomas, W.A., C. Boscher, Y.S. Chu, D. Cuvelier, C. Martinez-Rico, R. Seddiki, J. Heysch, B. Ladoux, J.P. Thiery, R.M. Mege, and S. Dufour. 2013. α-Catenin and vinculin cooperate to promote high E-cadherin-based adhesion strength. *J. Biol. Chem.* 288:4957–4969. http://dx.doi.org/10.1074/jbc.M112.403774

Thoumine, O., M. Lambert, R.M. Mège, and D. Choquet. 2006. Regulation of N-cadherin dynamics at neuronal contacts by ligand binding and cytoskeletal coupling. *Mol. Biol. Cell.* 17:862–875. http://dx.doi.org/10.1091/mbc.E05-04-0335

Tinazli, A., J. Tang, R. Valiokas, S. Picuric, S. Lata, J. Piehler, B. Liedberg, and R. Tampé. 2005. High-affinity chelator thiols for switchable and oriented immobilization of histidine-tagged proteins: a generic platform for protein chip technologies. *Chemistry.* 11:5249–5259. http://dx.doi.org/10.1002/chem.200500154

Troyanovsky, R.B., I. Indra, C.S. Chen, S. Hong, and S.M. Troyanovsky. 2015. Cadherin controls nectin recruitment into adherens junctions by remodeling the actin cytoskeleton. *J. Cell Sci.* 128:140–149. http://dx.doi.org/10.1242/jcs.161588

Truong Quang, B.A., M. Mani, O. Markova, T. Lecuit, and P.F. Lenne. 2013. Principles of E-cadherin supramolecular organization in vivo. *Curr. Biol.* 23:2197–2207. http://dx.doi.org/10.1016/j.cub.2013.09.015

Vedula, S.R.K., M.C. Leong, T.L. Lai, P. Hersen, A.J. Kabla, C.T. Lim, and B. Ladoux. 2012. Emerging modes of collective cell migration induced by geometrical constraints. *Proc. Natl. Acad. Sci. USA.* 109:12974–12979. http://dx.doi.org/10.1073/pnas.1119313109

Vedula, S.R.K., H. Hirata, M.H. Nai, A. Brugués, Y. Toyama, X. Trepat, C.T. Lim, and B. Ladoux. 2014. Epithelial bridges maintain tissue integrity during collective cell migration. *Nat. Mater.* 13:87–96. http://dx.doi.org/10.1038/nmat3814

Weikl, T.R., D. Andelman, S. Komura, and R. Lipowsky. 2002. Adhesion of membranes with competing specific and generic interactions. *Eur Phys J E Soft Matter.* 8:59–66. http://dx.doi.org/10.1140/epje/i2002-10008-2

Wu, Y., J. Vendome, L. Shapiro, A. Ben-Shaul, and B. Honig. 2011. Transforming binding affinities from three dimensions to two with application to cadherin clustering. *Nature.* 475:510–513. http://dx.doi.org/10.1038/nature10183

Wu, Y., B. Honig, and A. Ben-Shaul. 2013. Theory and simulations of adhesion receptor dimerization on membrane surfaces. *Biophys. J.* 104:1221–1229. http://dx.doi.org/10.1016/j.bpj.2013.02.009

Wu, Y., P. Kanchanawong, and R. Zaidel-Bar. 2015. Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions. *Dev. Cell.* 32:139–154. http://dx.doi.org/10.1016/j.devcel.2014.12.003

Yonemura, S., Y. Wada, T. Watanabe, A. Nagafuchi, and M. Shibata. 2010. α-Catenin as a tension transducer that induces adherens junction development. *Nat. Cell Biol.* 12:533–542. http://dx.doi.org/10.1038/ncb2055