



Regulation of the Akt/mTORC1 Pathway by HIV Transcriptional Activator Tat in B Cells

Burkitkan Akbay

► To cite this version:

Burkitkan Akbay. Regulation of the Akt/mTORC1 Pathway by HIV Transcriptional Activator Tat in B Cells. Cancer. Université Paris-Saclay; Al-Farabi Kazakh National University (Almaty, Kazakhstan), 2021. English. NNT : 2021UPASL026 . tel-03216741

HAL Id: tel-03216741

<https://theses.hal.science/tel-03216741>

Submitted on 4 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Regulation of the Akt/mTORC1 pathway by HIV transcriptional activator Tat in B cells

Régulation de la voie Akt / mTORC1 par l'activateur
transcriptionnel du VIH Tat dans les cellules B

**Thèse de doctorat en cotutelle de l'Université Paris-Saclay et Al-
Farabi Kazakh National Université préparée à l'Université Paris-
Saclay**

École doctorale n°582 : cancérologie : biologie - médecine – santé (CBMS)

Spécialité de doctorat: Aspects moléculaires et cellulaires de la Biologie

Unité de recherche : Université Paris-Saclay, CNRS, Institut Gustave Roussy, Aspects
métaboliques et systémiques de l'oncogénèse pour de nouvelles approches
thérapeutiques, 94805, Villejuif, France

Référent : Faculté de médecine

**Thèse présentée et soutenue en Visio conférence totale, le 29 Mars
2021, par**

Burkitkan AKBAY

Composition du Jury

Murat SAPARBAEV

Directeur de Recherche, CNRS, Université
Paris-Saclay

Président

Valery FILONENKO

Professeur, National Academy of Sciences of
Ukraine

Rapporteur

Isabelle VERGNE

Chargée de Recherche, CNRS

Rapporteur

Audrey ESCLATINE

Directrice de Recherche, CNRS, Université
Paris-Saclay

Examinatrice

David BOUTBOUL

Maitre de conférence, Hôpital Saint-Louis

Examineur

Direction de la thèse

Yegor VASSETZKY

Directeur de Recherche, CNRS

Directeur de thèse

Svetlana DOKUDOVSKAYA

Directrice de Recherche, CNRS

Co-directrice de thèse

Amangeldy BISSENBAEV

Professeur, Al-Farabi Kazakh National
University

Co-directeur de thèse

Acknowledgements

I cannot begin to express my deepest appreciation to my esteemed supervisors, Dr. Svetlana Dokudovskaya, Dr. Yegor Vassetzky and Pr. Amangeldy Bissenbaev. I am extremely grateful to them for their invaluable supervision, unwavering guidance and continuous support. They showed profound belief in my abilities. Their immense knowledge and plentiful experience have encouraged me in all the time of my thesis project. They taught me to be professional, to be critical, and to be scientific wise. I truly learned from them how to be a scientist. I'm deeply indebted to Dr Svetlana Dokudovskaya for her patience which I appreciated throughout my thesis especially during the long processes of writing papers and thesis. She transmitted to me her professional passion and her knowledge in various fields. I am very proud and satisfied to be a doctoral student in her team. I am deeply thankful to Dr Yegor Vassetzky who is the first person I contacted and gave me the opportunity to start my doctoral study life in the team. I would also like to extend my deepest gratitude to Dr. Diego Germini for his practical suggestions and helpful advices. I thank them for their wise advices and insightful suggestions in difficult times and for guiding me throughout this thesis. Without their persistent help, the goal of my thesis would not have been realized.

I would like to express my deepest appreciation to the jury members who accepted to witness and assess my work. I wish to show my gratitude to Dr. Valery Filonenko and Dr. Isabelle Vergne for their time and energy contributed to read and examine my thesis manuscript. I am extremely thankful for their constructive suggestions. I would also like to extend my sincere thanks to Dr. Murat Saparbaev who accepted to be the examiner and the president of the jury. I am also grateful to Dr. Audrey Esclatine and Dr. David Boutboul who accepted to be the examiners. Special thanks to Dr. Audrey Esclatine who was also the member of "le comité de suivi individuel du doctorant" to assess the progress of my thesis, where she gave her valuable suggestions and advices.

I also had great pleasure of working with Dr. Eugene Sheval and Dr. Yana Musinova for the fruitful collaborations which gave us the opportunity to generate the cell lines that were further validated and used throughout my thesis.

I would also express my gratitude to the French Embassy in Kazakhstan and Campus France in Paris for their support and funding. My gratitude extends to l'Etablissement Français du Sang - Hôpital Saint Louis for facilitating our study.

I would also like to express my gratitude to all the members of previous UMR 8126 and current UMR 9018 - Aspects métaboliques et systémiques de l'oncogenèse pour de nouvelles approches thérapeutiques (METSY), a research unit rich in themes and nationalities. I'm proud to be the part of this research unit. I

especially thank each person from the " Dynamique de la chromatine et métabolisme dans le cancer" team, most notably Alberto, Yingxing, Fabi, and Reynand, with whom I spent beautiful times in the lab which will remain as a beautiful memory forever.

Finally, I am extremely grateful to my parents. Definitely words will never be enough to express my thanks to them. Thank you for many years of unconditional support, trust, and love. Since my childhood you have given me the opportunity, at any cost, to make my dream come true, even if you were living a harsh nomadic life in the steppe. Despite all the difficulties you have always made my study life easier for me. It will never be possible for me to return everything that you have offered to me. Thank you for my sister Zhaynakhan, and my brother Kenzhebek for helping me, believing in me and always pushing me for the best, with their support, their sacrifices, and their prayers. My dear brother Kenzhebek thank you very much for looking after our parents, which I couldn't be able to do since I am away from you all for so many years to pursue my dream of studying.

I wish to acknowledge the support and great love of my wife, Gulgina, who has been taking care of my two cute sons, Rassul and Amire. Even when I am away from you all, you stayed alone to take care of them without any complains, even though you faced a lot of difficulties. Hope we will go through life adventures together in the future. Hope our children stay healthy. We will do all to our children's future as our parents did to us. I do believe that we have a bright future.

God protects all of us.

Synthèse

Malgré l'avènement de la trithérapie antirétrovirale (ARV), l'incidence des lymphomes agressifs à cellules B reste élevée chez les personnes vivantes avec le VIH-1 (PVVIH), ces lymphomes constituant ainsi une des principales causes de décès chez ces patients. Pourtant, les cellules B ne sont pas la cible d'infection par le virus et les mécanismes exacts du développement de ces lymphomes ne sont pas encore totalement connus. Plusieurs hypothèses ont cependant été proposées pour expliquer les mécanismes de la lymphomagenèse chez les PVVIH: 1) les lymphocytes T infectés par le VIH-1 et qui échappent à l'élimination dans les centres germinatifs pourraient provoquer une expansion inhabituelle des cellules B; 2) des hypermutations somatiques aberrantes sur les gènes d'immunoglobuline pourraient favoriser la survenue de translocations chromosomiques conduisant à une transformation maligne; 3) une inflammation chronique pourrait être le lit d'une lymphoprolifération. Une autre hypothèse sur le processus de lymphomagenèse B chez les PVVIH est l'implication directe des protéines du VIH-1 qui peuvent être sécrétées par les cellules infectées. Parmi elles, nous avons la protéine Tat qui, après sécrétion par les cellules infectées, peut pénétrer dans d'autres cellules non infectées, y compris les cellules B.

Tat est un activateur transcriptionnel du VIH-1. C'est l'une des premières protéines virales à être exprimée juste après l'infection. Grâce à sa taille et de ses structures telles que le domaine de transduction protéique (PTD) et le signal de localisation nucléaire (NLS), il peut pénétrer dans les cellules voisines non infectées. Par conséquent, Tat est impliqué dans l'oncogenèse de nombreux cancers incluant les cancers colorectaux, les cancers du col de l'utérus, le sarcome de Kaposi associé au SIDA et les lymphomes à cellules B. Les mécanismes exacts de l'oncogenèse induite par Tat, en particulier celle des lymphomes à cellules B, restent cependant à être élucidés. Des études antérieures de notre équipe ont montré d'une part, que dans les cellules B, Tat induisait une production d'espèces réactives de l'oxygène (ROS), augmentant ainsi les dommages oxydatives à l'ADN, source d'instabilité génomique. D'autre part, Tat favorisait également dans les cellules B, une proximité des loci MYC et IGH (normalement situés dans des compartiments nucléaires différents) et la surexpression du gène AICDA (activation-induced cytidine deaminase), ce qui conduit vraisemblablement à la translocation oncogénique t(8 ;14) entre MYC et IGH caractéristique du lymphome de Burkitt. Cependant, la manière dont ces événements sont liés n'est pas bien comprise.

Parmi les voies de signalisations cellulaires, la voie AKT/mTORC1 apparaît comme un intégrateur central de nombreux signaux intra et extracellulaires, y compris dans l'infection virale et les dommages à l'ADN. Des études ont montré que le VIH-1 et ses protéines Tat, Env et Nef pouvaient réguler la voie AKT/mTORC1. En revanche, à notre connaissance, aucune étude ne s'est encore intéressée à la régulation de la voie AKT / mTORC1 par Tat dans les cellules B.

Compte tenu de l'importance de la voie de AKT / mTORC1 dans l'oncogenèse et en combinant les résultats des travaux précédents de notre équipe sur Tat, nous avons émis l'hypothèse selon laquelle la protéine Tat du VIH-1 pourrait induire dans les cellules B les effets oncogènes sus-mentionnés en modulant la voie de signalisation AKT / mTORC1. Nous nous sommes essentiellement focalisés sur les mécanismes de régulation de l'expression du gène AICDA par Tat non seulement parce que son expression est associée à la voie mTORC1, mais aussi parce que Tat induit une expression aberrante d'AICDA dans les cellules B qui est étroitement associée à la lymphomagenèse B.

Une grande partie de ma thèse portait sur la régulation de la voie AKT / mTORC1 par Tat dans les cellules B. Nous avons utilisé différentes lignées cellulaires B : i) des cellules B primaires issues de donneurs sains traitées ou pas avec une protéine Tat purifiée ; ii) des cellules d'une lignée lymphoblastoïdes RPMI8866 traitées ou pas avec une protéine Tat purifiée ; et iii) des cellules de RPMI8866 exprimant la protéine Tat de façon stable mais inductible par la doxycycline. Cette dernière lignée a été développée avec nos collaborateurs et une procédure détaillée décrivant la construction de cette lignée cellulaire est publiée dans le Russian Journal of Developmental Biology. En utilisant ces différents modèles cellulaires, nous avons étudié en présence et en l'absence de Tat, l'activation de la voie AKT/mTORC1, l'autophagie, les dommages d'ADN et la production de ROS dans les cellules B. Nous avons ensuite analysé, en fonction du profil d'activation de la voie mTORC1 par Tat, l'expression du gène AICDA en présence et en l'absence de ses activateurs et répresseurs transcriptionnels.. Tous les résultats obtenus sont décrits en détail dans notre article publié dans le numéro spécial «mTOR Signalling network in Cell Biology and Disease» du Journal of International Molecular Sciences.

Nos résultats montrent, en présence de Tat, une activation de la voie Akt / mTORC1, conséquence des dommages à l'ADN induits par Tat via la production de ROS. mTORC1 activé va à son tour inhiber l'expression de cMYB et E2F8, des inhibiteurs transcriptionnels de l'AICDA, conduisant ainsi à sa surexpression. Tat semble aussi inhiber l'autophagie, cependant, cette régulation de l'autophagie devra être explorée plus en détail.

En conclusion, la protéine Tat libérée dans le milieu extracellulaire par les cellules infectées, pénètre dans les cellules B et y induit la production de ROS conduisant à des dommages à l'ADN, qui sont en outre détectés par la voie AKT / mTORC1. Une fois activé, mTORC1 inhibe l'expression des répresseurs transcriptionnels d'AICDA, Cmyb et E2F8, favorisant ainsi la surexpression d'AICDA. Ces perturbations peuvent finalement conduire à une instabilité génomique accrue qui constitue une des principales caractéristiques de l'oncogenèse, y compris dans les cellules B.

Table of Contents

Introduction	13
1. Viral infection	13
1.1. Attachment	14
1.2. Entry	14
1.3. Replication, Assembly and Release	14
2. Human immunodeficiency virus type - 1	17
2.1. Structure of the HIV-1 virion	17
2.2. Life cycle of HIV-1	18
3. HIV-1-Tat	21
3.1. Structure of Tat.....	21
3.2. Functions of Tat	22
3.3. Tat in HIV-1 transcription.....	22
3.4. Tat in host cell gene expression	22
3.5. Tat secretion and penetration in bystander cells	24
4. HIV-associated cancers and related diseases.....	25
5. The mammalian target of rapamycin complex.....	28
5.1. Upstream regulation of mTORC1	30
5.1.1. PI3K-AKT signaling to mTORC1	30
5.1.2. Amino acid signaling to mTORC1.....	32
5.1.3. DNA damage response and mTORC1	35
5.2. Downstream targets of mTORC1	39
5.3. mTORC1 and the Immune System	41
5.3.1. mTORC1 in the innate immunity.....	41
5.3.2. mTORC1 in adaptive immunity.....	43
5.3.2.1. mTORC1 in T lymphocytes	44
5.3.2.2. mTORC1 in B lymphocytes.....	45
5.4. mTORC1 and viruses.....	48
5.4.1. Regulation of mTORC1 by HIV-1	49
5.4.2. Regulation of Autophagy by HIV-1	51
5.4.3. mTORC1 in HIV-1 Latency	52
Objectives of the thesis.....	73

Materials and Methods	74
1. Isolation of the peripheral blood mononuclear cells	74
2. Purification of B lymphocytes	74
3. Cell lines	74
4. Tat treatment and expression induction	75
5. Inhibition of AKT/mTORC1 pathway by MK-2206 and Rapamycin treatments	75
6. Western Blot	76
7. Monitoring ROS production	76
8. qRT-PCR	77
9. DNA damage detection with immunofluorescence.....	78
10. Statistical analyses.....	79
Results	80
1. Generation of B cell lines providing inducible and constant Tat expression.....	80
2. HIV-1 Tat activates AKT/mTORC1 pathway and AICDA expression by downregulating its transcriptional inhibitors in B cells	87
2.1. Increased <i>AICDA</i> expression by Tat is not prevented by AKT inhibition	103
2.2. Exogenous Tat differently regulates downstream targets of mTORC1, p70S6K and 4E-BP1	104
2.3. HIV-Tat inhibits starvation-induced autophagy in B cells.....	105
Discussion	106
Conclusions and perspectives	110
Conferences and Publications	111
1. Conferences.....	111
2. Publications.....	111
References	112

List of abbreviations

ICTV	International Committee on Taxonomy of Viruses
4E-BP1	Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1
5'UTR	5' untranslated region
aa	Amino acid
ADE	Antibody-dependent enhancement
AICDA	Activation-induced cytidine deaminase
AIDS	Acquired Immunodeficiency Syndrome
AMPK	AMP-activated protein kinase
ARL	AIDS-related lymphomas
ART	Antiretroviral therapy
ATF4	Activating transcription factor 4
ATG13	Autophagy-related 13
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia- and RAD3-related
ATRIP	ATR-interacting protein
BACE1-AS	BACE1-antisense transcript
BCR	B cell receptor
BER	Base excision repair
BL	Burkitt's lymphomas
CAD	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, dihydroorotase
cART	Combination antiretroviral therapy
CASTOR1	Cellular Arginine Sensor for mTORC1 1
CASTOR2	Cellular Arginine Sensor for mTORC1 2
CCR5	C-C chemokine receptor type 5
CDK	Cyclin-dependent kinase
CDK9	Cyclin-dependent kinase 9
Chk1	Checkpoint kinase 1
Chk2	Checkpoint kinase 2
CREB	CAMP-responsive element-binding protein
CTL	Cytolytic T lymphocytes
CXCR4	C-X-C chemokine receptor type 4
CycT1	Cyclin T1
DC	Dendritic cell
DDR	DNA damage response
DEPDC5	DEP domain-containing 5
DEPTOR	DEP domain containing mTOR-interacting protein

DLBCL	Diffuse large B cell lymphomas
DNA-PK	DNA-dependent protein kinase
DSB	Double strand break
dsDNA	Double stranded DNA
E2F3	E2F transcription factor 3
EBV	Epstein–Barr virus
env	Envelope
ESCRT	Endosomal sorting complexes required for transport
FAT	Focal adhesion targeting
FAT	FRAP-ATM-TRRAP
FATC	FAT carboxy-terminal
FIP200	Focal adhesion kinase family interacting protein of 200kDa
FLCN	Folliculin
FNIP	FLCN-interacting protein
FOXO	Forkhead family of transcription factors
FRAP	FK506-binding protein-rapamycin-associated protein
FRB	FKBP-rapamycin-binding
gag	Group-specific antigen
GAP	GTPase-activating protein
GEF	Guanine nucleotide exchange factor
gp120	Glycoprotein 120
gp41	Glycoprotein 41
GSK3	Glycogen synthase kinase 3
HAART	Highly active antiretroviral therapy
HAND	HIV-associated neurocognitive disorder
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHV8	Human herpesvirus 8
HIV-1	Human immunodeficiency virus type-1
HIVAN	HIV-associated nephropathy
HL	Hodgkin's lymphomas
HPV	Human papillomavirus
HR	Homologous recombination
HSC	Hematopoietic stem cells
hSMG-1	Human suppressor of morphogenesis in genitalia 1
HSPGs	Heparan sulfate proteoglycans
IL-1b	Interleukin 1 beta
IL-6	Interleukin 6
IN	Integrase

KS	Kaposi's sarcoma
LEL	Late endosomes/lysosomes
LITAF	Lipopolysaccharide-induced tumor necrosis factor
lncRNA	Long non-coding RNA
LRP	Lipoprotein receptor-related protein
LRS	Leucyl-tRNA synthetase
LTR	Long terminal repeats
MA	Matrix
MCD	Multicentric Castleman's disease
MDM2	Mouse double minute 2 homolog
MHC	Major histocompatibility complex
MIOS	Meiosis regulator for oocyte development
MITF	Microphthalmia transcription factor
mLST8	Mammalian lethal with sec-13 protein 8
MMR	Mismatch repair
MRN	Mre11-Rad50-Nbs1
mSin1	Mammalian stress-activated map kinase-interacting protein 1
MTHFD2	Mitochondrial tetrahydrofolate cycle enzyme methylenetetrahydrofolate dehydrogenase 2
mTOR	Mammalian TOR
mTORC	Mammalian target of rapamycin complex
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
NC	Nucleocapsid
nef	Negative regulating factor
NER	Nucleotide excision repair
NF-κB	Nuclear factor of κ B
NHEJ	Non-homologous end joining
NHL	Non-Hodgkin's lymphomas
NK	Natural killer
NLS	Nuclear localization signal
NPRL2	Nitrogen permease regulator-like 2
NPRL3	Nitrogen permease regulator-like 3
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
p70S6K	70 kDa ribosomal protein S6 kinase 1
PBL	Plasmablastic lymphoma
PBMC	Peripheral blood mononuclear cells
PCNSL	Primary central nervous system lymphoma
PKD1	3-phosphoinositide-dependent kinase 1

PEL	Primary effusion lymphoma
PI3K	Phosphatidylinositol 3-kinase
PIKK	Phosphatidylinositol 3-kinase related kinases
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
PKC	Protein kinase C
pol	Polymerase
PP2	Protein phosphatase 2
PR	Protease
PRAS40	Proline-rich AKT substrate 40 kDa
PRD	PIKK-regulatory domain
Protor	Protein observed with rictor
PTD	Protein transduction domain
PTEN	Phosphatase and tensin homolog
RAFT	Rapamycin and FK506-binding protein target
RAPT	Rapamycin target
Raptor	Regulatory-associated protein of mammalian target of rapamycin
RBD	RNA binding domain
rev	RNA splicing-regulator
RGD	Arginine-glycine-aspartic acid
RHEB	Ras homologue enriched in brain
Rictor	Rapamycin-insensitive companion of mTOR
ROS	Reactive oxygen species
RT	Reverse transcriptase
RTK	Receptor tyrosine kinases
S6K1	Ribosomal protein S6 kinase beta-1
SAMTOR	S-adenosylmethionine sensor upstream of mTORC1
SEH1L	Seh1 like nucleoporin
Ser	Serine
SGK1	Serum- and glucocorticoid-induced protein kinase 1
SHM	Somatic hyper mutation
SMAD2	SMAD Family Member 2
SREBP1/2	Sterol regulatory element binding protein 1/2
SSB	Single strand break
Superoxide Dismutase 2	SOD2
TAR	Transactivation response
tat	Transactivator of transcription
TCR	T cell receptors

TFE3	Transcription factor E3
TFEB	Transcription factor EB
Tfh	Follicular helper T cells
Th	T helper
TNF-β	Tumor necrosis factor beta
TopBP1	Topoisomerase II Binding Protein 1
TOR1	Target of rapamycin 1
TOR2	Target of rapamycin 2
TOS	TOR signaling
Treg	Regulatory T cell
TRRAP	Transformation/transcription associated protein
TSC	Tuberous Sclerosis Complex
Twist1	Twist-related protein 1
ULK1	Unc-51-like autophagy-activating kinase 1
UNG	Uracil nucleoside glycosylase
UPR	Unfolded protein response
vif	Viral infectivity factor
vpr	Virus protein r
vpu	Virus protein unique
vRNA	Viral RNA
WDR24	WD repeat domain 24
WDR59	WD repeat domain 59
ZEB1	Zinc finger E-box binding protein 1

List of Figures

Figure 1	Basic structure of a virus
Figure 2	Viral attachment and entry into the host cells
Figure 3	Viral replication, assembly and release
Figure 4	Structure of HIV-1 virion
Figure 5	Life cycle of HIV-1
Figure 6	Structure of HIV-1 Tat protein
Figure 7	Tat regulates gene expression of host and bystander cells
Figure 8	HIV-associated cancers and other diseases
Figure 9	Mechanisms of development of AIDS-related B cell lymphomas
Figure 10	Structure of mTOR protein and its complexes
Figure 11	PI3K-AKT signaling pathway
Figure 12	Activation of the small GTPases
Figure 13	Regulation of mTORC1 by growth factors, cytokines and amino acids
Figure 14	DNA damage signaling
Figure 15	Regulation of mTORC1 by DNA damage
Figure 16	Downstream targets of mTORC1
Figure 17	Role of mTORC1 in the innate immunity
Figure 18	mTORC1 in T lymphocytes
Figure 19	Function of mTORC1 in B cells
Figure 20	Regulation of mTORC1 by HIV-1
Figure 21	Implication of various HIV-1 proteins in modulation of autophagy in different cells
Figure 22	HIV-1 latency
Figure 23	Treatment with AKT/mTORC1 inhibitors as a promising approach for HIV-1 therapy
Figure 24	Inhibition of AKT by its specific inhibitor MK-2206
Figure 25	Inhibition of AKT was not able to prevent AICDA overexpression by Tat
Figure 26	P70S6K and 4E-BP1 are differently regulated by exogenous Tat treatment in B cells
Figure 27	Tat inhibits autophagy induction by amino acid starvation

List of Tables

Table 1	AIDS-related lymphomas and associated viruses
Table 2	List of primers used for qRT-PCR

Introduction

1. Viral infection

Discovery of viruses goes back to late nineteenth and early twentieth century with yellow fever virus known to be the first that infect humans (1,2). Since then more than 200 human viruses have been identified with a rate of three to four each year (1) with SARS-Cov-2 being the latest one threatening the globe.

An infectious viral particle or virion is a fully assembled virus (**Figure 1**), which consists of genetic material (**RNA or DNA**) protected by a protein coat, **capsid**, and often (but not always) an outer membrane, **envelope**, which are typically derived from proteins and phospholipid membranes of the host cell, but may also contain other viral glycoproteins. Proteins constructing the viral particle are called structural proteins. The main function of capsid and envelope is protection which enables the virus to be stable in various extra-cellular environments. Capsid and envelope also help the virus to enter and deliver its genome into the host cell. There are other proteins known as non-structural or regulatory proteins that control the expression of viral genome and modulate host immune responses (3–6).

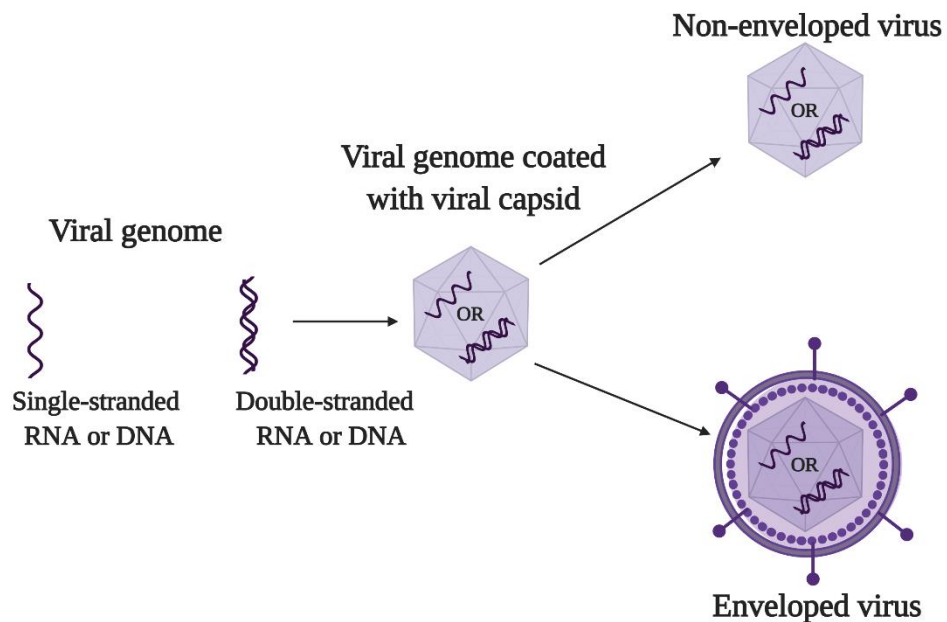


Figure 1. Basic structure of a virus: an infectious viral particle consists of viral genome (single- or double-stranded RNA or DNA) protected by the capsid, which can be non-enveloped or enveloped.

Viruses are named and classified by the International Committee on Taxonomy of Viruses (ICTV) (<https://talk.ictvonline.org/>). Due to the extreme diversity and complexity of viruses, their classification or grouping at different hierarchical levels of order, family, subfamily, genus, and species are based on a variety of parameters such as presence or absence of an envelope, structure of capsids, morphology, mode of replication and type of viral genome (DNA or RNA, linear or circular, single- (ss) or double-stranded (ds), positive or negative ssRNA, ssRNA requiring a DNA intermediate or dsDNA requiring an RNA intermediate) (5–8).

In general, most viral infections include attachment, entry, replication, assembly, and release cycle (**Figure 2, Figure 3**). Viruses must first recognize and attach to their host cells. After successful attachment viruses penetrate the cell membrane, upon which they copy their genome and make their own proteins inside the host cells. In order to survive within the host, viruses must escape from the destruction caused by host immune system so that they can infect innate immune cells or affect other cells (bystander cells).

1.1. Attachment

Many viruses use glycoproteins on the surface of the viral membrane or certain sites of viral capsid to bind to the molecules on the host cell called *viral receptors* (**Figure 2**). The receptors on cell surfaces are the molecules that normally have their own physiological functions. Viruses can have one single receptor or several receptors. As interactions between virus and receptors are specific, presence of receptors always determines which cell types can be infected. Receptors further facilitate various processes of viral entry by activating certain signaling pathways within the host cells or by inducing structural changes in the viral attachment proteins (9–11).

1.2. Entry

Viral entry is a process through which virus gains access to the sites of replication. Many viruses pass through the cellular membrane via either a process called *endocytosis* with further membrane fusion or via direct membrane fusion, while some other viruses (non-enveloped) enter the cells through a pore formed with viral capsid and cellular membrane after endocytosis (**Figure 2**) (12–16). Once inside of the host cells' cytoplasm, the viral capsid is degraded. The viral genetic material become available for further trafficking in order to be replicated, transcribed, translated, thus providing conditions for production of new virions.

1.3. Replication, Assembly and Release

The replication allows the virus to generate enough copies of viral genome to make new virions (**Figure 3**), which is essential for viral survival and infection of new hosts. The replication mechanism is

dependent on the type of viral genome. Viruses keep their genome either in the form of RNA or DNA. There are two ways of replication for RNA viruses: either RNA-dependent RNA synthesis or RNA-dependent DNA synthesis. In both cases, RNA viruses must encode their own polymerases and other necessary enzymes. In the RNA-dependent RNA synthesis process, RNA viruses first make a complementary RNA (cRNA) which is then used as template to make new viral RNA (vRNA) copies.

Viral mRNA is further transcribed with the help of viral polymerase in coordination with cellular RNA polymerase. In the RNA-dependent DNA synthesis process, virus use an enzyme called reverse transcriptase that converts viral RNA into viral DNA which is integrated into the host genome with the help of another enzyme called integrase. The integrated virus genome is then transcribed as a cellular gene. DNA viruses benefit from replication and transcription strategies of host cells.

Following replication and transcription, viral mRNA is exported to the cytoplasm to make viral proteins by the translational machinery of the host cell. Newly synthesized viral proteins with nuclear localization signal are imported to the nucleus to assist in the vRNA replication and viral mRNA transcription. (**Figure 3**). Some viruses replicate their genome in the nucleus while others - in the cytoplasm of the host cells. Viruses replicating in the nucleus transport their genome to the nucleus via nuclear pore complex, using host nuclear import machinery. Viruses replicating in the cytoplasm are assisted by organelle-like compartments: single/double membrane vesicles and invaginations formed by cellular membrane rearrangements (17–20).

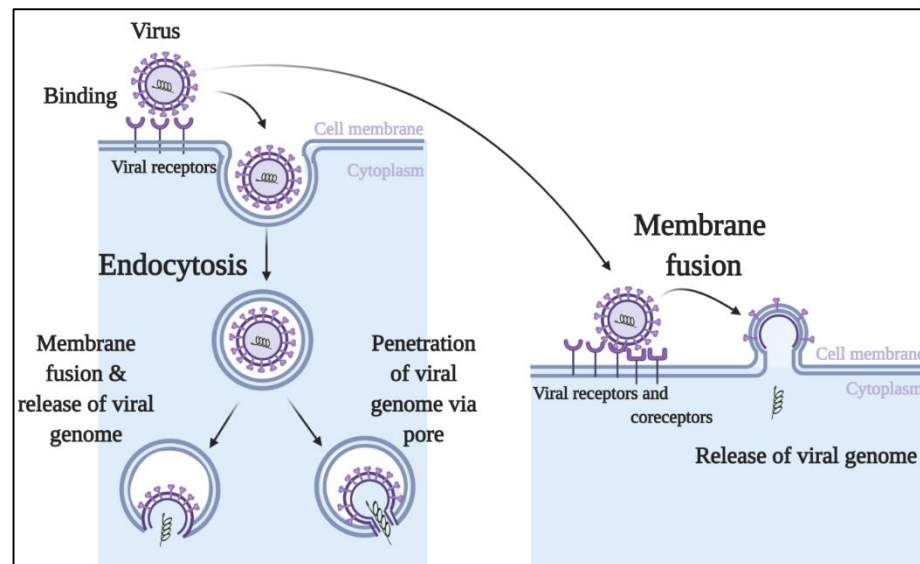


Figure 2. Viral attachment and entry into host cells. Viruses bind the receptors and coreceptors expressed on the host cell surfaces. Successful binding allows the viruses to enter the cells through the process of endocytosis followed by either further membrane fusion or formation of membrane pore (in the case of non-enveloped viruses) and direct membrane fusion. Once inside of the host cells' cytoplasm, the viral capsid is degraded and viral genetic material is released. The processes of viral replication, assembly and release of new virions start.

Replication and transcription of viral genome, followed by translation of viral mRNAs to produce proteins, provide all necessary components for assembly. Packaging signals or certain proteins that interact with viral genome with high specificity allow viral genome packaging into the capsid which in the case of enveloped viruses is further surrounded by an envelope (21–23). Assembled new virions are released from the host cells to infect new ones.

New virions are released from their specific host cells to be able to infect neighboring cells or new hosts and repeat the replication cycle (**Figure 3**). Some viruses are released after killing the host cells, while other viruses leave infected cells by budding through the membrane without killing the cells (20,23).

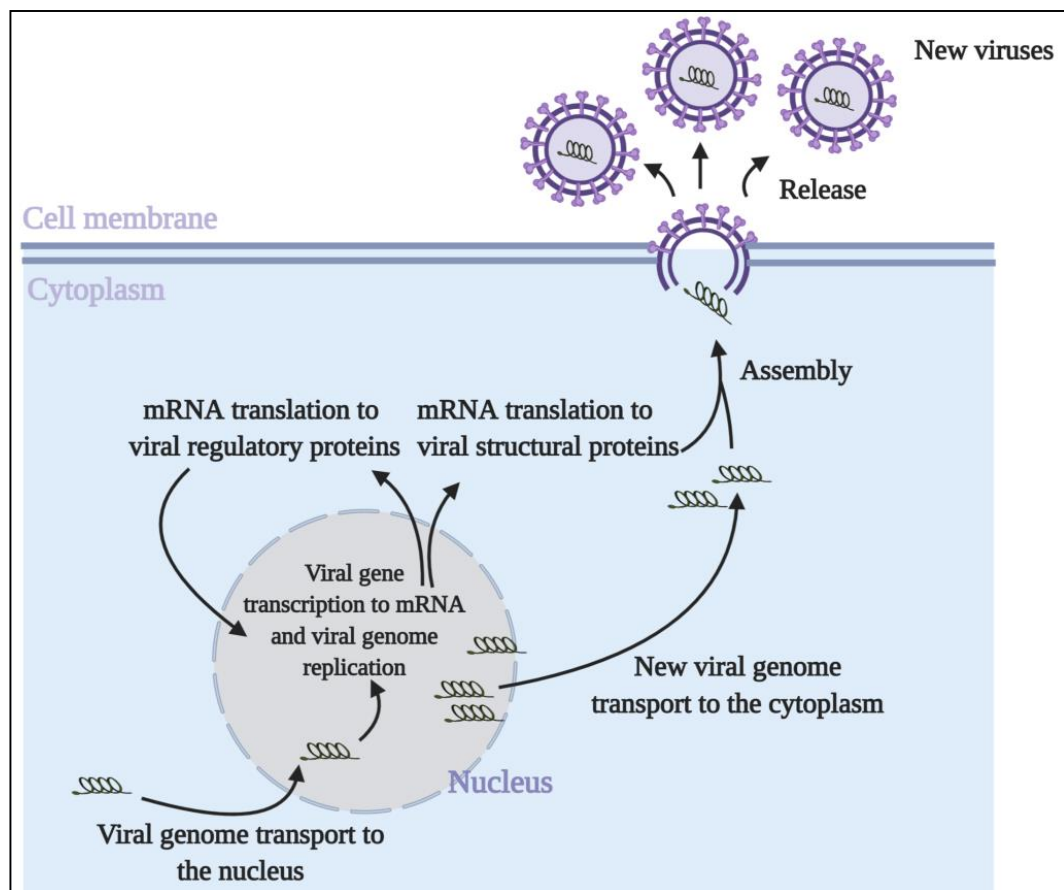


Figure 3. Viral replication, assembly and release. Following entry, viral genetic material in the cytoplasm is transported to the nucleus through host nuclear import machinery. With the help of host RNA polymerases and by synthesizing their own necessary enzymes, viruses produce new viral mRNA that is used to make structural proteins and regulatory proteins. Regulatory proteins translocate to the nucleus to further assist viral genome replication and synthesized viral genome is then transported to the cytoplasm, where the assembly process begins with viral genome packaging into viral capsid wrapped with an envelope in the case of enveloped virus. Finally, assembled new virions are released to infect new cells.

2. Human immunodeficiency virus type - 1

Human immunodeficiency virus type-1 (HIV-1) is the cause of the Acquired Immunodeficiency Syndrome (AIDS). HIV-1 was first isolated by research groups from Pasteur Institute in France and National Cancer Institute in USA in 1983 (24). HIV-1 belongs to the *Lentivirus* genus within the family of *Retroviridae* and subfamily *Orthoretrovirinae*. HIV-1 was transmitted to the human population from Central African chimpanzees in around 1920 (25,26). Since then more than 30 million people have died of AIDS. As of 2019, around 38 million people are living with HIV-1 and 1.7 million new infections occur annually in the world (UNAIDS, 2020), still remaining as a major health burden.

2.1. Structure of the HIV-1 virion

HIV-1 contains two positive-sense single strand RNAs encapsulated in a cone shaped capsid surrounded by an envelope. Also enclosed within the capsid are reverse transcriptase, integrase, and protease. The viral envelope contains an external surface glycoprotein (gp120) and a transmembrane glycoprotein (gp41) (**Figure 4**). HIV-1 genome is around 9.7 kB, flanked by a repeated sequence called long terminal repeats (LTR) at 5' and 3' ends. These LTRs are actively involved in the integration of viral genome into host chromosome and also serve as a promoter for the transcription of proviral DNA. HIV-1 genome has nine genes which code for the following 15 proteins (27,28) (**Figure 4**) :

Genes coding for structural proteins:

- *gag* (group-specific antigen) : codes for matrix protein (MA, p17), the capsid protein (CA, p24), and the nucleocapsid proteins (NC, p6 and p7).
- *pol* (polymerase): codes for reverse transcriptase (RT), protease (PR) and integrase (IN).
- *env* (envelope): codes for gp160, which is the precursor of the gp120 and gp41 proteins.

Genes coding for regulatory proteins:

- *tat* (transactivator of transcription): codes for Tat protein
- *rev* (RNA splicing-regulator): codes for Rev protein

Genes coding for accessory proteins:

- *nef* (negative regulating factor): codes for Nef protein
- *vif* (viral infectivity factor): codes for Vif protein
- *vpr* (virus protein r): codes for Vpr protein
- *vpu* (virus protein unique): codes for Vpu protein

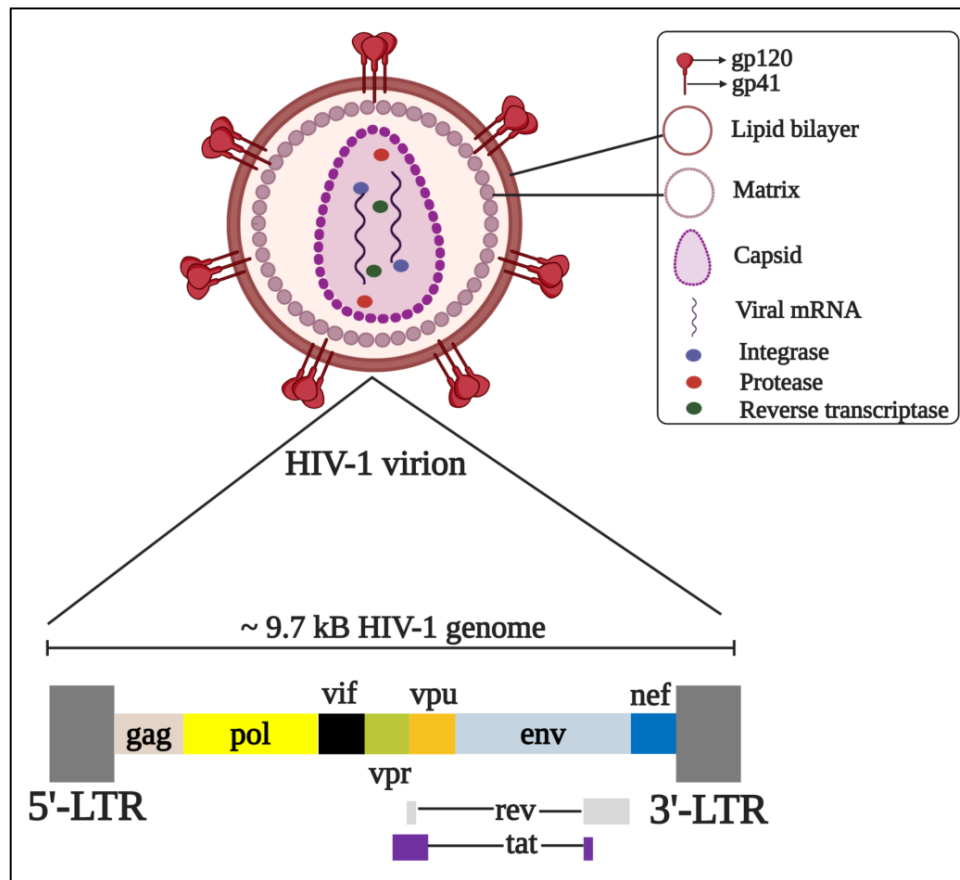


Figure 4. Structure of HIV-1 virion. HIV-1 virion consists of two single strand RNAs which are encapsulated in a capsid along with integrase, protease and reverse transcriptase. Matrix layer targets the RNA to the plasma membrane and mediates incorporation of envelope containing external surface glycoprotein (gp120) and a transmembrane glycoprotein (gp41). HIV-1 genome (9.7 kb) consists of 9 genes flanked by LTR (long terminal repeat) sequences at 5' and 3' ends.

2.2. Life cycle of HIV-1

Like many other viruses after infection HIV-1 viral particles proceed through the stages of attachment, entry, transcription, translation, assembly and release, with two more extra steps characteristic of the *Retroviridae* viruses family - reverse transcription and integration – to complete a life cycle (**Figure 5**).

Main targets of HIV-1 are T cells, macrophages and dendritic cells (DCs), because they express viral receptors essential for HIV-1 attachment (29,30). Envelope protein gp120 first binds to cluster of differentiation 4 (CD4) receptors, which causes a structural change in gp120 generating a binding site either for the C-C chemokine receptor type 5 (CCR5) or for the C-X-C chemokine receptor type 4 (CXCR4) co-receptors. Binding of the co-receptor causes further structural changes in gp41 protein, which triggers the

viral entry process (31,32). A series of conformational changes in gp41 protein caused by the trimeric complex of gp120/CD4/CCR5 or by CXCR4 expose the fusion domain on the N-terminus of gp41, which triggers the direct fusion of viral and cellular membranes and destabilizing the host cell membrane (32–34). Although main entry of HIV-1 is mediated by CD4/CCR5 or CXCR4, CD4/co-receptor independent entry into other host cells such as renal epithelial cells and astrocytes also takes place, which can occur either through direct cell-cell interaction or through certain receptors like C-type lectin DEC-205 and human mannose receptor (35–38).

After successful entry, the capsid disassembles (a process known as uncoating), single stranded viral RNA is released into the cytoplasm where it is converted into double stranded DNA (dsDNA) by the reverse transcriptase. Uncoating probably occurs in the cytoplasm in coordination with reverse transcription or at the nuclear envelope during nuclear import (39). Subsequently viral dsDNA uses the host nuclear import machinery to move to the host cell nucleus, where it integrates into the host DNA with the help of an integrase coded by HIV-1. Integrated viral DNA can also remain latent, without making new copies of HIV-1, which is also forming a barrier for successful treatment of HIV-1 infected patients (40–44). Remarkably, recent studies revealed that intact viral cores can enter to the nucleus and uncoat just before the integration to their chromosomal integration sites (39,45). Integrated dsDNA, also called proviral DNA, continues to transcribe the viral genome and encode necessary viral proteins.

Integrated proviral viral DNA uses host transcriptional machinery along with viral regulatory proteins to synthesize RNA copies of HIV-1 genome and viral mRNA, which is subsequently translated by host translational machinery into viral proteins. Transcription of the viral genome starts from 5' LTR region of the proviral DNA that acts as promoter which has binding sites for host transcription factors. Host transcription factors, however, mostly lead to the generation of short transcripts. Therefore, HIV-1 needs a viral protein called Tat (transactivator of transcription) that binds to transactivation response (TAR) RNA element which is located at the 5' end of all nascent viral transcripts and robustly increases the efficiency of polymerase II to make full-length viral transcripts (46).

After splicing three types of transcripts are formed: unspliced RNA, partially spliced RNA, and fully spliced RNA. Unspliced and partially spliced mRNAs are exported to the cytoplasm via Rev protein (regulator of expression of virion proteins), which binds to the unspliced intron sequences of viral mRNA and further connects with conventional mRNA export machinery, while fully spliced mRNAs are directly transported to the cytoplasm by the export machinery. In the cytoplasm, transported mRNAs are translated into viral proteins by host translational machinery. HIV-1 viral proteins can be classified as structural proteins (comprise virion particles), regulatory proteins (involved in transcription and translation process), and accessory proteins (not required for replication but critical virulence factors) (47–49).

Assembly of HIV-1 virion takes place at the plasma membrane, where the precursor polypeptides of Gag and Gag-Pol interact between themselves via Gag proteins to form an assembly platform. The Gag precursor has several domains namely matrix (MA), capsid (CA), nucleocapsid (NC) and p6 domains, along with two other spacer peptides SP1 and SP2. The MA domain of Gag binds to the plasma membrane and at the same time recruits Env glycoproteins while its CA domain promotes Gag multimerization and forms conical capsid. Two copies of viral mRNA are also recruited to the assembly platform via NC domain of Gag. At last, Gag p6 domain brings Vpr along with the ESCRT factors (endosomal sorting complexes required for transport) to sites of assembly which promotes membrane fission to facilitate the release of nascent viral particle, but at this stage it is an immature and noninfectious particle characterized by a thick layer of Gag and Gag-Pol precursors. Therefore, during or shortly after budding, viral protease is activated and cleaves Gag and Gag-Pol precursor, which results in mature infectious particle (virion) (50–52).

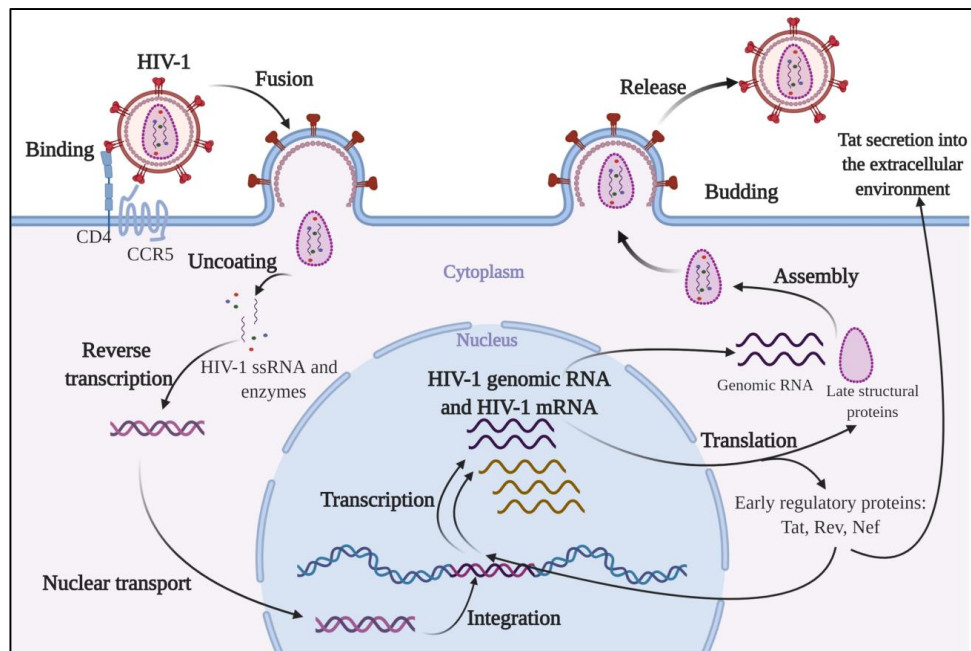


Figure 5. Life cycle of HIV-1. HIV-1 binds its receptors and co-receptors enabling viral fusion with cellular membrane and subsequent uncoating of its single strand RNA and associated enzymes (integrase, protease, and reverse transcriptase). With the help of reverse transcriptase, viral RNA is reverse transcribed into double stranded DNA that is transported into the nucleus by nuclear import machinery. In the nucleus, viral double stranded DNA is integrated into the host genome by integrase. After integration, a proviral DNA is transcribed into mRNA and transported into cytoplasm. A fraction of this mRNA pool serves as HIV-1 genomic RNA, another fraction is translated into early regulatory proteins and structural proteins. Regulatory proteins go back to the nucleus to increase the efficiency of proviral DNA transcription. Coordinated interactions between genomic RNA and all necessary HIV-1 proteins initiate the assembly of HIV-1 virion which is then released into the extracellular environment through budding process following virion maturation.

3. HIV-1-Tat

HIV-1 transactivator of transcription (Tat), is a HIV-1 regulatory protein essential for viral replication, establishment of infection and virus reactivation (53–57).

3.1. Structure of Tat

Tat is a small protein of 86-102 amino acids (aa) depending on the viral strains with a predicted molecular weight of 14-16 kDa. The 86 aa version of Tat is commonly referred to as the “full-length” Tat. Tat mRNA is composed of two exons (**Figure 6**). The first exon (aa 1-72) is highly conserved, while the second exon (aa 73-102) is less conserved. Tat protein has several domains which are mainly coded by the first exon. The first domain contains a proline rich acidic N-terminus (aa 1-21), a cysteine-rich region (aa 22-37), and a hydrophobic core region (aa 38-48), which are important for protein-protein interactions. Second domain is an arginine-rich basic domain (aa 49-58) comprising the protein transduction domain (PTD), the nuclear localization signal (NLS) and RNA binding domain (RBD). Glutamine-rich domain is required for Tat binding to TAR element and is important for the apoptotic function of Tat. Finally, C-terminal domain coded by the second exon contains arginine-glycine-aspartic acid (RGD) sequence (aa 73-86) necessary for Tat interaction with cell surface receptors (56,58) (**Figure 6**). Tat is a disordered protein, which gets more ordered structure through conformational changes following interaction with its target partners (59).

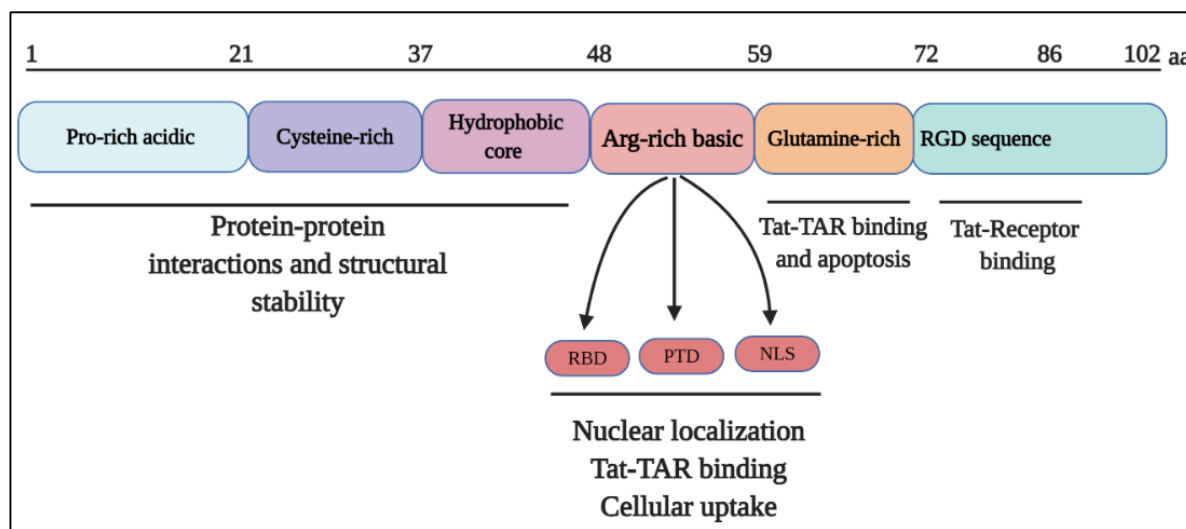


Figure 6. Structure of HIV-1 Tat protein.

3.2. Functions of Tat

Tat can be active both in cytoplasm and nucleus, but its main localization is in the nucleus. Two properties of Tat, the small molecular weight and NLS sequence, allows Tat to shuttle between the nucleus and the cytoplasm (**Figure 7**). Its small molecular weight assures the passive diffusion through nuclear pore complexes, while NLS sequence enables its nuclear import through classical nuclear import pathway (60). Tat can also be differently distributed within the nucleus itself. When Tat is abundant it localizes into the nucleoli, otherwise it localizes in the nucleoplasm (61).

3.3. Tat in HIV-1 transcription

Efficient HIV-1 gene expression depends on Tat. In the absence of Tat, viral gene transcription starts normally, but short, abortive viral transcripts are generated due to the inefficient activity of polymerase II. In order to overcome such unproductive transcription process, Tat expression starts early. Abortive viral transcripts are subsequently spliced and translated into Tat protein. Newly synthesized Tat enters to the nucleus (**Figure 7**) and directly binds to cyclin T1 (CycT1) subunit of pTEFb kinase complex consisting of CycT1 and cyclin-dependent kinase 9 (CDK9). Tat-pTEFB complex binds to TAR element in a Tat-dependent manner (62), CDK9 subunit of the complex phosphorylates C-terminal domain of RNA Polymerase II increasing its activity (63). pTEFB complex also recruits TATA box binding proteins to the LTR promoter while Tat recruits chromatin-modifying proteins to the promoter region, which dramatically promotes the transcription of HIV-1 RNA and stimulates the assembly of new transcription complexes (64,65).

3.4. Tat in host cell gene expression

Tat not only induces viral response, it can also generate host cell response by regulating a large number of genes coding for cytokines, cell cycle-related proteins, surface and chemokine receptors, mRNA processing factors, and proteins, involved in autophagy, DNA damage, and apoptosis (**Figure 7**). Tat can interact with promoter regions of hundreds of genes, involved in development, proliferation, growth, cellular organization, and intracellular signaling (66), showing its ability to significantly regulate gene expression in the host (67,68). How Tat modulates host gene expression is still not completely clear, yet several mechanisms of gene expression regulation by Tat have been suggested: i) binding to the TAR-like sequences at the 5'-untranslated regions of mRNA, ii) binding to the promoter region of target genes, iii) interacting with various transcriptional regulator proteins (56,69). These actions of Tat are ultimately favored by a variety of cellular signaling pathways.

Genes with TAR-like stem-loop structure on the 5' untranslated region (5'UTR) are one of the “favorite” targets of Tat. For example, proinflammatory cytokines interleukin 1 beta (IL-1b), interleukin 6 (IL-6) and tumor necrosis factor beta (TNF-β) proteins are overexpressed, when Tat binds to corresponding 5'UTR and up-regulates promoter activity, thus further increasing their expression (66,70,71).

Tat can also act through a TAR-independent way, due to its ability to bind to the promoter region of genes. For example, Tat binds to the promoters of *PTEN* (phosphatase and tensin homolog) and *PP2* (protein phosphatase 2), two upstream inhibitors of AKT pathway, and boosts their expressions. As a result, the activity of AKT signaling pathway is decreased.

Increased activity of key transcription factors is another proposed mechanism of Tat action (**Figure 7**). Tat increases the activity of NF-κB transcription factor and further promotes NF-κB signaling which leads to the increased BAG3 protein level, resulting in autophagy induction (see below) (72). Tat-activated NF-κB transcription factor is also involved in DNA damage further causing aberrant chromosomal translocations (73). Recent study showed that transcription factors Slug, Snail, Twist1 (Twist-related protein 1) and ZEB1 (Zinc finger E-box binding protein 1) were activated by Tat following the phosphorylation of SMAD2 (SMAD Family Member 2) transcriptional modulator through TGF-β and MAPK signaling pathways promoting epithelial–mesenchymal transition of mucosal epithelial cells (74). Tat up-regulates the expression of HIF-1α transcription factor and further favors its interaction with the long non-coding RNA (lncRNA) BACE1-antisense transcript (BACE1-AS), forming a HIF-1α/lncRNABACE1-AS/BACE1 axis that resulted in the accumulation of a toxic amyloid protein which contributes to the progressions of HIV-associated neurocognitive disorders (HANDs) (75).

Not only Tat can promote the expression of transcription factors, it can also interact with them and modulate their binding to the promoter region of genes. For example, Tat interacts with Sp-family transcription factors (Sp-1, Sp-3) and alters their binding to the promoter of SOD2 (Superoxide Dismutase) gene that may lead to altered oxidative stresses (76). Tat is not always enhancing the activity of transcriptional regulators, but it also might have suppressive effects on them. Tat inhibits the activity of transcription factor Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) along with its target genes (77). Expression and transcriptional activity of transcription factors like E2F3 (E2F transcription factor 3) and CREB (CAMP-responsive element-binding protein) is also hindered by Tat, due to the activation of p53 signaling pathway (78). Down-regulation of CREB activity by Tat can also happen via AKT signaling pathway (79).

3.5. Tat secretion and penetration in bystander cells

Tat can be secreted by the productively infected cells, accumulated in the extracellular environment in a soluble form, and enter the neighboring cells through cell surface receptors (**Figure 7**). Tat concentration in blood of HIV-1-infected patients can be as high as 500 ng/ml (73). Tat secreted from HIV-infected cells can perturb both HIV-infected and uninfected bystander cells in the surrounding microenvironment. The mechanism of Tat secretion is poorly understood, as it lacks secretion sequences, but it seems that Tat is released by an unconventional secretion pathway different from the conventional ER-to-Golgi secretory pathway (80,81). Several Tat secretion pathways have been described: oligomerization-mediated pore formation, spontaneous translocation, and incorporation into exosomes (82).

Released Tat remains in a soluble form which can circulate in the blood and bind to the cell surface receptors to enter different cells. Secreted Tat can transduce many cell types (monocytes, macrophages, microglia, B cells, CD4 T lymphocytes, astrocytes, neurons, cardiomyocytes, endothelial cells) due to its protein transduction domain (**Figure 7**). Circulating Tat has been detected in cerebrospinal fluid, sera and tissues of HIV-infected individuals, even in patients with no detectable viral load (80,83–85). PTD of Tat can be used as a highly efficient drug delivery peptide (86,87). As it is able to cross the biological membranes, PTD is conjugated to other therapeutic molecules as cell penetrating peptide, delivering them to their difficult-to-access targets (88).

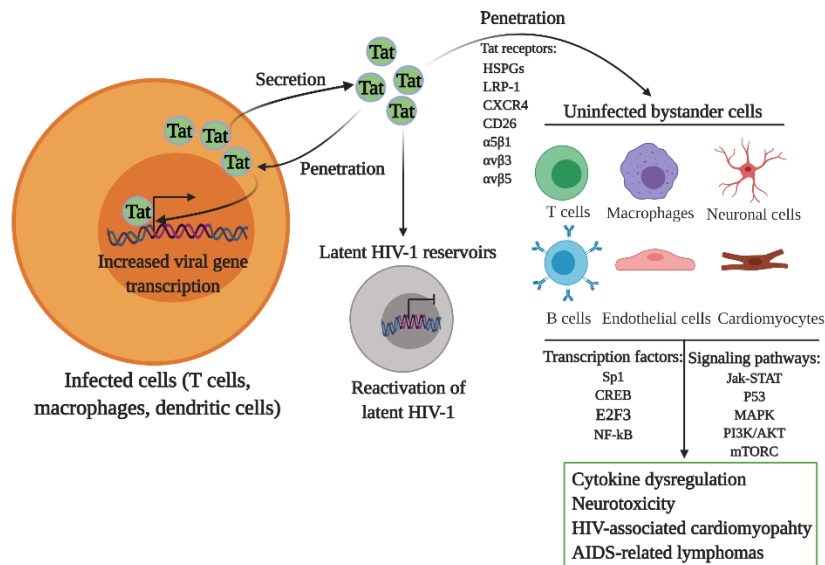


Figure 7. Tat regulates gene expression of host and bystander cells. Tat released from infected cells can go back to the nucleus of infected cells to promote HIV-1 gene transcription. Tat can reactivate latent HIV-1. Tat can enter uninfected cells through cell surface receptors. Tat regulates the expression of transcription factors and signaling pathways.

Tat entrance into bystander cells have been attributed to its different cell surface receptors - heparan sulfate proteoglycans (HSPGs), lipoprotein receptor-related protein (LRP), C-X-C chemokine receptor type 4 (CXCR4), dipeptidyl aminopeptidase IV (CD26), integrins ($\alpha 5\beta 1$, $\alpha v\beta 3$ and $\alpha v\beta 5$) (**Figure 7**) (83,89–92). Tat interacts with these receptors through its basic region and the RGD motif, which allows Tat to enter the cells *via* endocytosis (93). As receptors like CD26, the LRP and HSPGs are ubiquitous, they further increase the binding range of Tat. Moreover, as Tat receptors (LRP, CXCR4, and HSPGs) are endocytic receptors, they further facilitate the internalization of Tat (94). Once inside the host cell Tat enters the nucleus due to its nuclear localization signal and regulates the expression of host genes.

All together Tat is a flexible versatile small protein which is expressed in productively infected cells, and released into the extracellular environment. Tat may affect both infected and non-infected neighboring cells of any kind, which may influence the expansion of HIV-1 infection and trigger other diseases.

4. HIV-associated cancers and related diseases

Patients infected with HIV have a substantially elevated risk of developing so-called AIDS-defining cancers (Kaposi's sarcoma (KS), aggressive B-cell lymphoma and invasive cervical cancer) and non-AIDS defining cancers (Hodgkin lymphoma, anal cancer, liver cancer, and lung cancer) (**Figure 8**). Some of these cancers are caused by co-infection with other viruses, such as Epstein–Barr virus (EBV), human herpesvirus 8 (HHV8), human papillomavirus (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV) (95,96). Other than cancers, neuronal and kidney diseases related to HIV infection (HAND, HIV-associated neuronal disorders; HIVAN, HIV-associated nephropathy) are also among the major causes of mortality in people with HIV infection (97,98). Even though the introduction cART had dramatically reduced certain HIV-associated malignancies like KS, the incidence of AIDS-related lymphomas (ARL) has remained high in HIV patients (99). Thus, it is of great importance to understand the mechanisms of the development of HIV-1-related diseases, even in patients under cART.

Patients infected with HIV-1 are at high risk of developing both non-Hodgkin's (NHL) and Hodgkin's (HL) lymphomas collectively called ARL that are always of B cell origin. Some lymphoma types are common to both HIV-1 uninfected and infected patients in whom the incidence is much higher, while some other lymphomas preferentially develop in the context of HIV-1 infection and frequently tend to be associated with the co-infection with other oncogenic viruses, such as EBV and HHV8 (**Table 1**). ARLs can be divided into several subtypes: Diffuse large B cell lymphomas (DLBCLs), Burkitt's lymphomas (BL), PCNSL (primary central nervous system lymphoma), primary effusion lymphoma (PEL), plasmablastic lymphoma (PBL), lymphoma arising in KSHV-associated multicentric Castleman's disease (MCD).

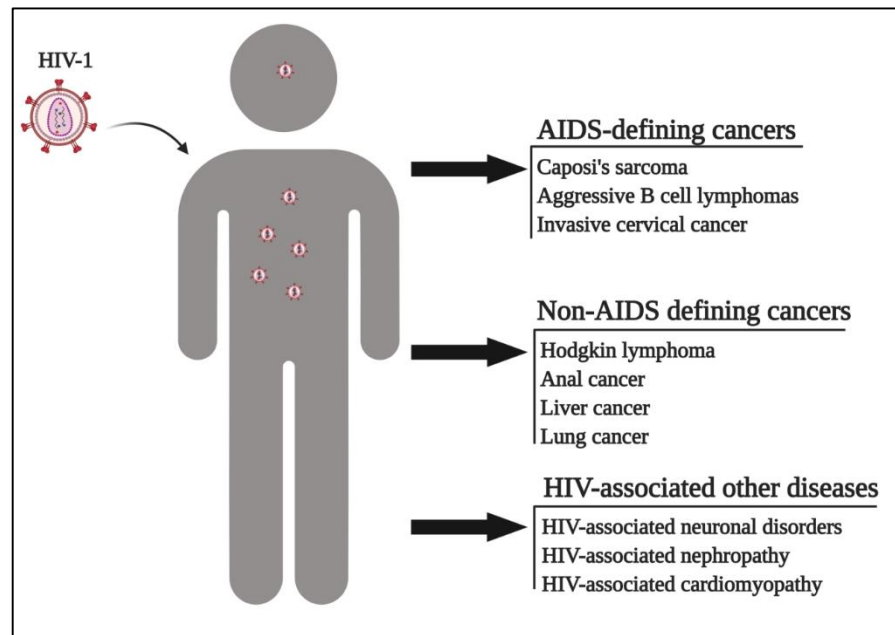


Figure 8. HIV-associated cancers and other diseases.

Though a decreased incidence of ARLs since the introduction of the HAART is reported in HIV-infected patients, ARLs still occur more frequently in HIV+ individuals than in general population. Moreover, survival rate of patients with NHL and HL is about two times lower than their HIV-uninfected counterparts (100–105).

Exact mechanisms of ARL pathogenesis are not very well understood, as B cells are not direct targets of HIV-1 and there is no evidence of B cell transformation due to HIV-1 infection. Several possible mechanisms, however, have been proposed: 1) secreted or transmitted viral proteins (Tat, p17) might have oncogenic effects on B cells (106,107); 2) aberrant somatic hypermutations of immunoglobulins might cause chromosomal translocations leading to malignant transformation (107); 3) infected T lymphocytes escaped from elimination in germinal centers could cause unusual B cell expansion (108); 4) chronic inflammation could lead to lymphoproliferation (109). These perturbations might generate an environment full of genetic abnormalities (chromosomal translocations, inactivation of p53, mutations), which might be further favored by co-infection with EBV and/or HHV8, leading to the emergence of ARL (**Figure 9**) (110–112).

Other possible mechanisms might involve deregulated cellular signaling pathways by HIV-1 or its viral proteins. Among many cellular signaling pathways, AKT/mTORC1 pathway plays an important role in ARL development, as various studies have shown the hyperactivation of this signaling pathway in different subgroups of ARL (106,113–118).

Table 1. AIDS-related lymphomas and associated oncogenic viruses

Name of lymphoma	Found in	Associated oncogenic virus
Diffuse large B cell lymphomas	HIV -/+ patients	Immunoblastic EBV 80-90% Centralistic EBV 30-40% (119)
Burkitt's lymphomas	HIV -/+ patients	EBV 25–40% (119,120)
Primary central nervous system lymphoma	HIV -/+ patients	EBV 80–100% (121)
Primary effusion lymphoma	Mainly HIV + patients	HHV8 (KSHV) 100% EBV 60–90% (122)
Plasmablastic lymphoma	HIV + patients	EBV 90–100% (123)
Lymphoma in KSHV-associated multicentric Castleman's disease	HIV+ patients	HHV8 100% (124)
Classical Hodgkin's lymphoma	HIV -/+ patients	EBV 80-100% (125)

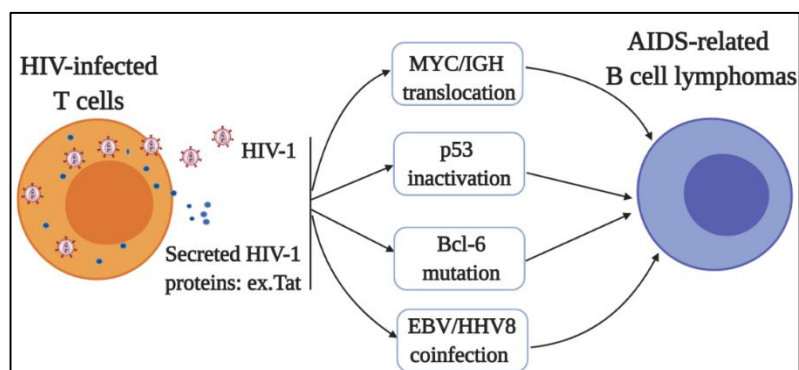


Figure 9. Mechanisms of development of AIDS-related B cell lymphomas. Secreted HIV-1 proteins e.g. Tat and infected T lymphocytes escaped from immune destruction cause a series of events including chromosomal translocations, inactivation of p53, mutations in Bcl6 gene, and coinfection with other viruses, which eventually lead to the development of AIDS-related B cell lymphomas.

5. The mammalian target of rapamycin complex

mTOR stands for the “mammalian (or mechanistic) target of rapamycin”, and, as the name indicates, its characterization is related to the discovery of rapamycin during a Canadian expedition to Rapa Nui island in 1964. Soil samples collected from this island had led to the isolation from the bacterium *Streptomyces hygroscopicus* of a macrolide having antifungal activity. Thus, the name “rapamycin” was given to this molecule because of the relation to Rapa Nui island (126). Later rapamycin was also found to have immunosuppressive and antitumor effects (127,128). Its mechanism of action remained unanswered till early 1990s when Michael Hall and his colleagues identified in *Saccharomyces cerevisiae* yeast cells two genes related to the rapamycin resistance, *TOR1* (target of rapamycin 1) and *TOR2* (target of rapamycin 2) (129). In 1994 mammalian orthologue of yeast TOR was identified (130–135). Even though two *TOR* genes exist in yeast *S.cerevisiae*, there is only one *TOR* gene in higher eukaryotes (136). In human *TOR* gene is localized to the chromosome region 1p36.22. Different groups that identified TOR gave different names to this mammalian orthologue of yeast TORs: FRAP (FK506-binding protein-rapamycin-associated protein, RAFT (rapamycin and FK506-binding protein target), RAPT (rapamycin target) and mTOR (mammalian TOR). The name mammalian TOR or mTOR was prevailed in the TOR community. In 2009, however, modification 'mechanistic TOR' or MTOR was adopted by the HUGO gene nomenclature committee as the official name (137).

mTOR protein (~280 kDa) is an evolutionarily conserved serine-threonine protein kinase, which belongs to the phosphatidylinositol 3-kinase related kinase family. mTOR has several conserved structural domains: 20 tandem HEAT repeats on the N-terminus followed by focal adhesion targeting (FAT) domain, kinase domain, FKBP-rapamycin-binding (FRB) domain and FAT carboxy-terminal (FATC) domain (**Figure 10**). Rapamycin together with FKBP12 binds to the FRB domain of mTOR and thus inhibits its activity (135).

mTOR kinase forms two different macromolecular protein complexes mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (**Figure 10**), which differ in their composition, downstream targets and regulation. mTOR complex 1 (mTORC1) comprised from three core components - mTOR, regulatory-associated protein of mammalian target of rapamycin (Raptor), mammalian lethal with sec-13 protein 8 (mLST8, also known as GβL) – and two inhibitory subunits: proline-rich AKT substrate 40 kDa (PRAS40) and DEP domain containing mTOR-interacting protein (DEPTOR). Raptor strongly interacts with N-terminal region of mTOR (138), but also binds S6K1 (Ribosomal protein S6 kinase beta-1) and 4E-BP1 (Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1), substrates of mTORC1, through their TOR signaling motifs that further ensures an efficient substrate phosphorylation event (139). mLST8 maintains the stability of mTOR/Raptor interaction and promotes the kinase activity of mTOR (140). Interaction of PRAS40 with mTOR is mediated by Raptor, but PRAS40 inhibits mTOR autophosphorylation

and further inhibits its kinase activity (141). Second inhibitory component DEPTOR through its PDZ domain binds to FAT domain of mTOR which sequesters mTORC1 away from its active pool and leads to the inhibition of mTOR kinase activity (142,143).

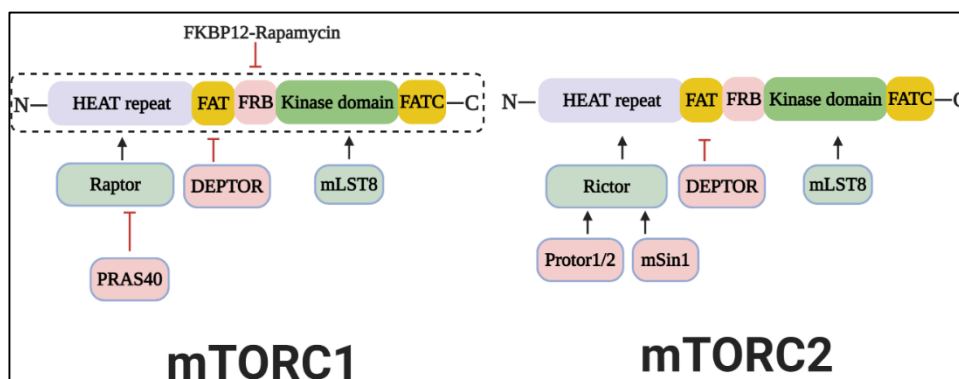


Figure 10. Structure of mTOR protein and its complexes. mTOR is comprised of HEAT repeat, FAT and FRB, kinase and FATC domains. FKBP12-Rapamycin complex binds mTOR through FRB domain and inhibits the activity of mTORC1. mTORC1 is comprised of Raptor, mLST8, PRAS40, and DEPTOR that interact with mTOR protein. Raptor interacts with N-terminal region and mLST8 - with kinase domain of mTOR, while interaction of the inhibitory subunit PRAS40 is mediated by Raptor and secondary inhibitory component DEPTOR interacts with FAT domain. DEPTOR, mLST8, Rictor, mSin1, and Protor 1/2 interact with mTOR to form mTORC2.

mTOR complex 2 (mTORC2) consists of six proteins: rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated map kinase-interacting protein 1 (mSin1), protein observed with Rictor (Protor) 1/2, and mTOR, mLST8, DEPTOR commonly shared between two complexes (144–146).

mTORC1 is sensitive, while mTORC2 is much less responsive to the rapamycin (Sirolimus®), which suppresses T and B cell activation by inhibition of the cell cycle. Various analogues of rapamycin, so called rapalogues (Everolimus®, Temsirolimus®), are also frequently used in clinics for immunosuppression. In addition, a number of alternative mTOR inhibitors have been developed. These inhibitors block both mTORC1 and mTORC2 (pan-inhibitors or TOR-KIs, *i.e.*, INK128) or act on mTOR kinase and another protein (dual inhibitors), most often targeting a network upstream of mTORC1/2 (147).

Rapamycin first forms a complex with FKBP12, which then directly binds to the FRB domain of mTOR (**Figure 10**). This interaction results in the changes in FRB domain, thereby allosterically inhibiting the kinase activity of mTOR (135,148), challenged by *in vitro* studies describing that rapamycin inhibits mTOR function by dissociating Raptor from mTOR but without changing its kinase activity (149), as Raptor plays essential role in mTORC1 activation (150). Recent 3.2 Å resolution structure of mTORC2 revealed that

C-terminal domain of RICTOR masks FRB domain in mTOR, explaining the rapamycin insensitivity of mTORC2 (151).

mTORC2 regulates many biological processes including cell survival, proliferation, metabolism, and cytoskeletal organization. Growth factors are found to be the most important upstream signals to activate mTORC2. AKT, serum- and glucocorticoid-induced protein kinase 1 (SGK1) and protein kinase C (PKC) are the best characterized substrates of mTORC2. mTORC2 regulates cell survival, proliferation and metabolism mainly through AKT and SGK1. mTORC2 phosphorylates AKT on Ser473 (**Figure 11**) and SGK1 on Ser422 (152–155). mTORC2 regulates FoxO transcription factors which are known substrates of AKT and SGK1 (154,156,157). Besides, mTORC2 controls cytoskeletal organization through PKC (158). Activation of AKT is also found to be necessary for the induction of cytoskeletal reorganization by mTORC2 (159).

mTORC1 integrates signals from many intracellular and extracellular cues: growth factors, amino acids, energy, oxygen, DNA damage and infectious agents, including viruses. Depending on the nature of the signal, its duration, cell type and many other factors, mTORC1 will “determine” the subsequent cell fate.

5.1. Upstream regulation of mTORC1

5.1.1. PI3K-AKT signaling to mTORC1

Growth factors, cytokines, immune cell receptors (e.g. TCR) and co-receptors activate mTORC1 through PI3K-AKT signaling pathway (160,161). The phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that phosphorylate phosphatidylinositol and phosphoinositides to activate many intracellular signaling pathways (162). PI3K is activated by signals from growth factor receptor tyrosine kinases (RTKs). In response to growth factor stimulation and the subsequent activation of RTKs, its substrate phosphatidylinositol 4,5-bisphosphate (PIP2) at the plasma membrane is converted to phosphatidylinositol-3,4,5-trisphosphate (PIP3), which in turn activates downstream signaling pathways (**Figure 11**). On the other hand, the cellular level of PIP3 is tightly regulated by phosphatase and tensin homolog (PTEN), a tumor suppressor, which antagonizes PI3Ks activity by converting PIP3 back to PIP2 via its intrinsic lipid phosphatase activity (**Figure 11**) (163).

PIP3 acting as second messenger activates, among multiple downstream signaling pathways, the protein serine/threonine kinase AKT (also known as PKB). Binding of PIP3 to AKT leads to its membrane recruitment. Subsequently Thr308 and Ser473 residues are phosphorylated by distinct kinases. Thr308 is phosphorylated by 3-phosphoinositide-dependent kinase 1 (PDK1), while Ser473 is phosphorylated by mTORC2. In the response to DNA damage AKT is phosphorylated at Ser473 by three major DNA damage sensors - ATM, ATR and DNA-PK kinases (see below). Once phosphorylated and activated, AKT in its turn

either activates or inhibits many downstream proteins such as glycogen synthase kinase 3 (GSK3), the forkhead family of transcription factors (FOXOs), nuclear factor of κ B (NF- κ B), and mouse double minute 2 homolog (MDM2) as well as various signaling pathways including mTORC1 pathway (see below) (**Figure 11**), thereby regulating a wide range of cellular functions including protein synthesis, cell survival, proliferation, and metabolism (164).

Active AKT phosphorylates and inactivates TSC2 component of TSC (Tuberous Sclerosis Complex) consisted of TSC1, TSC2, TBC1D7 (**Figure 13**) (165). TSC2 has a GTPase activating protein activity (**Figure 12**) for the small GTPase RHEB (Ras homologue enriched in brain), converting RHEB-GTP to the inactive RHEB-GDP. In its GTP form, RHEB is an activator of mTORC1. Thus, through its GAP activity, TSC2 represses mTORC1 by inactivating RHEB. Phosphorylation of TSC2 by AKT relieves this inhibitory action on mTORC1 (166). AKT can also activate mTORC1 directly, in a TSC-independent way, by phosphorylating and inactivating the mTORC1 inhibitory component PRAS40 (**Figure 13**) (167).

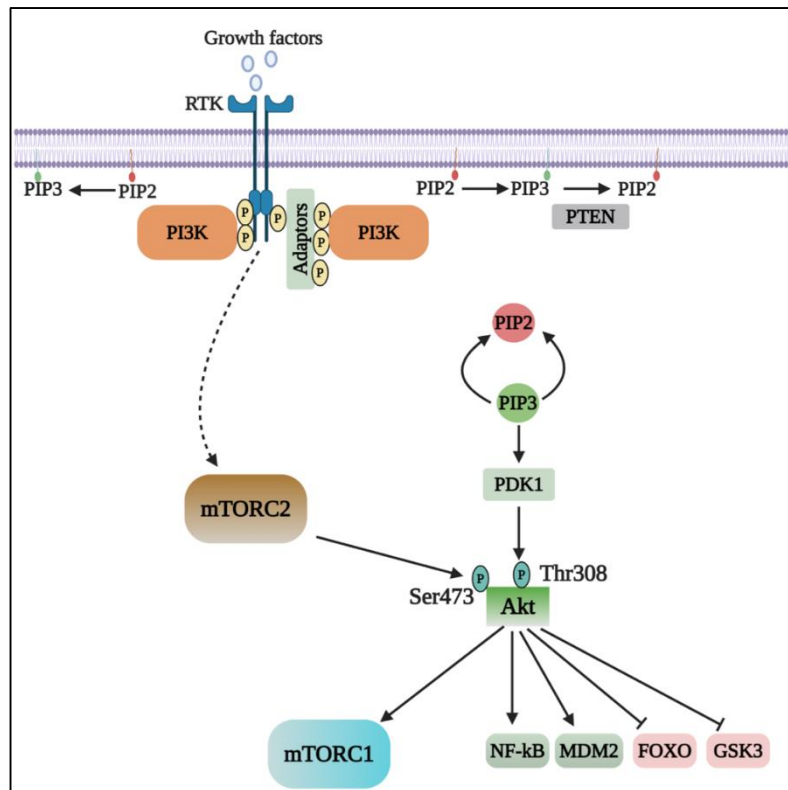


Figure 11. PI3K-AKT signaling pathway. Class IA PI3K receives signals from growth factors via receptor tyrosine kinase (RTK). Catalytic p110 subunit converts PIP2 to PIP3 which subsequently activates AKT by phosphorylating it on Thr308 via 3-phosphoinositide-dependent kinase 1 (PDK1) and on Ser473 via mTORC2. Phosphatase and tensin homolog (PTEN) antagonizes this activity by converting PIP3 back to PIP2. Phosphorylated AKT regulates a large number of downstream targets including mTORC1, nuclear factor of κ B (NF- κ B), mouse double minute 2 homolog (MDM2), forkhead family of transcription factor (FOXO), and glycogen synthase kinase 3 (GSK3).

5.1.2. Amino acid signaling to mTORC1

Nutrient sensing and response to nutrient availability is one of the principal functions of mTORC1. Transmission of information about presence and quality of nutrients requires the coordinated interaction of sensors and regulators through the vast, complex signaling network upstream of the mTORC1. I will outline the main principles of sensing and responding within the mTORC1 pathway on the example of amino acid signaling to mTORC1.

Amino acids enter the cells via specific transporters. For example, solute carrier family 7 member 5 (SLC7A5)/SLC3A2, a heterodimeric bidirectional transporter, transports extracellular leucine into the cells in exchange for intracellular L-glutamine (168). Within the cells, lysosome is considered as the key site of amino acid sensing. Lysosomes are the organelles responsible for degrading macromolecules which are exported to the cytosol and reused in the cellular metabolism. Even in the absence of extra cellular amino acids, mTORC1 can be activated by intracellular amino acid pools (169) which are most probably provided by lysosomes. Amino acid sensing is even believed to be initiated from within lysosomal lumen via so-called “inside-out mechanism” (170). mTORC1 can also sense amino acids through a number of individual amino acid sensors. For example, cytosolic leucine is detected by Leucyl-tRNA synthetase (LRS) (171) and Sestrin 2 (172–174). SLC38A9, a lysosomal amino acid transporter, is also a lysosomal arginine sensor (175). Cytosolic arginine in contrast is detected by CASTOR1 and CASTOR2 (Cellular Arginine Sensor for mTORC1 1 and 2) (176). Finally, SAMTOR (S-adenosylmethionine sensor upstream of mTORC1) is a S-adenosylmethionine sensor.

In order to be activated, mTORC1 needs to be located to the lysosomal surface, where RAG GTPase family proteins play a key role in mTORC1 activation. In mammals four Rag proteins, RAGA, RAGB, RAGC, and RAGD form heterodimers RAGA/B, RAGC/D (**Figure 13**). In order activate mTORC1 in the presence of amino acids, RAGA/B have to be bound with GTP, while RagC/D - with GDP (**Figure 13**). Rag GTPase activators - guanine nucleotide exchange factors (GEFs) - and inhibitors - GTPase-activating proteins (GAPs) - modulate GTP/GDP nucleotide bound state of each heterodimer (**Figure 13**).

A number of GAPs and GEF has been identified recently. For example, Folliculin (FLCN), in a complex with FLCN-interacting proteins (FNIPs) (177,178) exerts GAP activity toward RagC/D, thereby activating mTORC1 (179) (**Figure 13**).

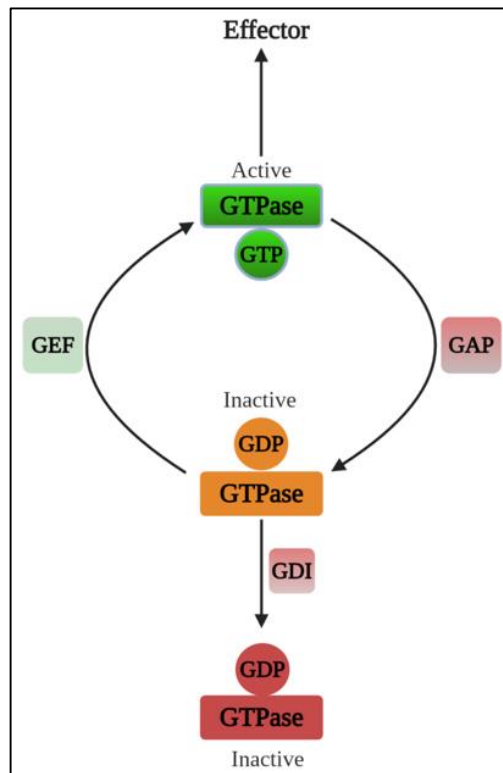


Figure 12. Small GTPases. GTPases are active when bound to GTP and inactive when bound to GDP. The activity of GTPases is regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and GDP dissociation inhibitors (GDIs). GEFs promote the exchange of GDP for GTP and GAPs hydrolyze GTP to GDP. GDIs inhibit GDP dissociation from GTPases.

The multiproteins complex called SEA in yeast and GATOR in mammalian cells has GAP activity towards RAGA/B thereby inhibiting mTORC1 (180,181). GATOR is comprised of two subcomplexes GATOR1 and GATOR2 (181). GATOR1, which has GAP activity towards RAGA/B, contains DEPDC5 (DEP domain-containing 5), NPRL2 (nitrogen permease regulator-like 2), and NPRL3 (nitrogen permease regulator-like 3) (**Figure 13**). GATOR2 consists of MIOS (meiosis regulator for oocyte development), WDR24 (WD repeat domain 24), WDR59 (WD repeat domain 59), SEH1L (Seh1 like nucleoporin) and SEC13 (Sec13 homolog nuclear pore and COPII coat complex component). GATOR2 activates mTORC1 by inhibiting GATOR1 via an unclear mechanism (**Figure 13**). GATOR1 can also interact with KICSTOR complex, composed of KPTN, ITFG2, C12orf66 and SZT2. In response to amino acid or glucose deprivation, KICSTOR localizes to the lysosomes, binds with GATOR1, and recruits it to the lysosomal surface, which leads to the inhibition of mTORC1 signaling (**Figure 13**).

GATORS are closely communicating with amino acid sensors to transfer the information about amino acid availability to mTORC1. In the absence of leucine, Sestrin2 binds to and inhibits GATOR2 which

further dampens mTORC1 activity (172–174) (**Figure 13**). In response to prolonged leucine deprivation mTORC1 presented triphasic response: an initial inhibition, a transient reactivation, and a subsequent inhibition. In the presence of leucine Sestrin2 liberates GATOR2 and activates mTORC1 (182,183). SAMTOR seems to need both GATOR1 and KICSTOR to exert its inhibitory activity against mTORC1 (**Figure 13**). CASTORs bind and inhibits GATOR in the absence of arginine to inhibit mTORC1 activity (**Figure 13**), and dissociate from GATOR2 in the presence of arginine enabling mTORC1 activation (176).

RAG GTPases are tethered to the lysosomal membranes with the help of a lysosome-resident pentameric protein complex Ragulator (184), which consists of p18(LAMTOR1), p14 (LAMTOR2), MP1 (LAMTOR3), C7orf59 (LAMTOR4)) and HBXIP (LAMTOR5) (184,185). The activation of RAGs leads to thier interaction with RAPTOR and subsequent recruitment of mTORC1 to the lysosome, where mTORC1 then fully activated by RHEB-GTP (184,186). When Ragulator components are depleted, RAGs and mTORC1 are no longer tethered to the lysosomal membrane resulting in mTORC1 inactivation (184). Ragulator also has GEF activity, towards RAGA/B, which occurs in amino acid- and vacuolar (V-)ATPase - dependent manner (**Figure 13**) (185). The v-ATPase, an ATP driven proton pump, plays important role in lysosomal lumen acidification (187). Accumulated amino acids inside the lysosomal lumen promotes v-ATPase-Ragulator interaction which further sends an activation signal to Rag GTPases to mTORC1 activation (**Figure 13**) (188).

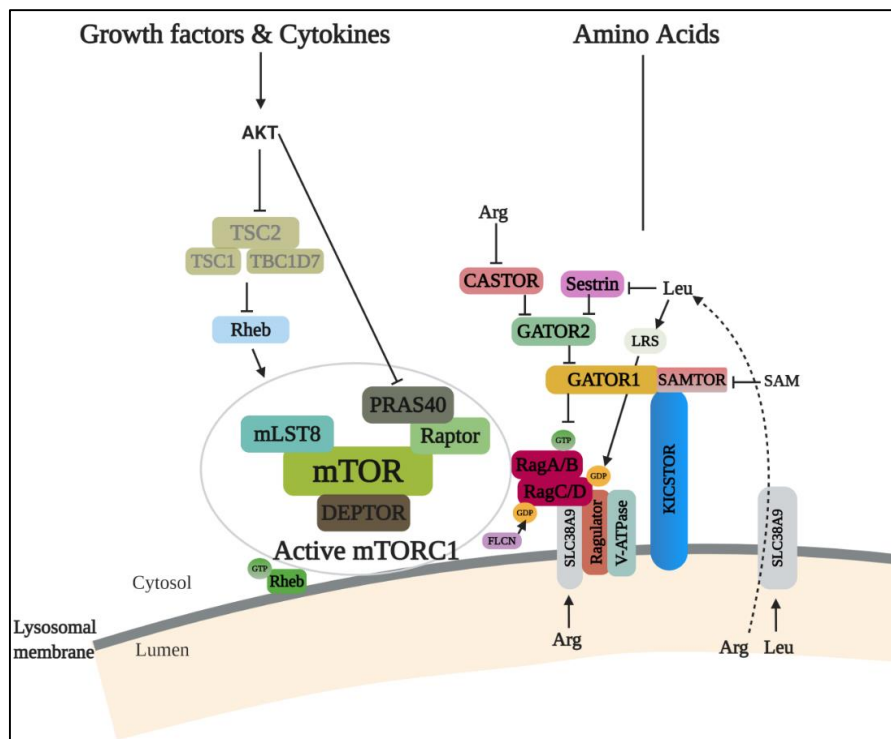


Figure 13. Regulation of mTORC1 by growth factors, cytokines and amino acids.

5.1.3. DNA damage response and mTORC1

A growing body of evidences indicates that mTOR signaling is closely related to another cellular protection mechanism – DNA damage response (DDR). Many factors important for DDR are also involved in the functioning of mTOR pathway.

mTOR belongs to the family of phosphatidylinositol 3-kinase related kinases (PIKKs). These giant (289 kDa – 470 kDa) Ser/Thr protein kinases do not have lipid kinase activity in contrast to other PI3Ks. Members of this family are mTOR, ataxia telangiectasia mutated (ATM), ataxia telangiectasia- and RAD3-related (ATR), DNA-dependent protein kinase (DNA-PK), human suppressor of morphogenesis in genitalia 1 (hSMG-1), and transformation/transcription associated protein (TRRAP) that is the only member without kinase activity (189,190). They share similar domain organization including kinase domain (except hSMG-1), FRAP-ATM-TRRAP (FAT) domain, the PIKK-regulatory domain (PRD) and the short FAT C-terminal motif (FATC).

ATM, ATR and DNA-PK collaboratively respond to DNA damage signals such as ionizing radiation (IR), chemotherapeutic drugs, reactive oxygen species (ROS) etc., which lead to single and double strand breaks (SSBs and DSBs) (191–195). DSBs also occur in the physiological conditions, for example during V(D)J recombination in B- and T- lymphocytes, which provides the basis for antigen binding diversity of immunoglobulins and T cell receptor proteins (196–198).

DSBs and SSBs trigger a series of post-translational modifications including, but not limited to phosphorylation, which are termed DNA damage responses (DDR). In DDR, recruitment of PIKKs to the sites of damage is the principal step of their activation. DNA-PK and ATM are recruited to DSBs, while ATR is recruited to SSBs. The kinases are further activated through their respective DNA-damage sensor proteins: Ku70/Ku80 heterodimer for DNA-PK, Mre11-Rad50-Nbs1 (MRN) complex for ATM, and ATR-interacting protein (ATRIP) for ATR (**Figure 14**). Once activated, they phosphorylated a large number of overlapping substrates that also include themselves (inter-molecular autophosphorylation and trans-phosphorylation) (191,199).

In response to DNA damage, cooperating checkpoint pathways are activated to block S-phase entry (the G1/S-phase checkpoint), to slow S-phase progression (the intra-S or S-phase checkpoint), or to prevent mitotic entry (the G2/M-phase checkpoint), which initiates repair mechanisms: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), DSB homologous recombination (HR) repair, or non-homologous end joining (NHEJ) (200). ATM, ATR, and DNA-PK often cooperate to regulate HR and NHEJ. If damage repair fails, checkpoints trigger cell death by apoptosis (201).

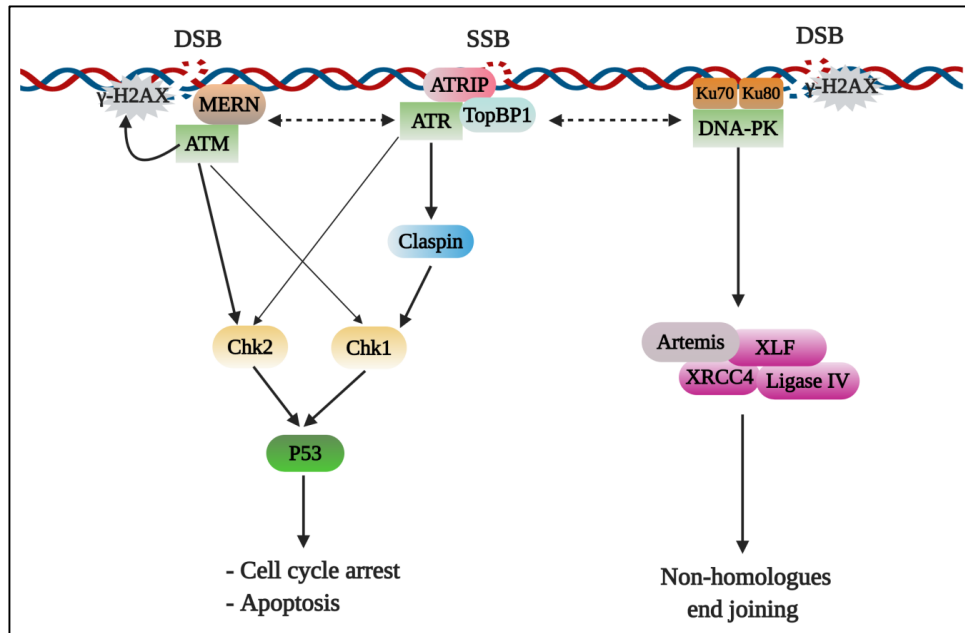


Figure 14. DNA damage signaling. ATM, ATR, and DNA-PK are key players in DNA damage signaling. In response to DSB and SSB, they are recruited to the damage sites through MRN, ATRIP, and Ku70/Ku80 respectively. After activation, ATM/Chk2 and ATR/Chk1 signaling cascades induce tumor suppressor p53, which leads to either cell cycle arrest or apoptosis. The non-homologues end joining NHEJ is controlled by DNA-PK together with XRCC4, XLF, DNA ligase IV and artemis.

ATM is activated by autophosphorylation and MRN interaction upon its recruitment to DSBs. After activation, ATM phosphorylates histone H2AX, forming the DNA damage-associated γ -H2AX histone, the golden marker of DNA damage (**Figure 14**) (202,203). ATM also phosphorylates and activates key downstream effector kinases, including a checkpoint kinase 2 (Chk2) at threonine 68 (204). Activated Chk2 phosphorylates Ser20 on p53 tumor suppressor, thereby activating G1 and S phase checkpoints (**Figure 14**) (205–207).

Unlike ATM and DNA-PK, ATR is recruited to the SSBs. Simple recruitment to the site is not sufficient for its optimal activation. DNA Topoisomerase II Binding Protein 1 (TopBP1) stimulates ATR kinase activity (**Figure 14**) (208). Activated ATR phosphorylates checkpoint kinase 1 (Chk1) at multiple sites, notably serines 317 and 345 (209,210). Chk1 activity is tightly associated with Claspin serving as mediator between ATR and Chk1. ATR-dependent phosphorylation of Claspin on its Chk1-binding domain facilitates its interaction with Chk1 leading to sustained Chk1 activation (**Figure 14**) (211). Activated Chk1 acts on the substrates involved in cell cycle control including Cdc25A, Cdc25C, and Wee1, decreasing the activity of cyclin-dependent kinase (CDK), thereby slowing cell-cycle progression (212–214).

The major role of DNA-PK in DDR is to initiate NHEJ repair of DSBs. DNA-PK gets activated when it is recruited to the site of DSBs through its Ku partner (215). Following the binding of Ku and DNA-PK complexes to DSBs, DNA-PK promotes DNA-end tethering and recruits additional NHEJ core factors XRCC4, XLF, and DNA ligase IV (216–219) (**Figure 14**). In addition, DNA-PK recruits other accessory proteins like Artemis, an endonuclease, to promote DNA-end processing (**Figure 14**) (220). In response to DNA damage, DNA-PK may also phosphorylates Chk2 at Thr68 residue *in vitro* (221). DNA-PK is required for the maintenance of Chk1-Clapsin stable interaction and absence of DNA-PK compromises Chk1 activity (222).

Phosphorylation of Chk1 by ATR and Chk2 by ATM, are not independent branches of the DNA damage response. Instead they demonstrate a high degree of cross-talk and connectivity where Chk1 and Chk2 phosphorylation can be triggered by DNA-PK, ATR and ATM (221–224). Moreover, DNA-end resection resulted from ATM-dependent DDR generates single stranded DNA signals for ATR which is translated as ATM-dependent ATR activation (225). ATR can phosphorylate ATM in response to UV exposure (226) and phosphorylation of ATM by DNA-PK is also described (227). Both ATM and ATR can phosphorylate DNA-PK in their turns (228,229) (**Figure 14**).

Activation of ATM in response to ROS (Reactive oxygen species) (230,231) or under hypoxic conditions (232) leads to repression of mTORC1. The transduction of the signal from ATM to mTORC1 is mediated *via* the AMPK (AMP-activated protein kinase) pathway, through TSC (233–237). DDR to DSBs mediated by ATM or ATR can transiently induce mTOR protein levels and kinase activity, although prolonged treatment with DSB-inducing drugs results in mTORC1 inhibition (238). Inactivation of mTOR with siRNA or its pharmacological inhibition with dual mTORC1/2 drugs inhibits S and G2/M cell cycle arrest provoked by a DSB-inducing agent etoposide. Interestingly, this effect is mediated by CHK1 activation and is dependent on mTORC2, while mTORC1 is dispensable for this process in breast cancer cells (239). Another mechanism of mTORC1 checkpoint regulation might involve the GATOR1 complex. Overexpression of the GATOR1 component NPRL2 leads to G1/S arrest in p53 positive cells and to CHK2-dependent S or G2/M arrest in p53 negative cancer cell lines (240). Finally, the connection between ATM and mTORC1 is even more complex, since mTORC1 can also negatively regulate ATM. A negative feedback loop between ATM and the mTORC1 pathway was described in the context of childhood sarcoma (241). mTORC1 suppresses ATM via S6K1/2 signaling by up-regulating two miRNAs (miR-18a and miR-421) targeting *ATM* mRNA. These miRNAs are under the control of the MYCN transcription factor, and one of the mechanisms by which mTORC1 can suppress ATM is through sustaining of MYCN by S6K1 signaling. mTORC1-S6K1 signaling can also control transcription of *CHK1* by regulating cyclin dependent kinases D and E. Recent studies demonstrate that mTOR inhibition results in downregulation of CHK1 and slow S-

phase progression following DNA damage both in cell culture and mouse rhabdomyosarcoma xenografts (242). These results suggest that when mTOR is not suppressed by DNA damage (e.g. in cancer cells), CHK1 activity is maintained and may contribute to survival.

p53 is one of the most powerful mTORC1 antagonists. In response to genotoxic stress, p53 suppresses cellular proliferation and restrains mTORC1 to allow activation of the DDR. Conversely, in a negative feedback loop, mTORC1 can increase p53 activity when energy levels are high. p53 phosphorylation by Chk2 and Chk1 enables its stabilization and activation. Activated p53 moves to the nucleus and targets multiple genes including AMPK regulatory subunit β , PTEN, and TSC2, which all negatively regulate mTORC1 (**Figure 15**) (243).

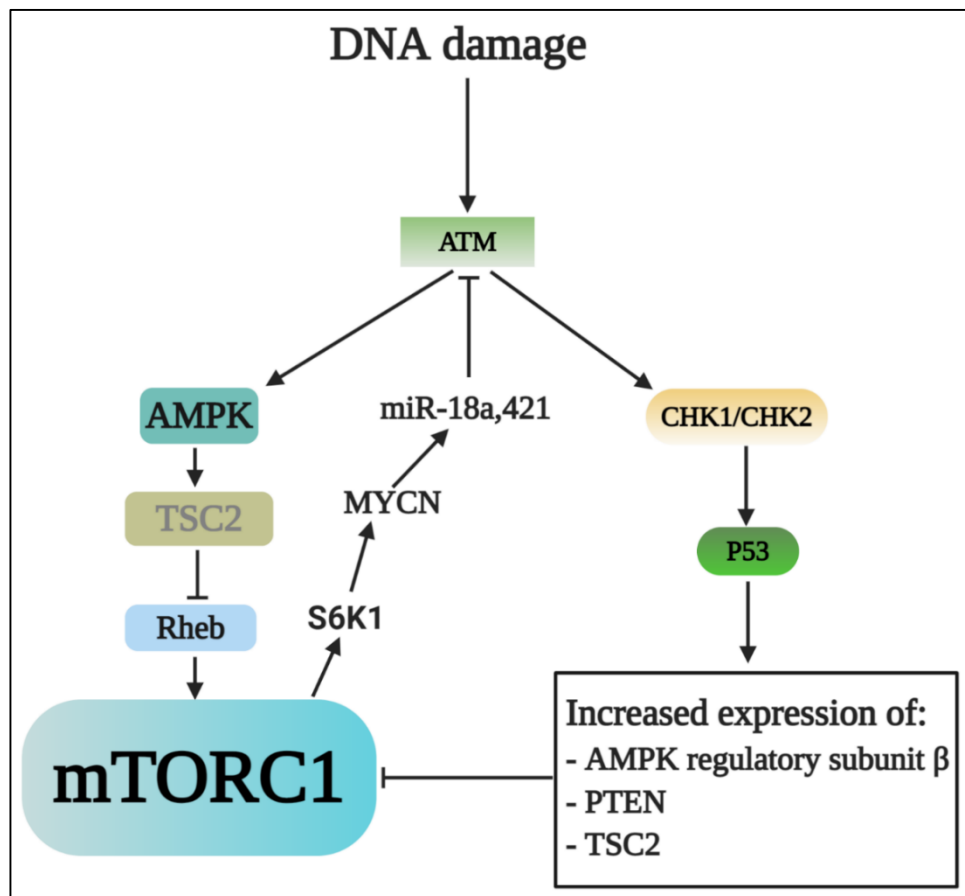


Figure 15. Regulation of mTORC1 by DNA damage. In response to the DNA damage ATM activates AMPK pathway which increases the activity of TSC, resulting in the inhibition of mTORC1. CHK1/CHK2 signaling kinases increase p53 activity and promote inhibitory signal towards mTORC1 through AMPK regulatory subunit β , PTEN and TSC2. A negative feedback loop from mTORC1 passes through S6K1 which promotes MYCN activity leading to the upregulation of miR-18a and 421 inhibiting ATM.

5.2. Downstream targets of mTORC1

In response to the signals which are delivered by its upstream regulators, mTORC1 phosphorylates its downstream substrates which participate in the synthesis of three major cell constituents: proteins, nucleotides, and lipids, favoring a growth regime featured by an increase in cell size as well as in cell number (244–246).

Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and 70 kDa ribosomal protein S6 kinase 1 (p70S6K, also known as S6K1) are the key targets phosphorylated by mTORC1 (**Figure 16**). Phosphorylation of p70S6K was the first observation linking the mTORC1 signaling to the amino acid signaling (247,248). Later studies identified a TOR signaling (TOS) motif in the N-terminus of p70S6K and in the C-terminus of 4E-BP1. RAPTOR component of mTORC1 binds to TOS motif and functions as a scaffolding protein, bringing mTOR in close proximity to 4E-BP1 and p70S6K to drive their phosphorylation at multiple sites (249,250). 4E-BP1 has six phosphorylation sites, among them Thr37, Thr46, Ser65, and Thr70 are known to be phosphorylated by mTORC1 (251,252). Phosphorylation by mTORC1 inhibits 4E-BP1, which promotes its dissociation from translational initiation factor eIF4E, allowing 5' cap-dependent mRNA translation (253). S6K1 phosphorylation by mTORC1 stimulates its kinase activity. Following its activation, S6K1 phosphorylates a number of its own downstream targets (such as SKAR and PDCD4) promoting the initiation of translation (**Figure 16**) (254,255).

mTORC1 participates in nucleotide synthesis through S6K1 phosphorylation, which increases ATF4 (the activating transcription factor 4)-dependent expression of MTHFD2 (mitochondrial tetrahydrofolate cycle enzyme methylenetetrahydrofolate dehydrogenase 2) supplying carbon units for purine synthesis (256). Through S6K1, mTORC1 also phosphorylates and activates CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, dihydroorotase), a rate-limiting enzyme in pyrimidine synthesis, favoring *de novo* pyrimidine synthesis (**Figure 16**) (257).

mTORC1 stimulates lipid synthesis through transcription factors SREBP1/2 (sterol regulatory element binding protein 1/2). In response to low sterol conditions, the SREBPs up-regulate genes necessary for *de novo* lipid and cholesterol synthesis. mTORC1 signaling can regulate SREBPs through a S6K1-dependent mechanism (258), which activate SREBPs as well as through the direct phosphorylation of LIPIN1, an inhibitor of SREBPs (**Figure 16**) (259).

mTORC1 regulates two major catabolic processes: protein degradation by ubiquitin-proteasome system and autophagy (**Figure 16**). Proteasome-dependent proteolysis can be boosted by mTORC1 inhibition which leads to either the increase of proteasomal chaperone levels or to overall increase of protein ubiquitylation (260,261). The main effect of mTORC1 on catabolic process is, however, through the

regulation of autophagy. Autophagy is an intracellular “self-digestion” process which removes misfolded proteins, dysfunctional organelles, and invading microorganisms by sequestering them in double membrane structures called autophagosomes. Autophagosomes further fuse with lysosomes forming autolysosomes in which the sequestered materials are digested by the lysosome hydrolases. The resulted components are recycled back into cytoplasm and can be used in the various metabolic processes, contributing to the maintenance of cellular homeostasis (262). mTORC1 is a well-known negative regulator of autophagy. mTORC1 exerts its inhibitory function by phosphorylating ULK1 (unc-51-like autophagy-activating kinase 1) and ATG13 (autophagy-related 13), leading to the inhibition of ULK1/ATG13/FIP200 (focal adhesion kinase family interacting protein of 200kDa) complex formation that is crucial for autophagosome formation (263). mTORC1 also regulates autophagy at transcriptional level. mTORC1 controls nuclear localization and activity of transcription factors like TFEB (transcription factor EB), TFE3 (transcription factor E3), and MITF (the microphthalmia transcription factor). These transcription factors activate genes necessary for autophagy and lysosome functioning (264,265).

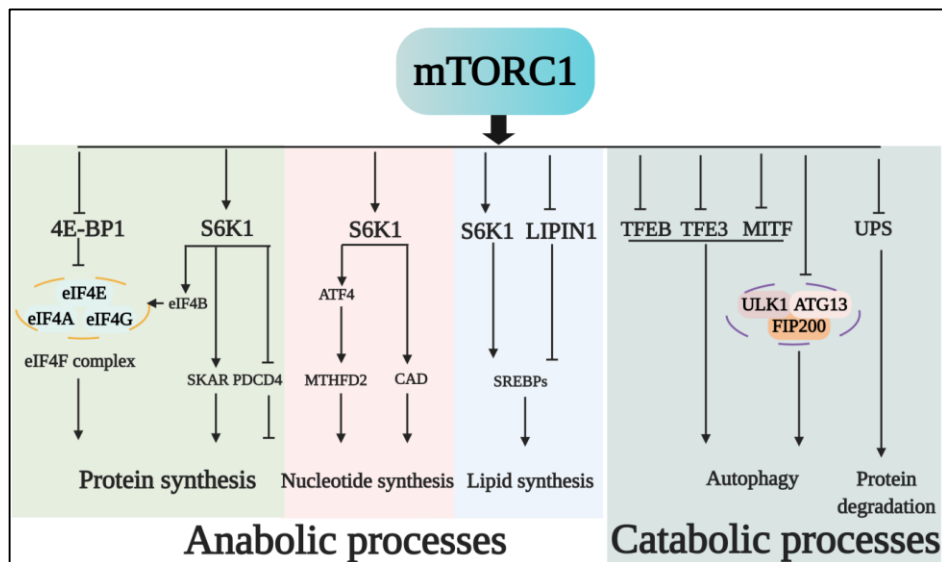


Figure 16. Downstream targets of mTORC1. mTORC1 regulates anabolic and catabolic processes. In the anabolic processes, protein synthesis, nucleotide synthesis, and lipid synthesis are promoted by mTORC1, while autophagy and protein degradation in the catabolic process are inhibited by mTORC1. 4E-BP1 and S6K1 are the well-known downstream targets of mTORC1. mTORC1 promotes protein synthesis by inhibiting 4E-BP1 and by activating S6K1. Moreover, S6K1 enhances protein synthesis independent of eIF4F complex by activating SKAR and inhibiting PDCD4, negative regulator of protein synthesis. mTORC1 induces nucleotide synthesis through S6K1 up-regulating ATF4-dependent expression of MTHFD2 and CAD activity as well as induces lipid synthesis by activating S6K1 and inhibiting LIPIN1 with ultimate increase in SREBPs activity. mTORC1 negatively regulates autophagy by inhibiting several factors including TFEB, TFE3, MITF, and ULK1, and prevents protein degradation by inhibiting ubiquitin-proteasome system.

Autophagy plays an important role in the carcinogenesis. Its regulation in B cell lymphomagenesis, however, seems to be paradoxical and complex. Early studies on tissue samples from patients had shown that B cell lymphomas with upregulated autophagy via beclin-1, a tumor suppressor, are more responsive to chemotherapy and present a favorable clinical outcome (266,267). Consistently, another study in a panel of cell lines derived from different B cell lymphomas described that the overexpressed BCL6 represses LITAF (lipopolysaccharide-induced tumor necrosis factor) gene, a possible tumor suppressor, resulting in the constitutive inhibition of autophagy which is suggested to be responsible for lymphomagenesis (268). Autophagy is also reported to be responsible for the apoptotic death of lymphoma cells. Study using DLBCL cell lines demonstrated that autophagy increased the degradation of antiapoptosis protein Survivin, thereby inducing the apoptosis in lymphoma cell lines (269). Autophagy-dependent apoptosis in B cell lymphomas was further supported by the studies in the light of finding potential therapeutic drugs (270,271). However, other recent studies have pointed out the prosurvival role of autophagy for B cell lymphomas. One recent study on the mechanisms of tumor suppressing function of miR-342-3p revealed that miR-342-3p carries out its tumor-suppressive role on B cell lymphoma cell lines via inhibiting the prosurvival autophagy (272). Protective role of autophagy is also reported in a study on DLBCL cell lines using autophagy inhibitors as well as knocking down key autophagy genes (273). Autophagy is also linked with the promotion of drug resistance in various B cell lymphoma cell lines and its inhibition increased the effects of anti-tumor agents (274). Therefore, researchers propose that targeting the autophagy with potent and selective inhibitors might be a novel promising strategy to overcome the chemoresistance in B cell lymphomas.

5.3. mTORC1 and the Immune System

mTORC1 plays a central role in metabolism, differentiation and effector functions of both innate and adaptive immune cells (275,276). The immune system protects an organism from various foreign pathogens and antigens. There are two main types of immune response: innate immunity and adaptive immunity. Innate immunity is the most immediate but non-specific defense mechanism against invading pathogens, which also has no immunologic memory. Adaptive immunity is slow but antigen-specific and produces immunologic memory which allows the organism to generate an efficient and rapid immune response upon exposure to the same antigen. Innate and adaptive immunity are complementary defense mechanisms (277,278).

5.3.1. mTORC1 in the innate immunity

Cells involved in innate immunity responses are macrophages, neutrophils, dendritic cells, mast cell, basophils, eosinophils, and natural killer (NK) cells. During infection and inflammation, a number of cytokines and chemokines are produced, which initiates a rapid recruitment of these cells to the appropriate sites to eliminate pathogens. Macrophages and neutrophils engulf the microbes and destroy them through

different pathways. In addition, macrophages are involved in the process of antigen presentation to the adaptive immune cells. In response to environmental stimuli, macrophages change their polarization status. Polarized macrophages are grouped into two subpopulations: (i) the M1-type formed by microbial products and pro-inflammatory cytokines, and (ii) the M2-type ultimately associated with tissue remodeling and repair. Dendritic cells also engulf pathogens and at the same time they act as antigen presenting cells to initiate adaptive immune response. Mast cells and basophils are mainly involved in the acute inflammatory responses in cases of allergy and asthma. Eosinophils are granulocytes also have phagocytic properties and always involved in the destruction of large parasites which are difficult to be phagocytosed. Together with mast cells and basophils, eosinophils also regulate mechanisms associated with allergy and asthma. NK cells kill virus-infected cells using proteins like perforins and granzymes by generating pores on the cell membranes of target cells. Moreover, NK cells produce cytokines like interferon-gamma that promotes the mobilization of antigen presenting cells and ensure effective anti-viral response (279–282).

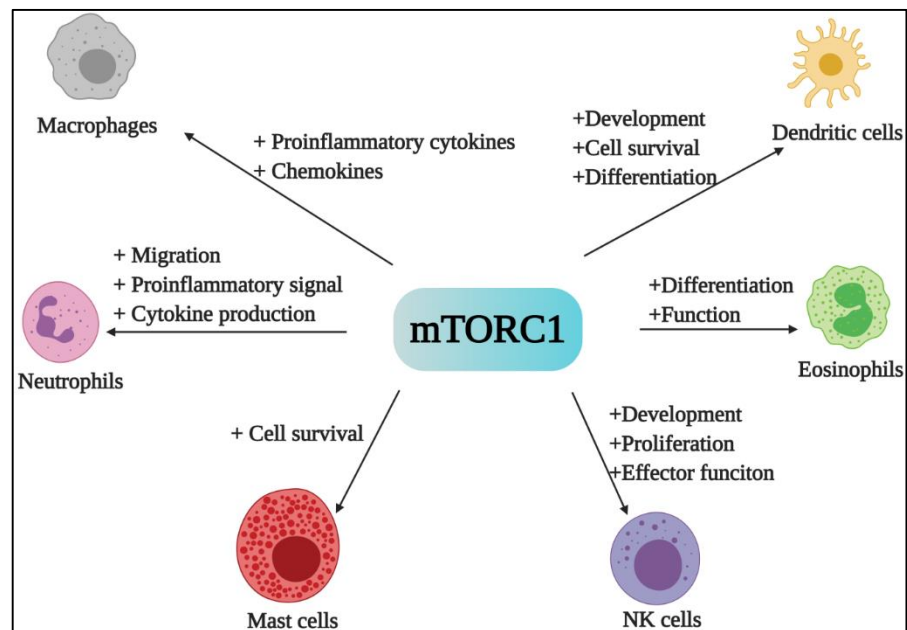


Figure 17. Role of mTORC1 in the innate immunity. In general, mTORC1 has positive effects on innate immune cells by increasing cytokine production, cell development, cell survival, differentiation, effector functions and migration.

mTORC1 plays an important role in the functioning of all innate immunity cells. Active mTORC1 drives macrophage differentiation into M1 macrophages (**Figure 17**), which are characterized by production of pro-inflammatory cytokines and chemokines that recruit other immune cells (283), while constitutively active mTORC1 (due to deletion of TSC1) disrupts macrophage differentiation to its M2 type (284).

Rapamycin completely blocked both neutrophils' chemotaxis (a directional cell movement as a response to a chemical) and chemokinesis (a random cell movement as a response to a chemical), due to the inhibition of actin polymerization, a hallmark of leukocyte migration (285). Neutrophils reaction to inflammatory signals and cytokine production capacity are also promoted by mTORC1 (286,287). mTORC1 is required for the survival of mast cells. Hyperactivated mTORC1, however, suppresses mast cells from producing cytokines and inhibits cell survival (288,289). mTORC1 can both positively (290) and negatively regulate the development of dendritic cells (291). mTORC1 hyperactivation due to the deletion of TSC1, negative regulator of mTORC1, inhibits dendritic cell development and changes their metabolic programming to glycolysis, mitochondrial respiration, and lipid synthesis which leads to the dendritic cell survival and differentiation (291). The effects of mTORC1 on dendritic cell function and immune responses by regulating their pro- and anti-inflammatory cytokines are well established (292–294). mTORC1 supports differentiation and function of mouse eosinophils (295), but the development of eosinophils is promoted by suppression of mTORC1 (296). mTORC1 is critical for the development of NK cells and plays important role during maturation by increasing the reactivity in a process called “NK cells education” to distinguish between normal and abnormal cells (297,298). NK cell proliferation and effector functions are favored by mTORC1 activation through the production of cytokines and the expression of granzyme B that is important effector molecule for killing the virus-infected cells, while mTORC1 inhibition disrupts these functions, leading to increased viral burden (**Figure 17**) (299,300).

5.3.2. mTORC1 in adaptive immunity

Adaptive immunity response plays critical role in eliminating infectious agents when innate immunity fails. Cells of the adaptive immune system are T lymphocytes and B lymphocytes. T cells originate from hematopoietic stem cells in the bone marrow. The progenitors of T cells migrate to the thymus, where they are also called thymocytes. In the thymus, thymocytes undergo a process of selection and maturation depending on the expression of different cell surface markers or receptors called T cell receptors (TCR). After a series of genomic rearrangements, cells that express TCR- β chain locus are selected. The β chain then couples with surrogate α chain, pre-Ta, creating pre-TCR, which forms a complex with CD3. Pre-TCR receptor signals cells to stop rearranging β chain, allowing the survival and proliferation through the expression of both CD4 and CD8 co-receptors. These so-called double positive cells further undergo a positive and negative selection. DP cells are presented to self-antigens by major histocompatibility complex (MHC) class I or class II molecules. Cells that bind with antigen/MHC with low avidity continue to survive, while cells that do not bind are eliminated. Survived cells then undergo negative selection, by interacting with self-MHC/antigens. Cells that interact too strongly with antigen die. Following selection and down-regulation

of either co-receptor, cells passing both positive and negative selection mature to be either CD8⁺ or CD4⁺ cells. Then they exit the thymus and migrate to the blood as fully differentiated but antigen-naïve T cells (301,302).

5.3.2.1. mTORC1 in T lymphocytes

CD8⁺ T cells are cytolytic T lymphocytes (CTLs) which kill the target host cells containing intracellular pathogens and transformed tumor cells. Cytotoxic T cells destroy target cells through the interaction of TCR with foreign cytosolic peptides from target cells bound to MHC class I molecules, which enables CTLs to form a close contact with the targets via an immunologic synapse. CTLs release granzymes and perforin, which induces apoptotic cell death. CTLs also express Fas ligand on their surfaces which further trigger apoptosis by binding to the target cell membrane. After clearing the infection, most of the CTLs are eliminated by phagocytes. Few CTLs, however, remain as memory immune cells, which can immediately differentiate into effector T cells in case of the invasion of the same pathogen in the future.

CD4⁺ cells are also called Th (T helper) cells, as these cells cannot directly kill infected cells or eliminate pathogens, but they provide other immune cells with signals via cellular contact or cytokine production to help them perform destruction tasks. Th cells are activated through their TCR recognizing antigens bound to MHC class II molecules. Once activated, Th cells produce different cytokines. There are various subtypes of Th cells with Th1, Th2 and Th17 being the most frequent ones. Each subtype is characterized by the cytokines produced. Th1 produces IFN- γ (Interferon gamma) which promotes the bactericidal activities of macrophages as well as activates anti-viral immunity. Th2 releases interleukin-4, 5, and 13 which enhances the development of B cells and also helps effective response against parasites by increasing the development and recruitment mast cells and eosinophils. Th17 cells make interleukin-17 family, which are involved in ongoing inflammatory responses, especially in chronic infection and disease. Most of Th cells die after handling the infection, but a few remains as Th memory cells. Regulatory T cells (Treg) negatively control both cytotoxic effector CD8⁺ cells and effector CD4⁺ cells. Tregs play important role in controlling autoimmune diseases as well as in developing tolerance to certain foreign antigens found in food. Subtypes of CD4⁺ cells also include Th9, Th22, and follicular helper T cells (Tfh). Like CTLs, a few Th cells remain as Th memory cells. Tfh cells help the development of germinal center B cells (302,303).

T lymphocytes in the resting state are catabolic and use autophagy to produce molecules necessary for protein synthesis and energy. After activation, T cells become anabolic and switch to glycolysis to produce energy and various substrates for proliferation. A transition from the resting to active state requires the up-regulation of metabolic pathways controlled by mTORC1. mTORC1 helps the differentiation process of CD4⁺ into Th1, Th2, and Th17 subtypes by promoting glycolysis and lipid biosynthesis (**Figure 18**) (304–307). mTORC1-deficient T cells have an impaired ability to differentiate (304). Similarly, T cells lacking the

mTORC1 activator RHEB also fail to differentiate into Th1 and Th17 (305). Alternatively, deletion of mTORC1 inhibitor TSC1, results in the enhanced mTORC1 activity, leading to elevated Th1 and Th17 differentiation and multiorgan inflammation in mice (308). In addition, a deficiency in the leucine transporter LAT1 (309) and glutamine transporter ASCT2 (310) impairs Th1 and Th17 differentiation in a mTORC1-dependent manner. mTORC1 is equally important in promoting Th2 cells differentiation (307). mTORC1 activity has negative effects on Treg generation (**Figure 18**) (311), while it is positively correlated with inhibitory functions of Tregs towards other T cells (312). Active mTORC1 promotes the conversion of Tregs to effector-like T cells and further impairs Treg stability and function (308,313,314). Toll-like receptor signals, which promote Treg proliferation, increase mTORC1 signaling, glycolysis and expression of glucose transporter Glut1 (313). mTORC1 is found to be indispensable for Tfh cell differentiation and germinal center formation (315). Increased mTORC1 activity is found to be beneficial for the generation of effector CD8⁺ T cells. In contrast, active mTORC1 down-regulates memory T cell formation resulting in a decreased response to secondary immunization (**Figure 18**) (316–318).

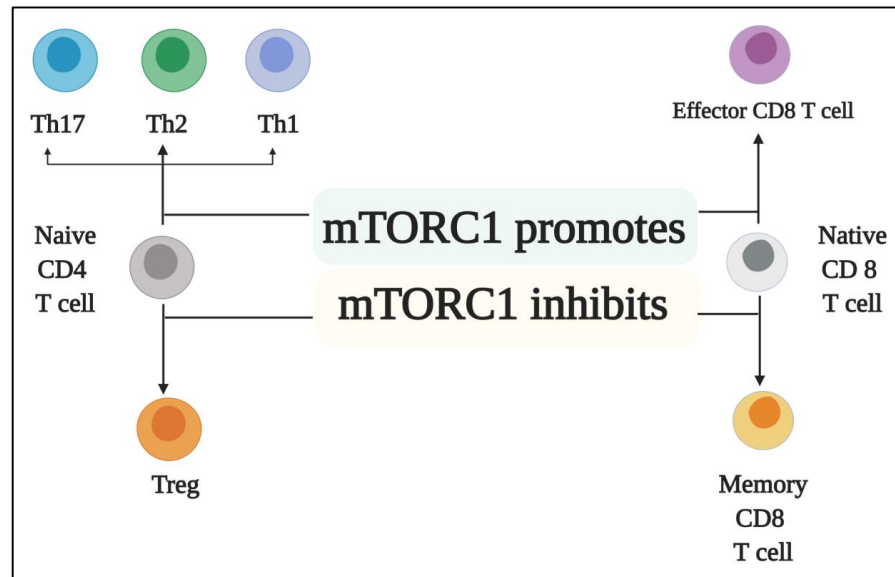


Figure 18. mTORC1 in T lymphocytes. mTORC1 promotes the differentiation process of CD4⁺ into Th1, Th2, and Th17 subtypes, while it down-regulates Treg generation and impairs their stability and function. Active mTORC1 promotes the generation of effector CD8⁺ T cells, but prevents the formation of memory T cells.

5.3.2.2. mTORC1 in B lymphocytes

B cells of adaptive immunity derive from hematopoietic stem cells (HSC) in the bone marrow where they pass through several development stages (**Figure 19**). Each stage of development is characterized by the

expression surface markers. At the antigen-independent development stage, B cells acquire surface marker immunoglobulins IgD and IgM (naïve B cells). Mature naïve B cells leave the bone marrow and migrate to the secondary lymphoid organs (lymph nodes, the spleen, and mucosal-associated lymphoid tissues), where they interact with other immune cells to be activated. The second phase of development is mainly driven by Th cells. Depending on the signal received they become antibody producing cells (plasma cells) or memory B cells.

In order to be activated, B cells need 2 types of signals: signal 1 and signal 2. Signal 1 is received by the recognition of antigen via B cell surface immunoglobulin receptors, which enables the interaction between B and T cells, generating signal 2 delivered by T cells. B cells in their turn act as antigen presenting cells providing peptides along with MHC class II molecules and directly contact with CD4⁺ T cells, which help to activate B cells. This interaction happens at the margin between B cell follicles and T cell areas in secondary lymphoid tissues. Activated B cells either migrate to the extrafollicular areas and become short-lived plasma cells secreting low-affinity antibodies or enter a follicle to establish a germinal center. In the germinal center B cells interact with T follicular helper cells and antigen-presenting cells (e.g., follicular dendritic cells) and further go through a process called class-switching, in which B cells change IgM and IgD production to other types of immunoglobulins like IgG, IgA and IgE (**Figure 19**).

Immunoglobulins are comprised of heavy chains and light chains assembled randomly from different segments. Heavy chains are formed from 4 segments (VH, D, JH and CH), while light chains are made from 3 segments (VL, JL, and CL). There are 9 different heavy chain types (IgM, IgD, IgG1-4, IgA1 and IgA2, and IgE) and 2 light chain types (k and l). Class-switching process takes place through a mechanism of gene segment rearrangements. A DNA sequence of VDJ unit and the genes coding for IgM and IgD is cut and ligated to a similar sequence in another C-region gene coding for any of the IgG, IgA, or IgE subtypes, resulting in the production of an antibody with new C-region isotype (319). While class-switching is occurring, another process called somatic hyper mutation (SHM) is activated. SHM generates point mutations in the V regions of immunoglobulin heavy and light chains with the help of activation-induced cytidine deaminase (AICDA) and uracil nucleoside glycosylase (UNG) enzymes which are also involved in the class switching process, leading to the production of antibodies with higher affinity for antigens termed affinity maturation (320). In the immune system, the first exposure to an antigen produces the primary immune response, which is slow and characterized by the production of IgM with low affinity followed by the productions of IgG, IgA or IgE with higher affinities. During the primary response, memory T and B cells are established. In a subsequent encounter with the same antigens, these cells are activated faster and high affinity IgG, IgA or IgE antibodies are produced more quickly in a process known as “secondary response” (302,303).

mTORC1 is highly active at the earliest stages of B cell development and its activity reduces upon B cell maturation (**Figure 19**) (321–323). Homozygous deletion of mTORC1 component RAPTOR prior to lineage specification results in a total lack of B cell maturation (321,322). Inhibition of mTORC1 at the transcriptional level in mice model has shown that loss of mTOR has led to a decrease in total T cell number and this effect is more pronounced in B cells with a block of B cell development in the bone marrow, decrease in proliferation, antibody production, and migration (245). Antibody production is also promoted by mTORC1 (**Figure 19**). Suppression of mTORC1 activity causes the destruction of plasma cells in the spleen and bone marrow as well as destroys germinal center B cells, leading to a profound decline in antibody production (324). Disruption of mTORC1 even reduces general populations of B cells including memory B cells, plasma cells, and germinal center B cells, leading to decreased expression of Bcl6 - a key transcription factor involved in germinal center B cell fate – and AICDA, which impairs generation of high affinity IgG1 antibody (322,323). mTORC1 prepares nascent plasma cells for antibody secretion even in advance by up-regulating UPR (unfolded protein response)-associated genes which help the newly formed plasma cells adapt to the increased antibody synthesis (**Figure 19**) (325).

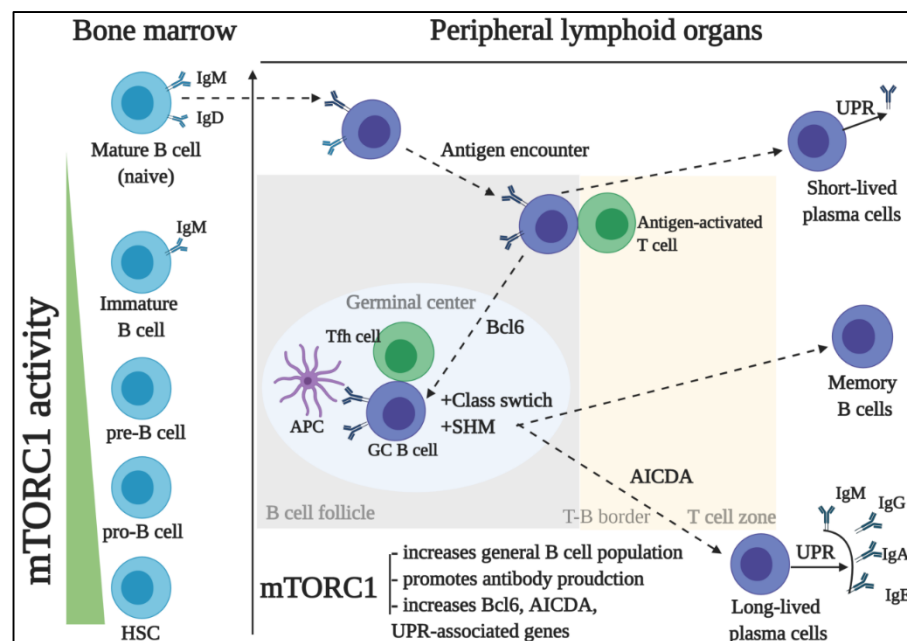


Figure 19. Function of mTORC1 in B cells. B cells derived from HSC pass through development stages in the bone marrow till mature naïve B cells which migrate from bone marrow to the peripheral lymphoid organs to be activated, and encounter antigens. These cells further interact with antigen-activated T cells at the border between B cell follicles and T-cell zone. Activated B cells migrate to the extrafollicular areas and become short-lived plasma cells or enter a follicle to establish a germinal center. In the germinal center B cells interact with Tfh cells as well as with APC cells and they go through class switch and SHM processes to become either memory B cells or long-lived plasma cells capable of producing high affinity antibodies (IgG, IgA and IgE). In the bone marrow, mTORC1 is highly active at the

early stages of B cell development and its activity reduces upon B cell maturation. In the peripheral lymphoid organs, mTORC1 promotes general B cell population and increases antibody production. In addition, mTORC1 promotes germinal center formation by up-regulating Bcl6, enhances class switch and somatic hyper mutation by AICDA facilitating the production of high affinity antibodies, and prepares newly formed plasma cells for antibody production by increasing the expression UPR-associated genes.

Considering the vital roles of mTORC1 in the B cell development, B cell fate decision in the germinal center, and promotion of antigen-specific memory B cells, clinical trials for mTORC1 inhibitors in controlling the severity of COVID-19 disease were recently initiated (326). Indeed, memory B cells generated by previous low-pathogenic coronaviruses which cause only cold-like symptoms rapidly produce cross-reactive antibodies, which leads SARS-Cov-2 to immune escape, resulting in antibody-dependent enhancement (ADE) and eventually causing cytokine storms considered as the major cause of critical illness and death related to COVID-19. Therefore, mTORC1 inhibitors like sirolimus might block memory B cells, prevent ADE, and reduce the disease severity and case mortality (326).

Taken together, mTORC1 senses and controls immune microenvironment in order to influence the development, maturation, differentiation, and production of immune cells.

5.4. mTORC1 and viruses

After viral infection, a stress response is initiated in the host system. Infected cells react to limit the viral spread by eliminating the invading viruses through induction of autophagy or apoptosis. Viruses, in their turn, use different strategies to escape from the destruction by the host system by hijacking host mechanisms like mTORC1 signaling pathway which not only inhibits apoptosis, but also suppresses autophagy, enabling viral protein synthesis and viral growth. Viruses can both activate and inhibit mTORC1 pathway, depending on their types, stage of infection, and cells in contact with (327).

Adenovirus use viral protein E4orf1 which mimics growth factor signaling leading to activation of PI3K/AKT/ mTORC1 pathway for viral replication, even under the absence of nutrients and growth factors (328). Epstein-Barr virus (EBV) activates PI3K/AKT/mTORC1 pathway by its latency protein LMP2A, which enhances EBV-driven cellular expansion (329). To benefit from the cap-dependent protein synthesis, West Nile virus activates mTORC1 early after infection to synthesize viral protein and to further increase viral growth (330). Viruses also interfere with the downstream part of mTORC1 pathway. For example, human cytomegalovirus directly affects the 4E-BP1 branch of mTORC1 by excluding 4E-BP1 from the

eIF4E complex thus supporting translation in human cytomegalovirus-infected cells (331). Hepatitis C virus induces 4E-BP1 expression and further increases its phosphorylation (332).

In response to viral infection, mTORC1 can also be inhibited, at least transiently. For example, influenza A virus temporarily inhibits mTORC1 signaling to promote viral replication effectively, but prolonged suppression of mTORC1 strongly impairs its replication (333). Classical swine fever virus inhibits mTORC1 at the early stage of infection to promote viral replication, but mTORC1 inhibition induces a feedback AKT/mTORC1 activation loop, maintaining a balance between host cell survival and viral replication (334).

Like other viruses, HIV-1 also regulates mTORC1 pathway to create a favorable environment for viral replication and transmission in stressful conditions.

5.4.1. Regulation of mTORC1 by HIV-1

One of the first evidences that mTORC1 was involved in HIV-1 infection came from the observation that treatment with rapamycin causes down-regulation of CCR5 expression in T cells (335). A number of studies that immediately followed confirmed that rapamycin possessed anti-HIV-1 properties both *in vitro* and *in vivo*, thus pointing to the mTORC1 importance during HIV-1 propagation (reviewed in (336,337)). Pan-inhibitors of mTORC1 block HIV-1 even more efficiently, interfering both with virus entry (by reducing CCR5 levels), and with basal and induced transcription, as shown in pre-clinical humanized mice models (338).

HIV-1 infection generally increases mTORC1 activity both in productively infected and bystander host cells thus promoting successful viral entry, integration and replication. Studies on peripheral blood mononuclear cells, memory T cells, dendritic cells, and tissue samples from patients as well as several model cell lines such as Jurkat cells, HeLa and HEK293 have shown that mTORC1 is generally activated following HIV-1 infection (339–344)

mTORC1 is involved in many steps of HIV-1 lifecycle (**Figure 20**). Inhibition of mTORC1 impairs HIV-1 entry by down-regulating CCR5 receptor and further decreases HIV-1 LTR transcription in peripheral blood mononuclear cells (PBMCs), CD4 lymphocytes, macrophages, and monocytes (345,346). Reverse transcription process is also promoted by mTORC1. Even in metabolically quiescent resting peripheral blood CD4 T cells HIV-1 promotes its reverse transcription through mTORC1 by increasing the production of dNTPs as well as stabilizing microtubules to transport the products of reverse transcription (347). Upon entry into the cells HIV-1 hyperactivates mTORC1 to promote nucleotide and protein synthesis in order to obtain enough amount of proteins, nucleotides and energy for efficient viral replication and even for reactivation (340,342). Inhibition of mTORC1 and AKT decreases HIV-1 viral replication (348).

HIV-1 not only regulates mTORC1 activity, but it can also regulate intracellular positioning of mTOR in response to other stress stimuli. When amino acids are present, GTP-loaded RAGA and RAGB induce mTORC1 translocation from the cytoplasm to late endosomes/lysosomes (LEs) (349). Even though nutrient starvation or oxidative stress results in the accumulation of a pool of mTOR-associated LELs at a juxtanuclear position, HIV-1 counteracts stress-induced trafficking of mTORC1 by maintaining the LELs throughout the cytoplasm (340), which is dependent on the RAGA/B. Ultimately, HIV-1 uses LEL trafficking through Rag GTPases for successful viral assembly and release. Interestingly, Gag, viral genomic RNA and several host proteins co-traffic on LEL during HIV-1 egress (350).

mTORC1 activity can be modulated individual proteins of HIV-1. For example, mTORC1 can be activated by treatment with HIV-1 Nef, or Env, or Tat proteins, resulting in efficient viral replication and generation of new virions (**Figure 20**) (341–343,351). In contrast, combined treatment of neuronal cells with HIV-1 Tat and methamphetamine (a stimulant drug, which increases the exposure to HIV-1) results in inhibition of mTORC1 activity (352). As Tat is present in high concentrations in patients' blood serum (112,353) and because of Tat's ability to enter almost any cell in a human body due to its cell penetration domain (59), this viral transactivator can probably remotely regulate mTORC1 activity in many non-infected cells.

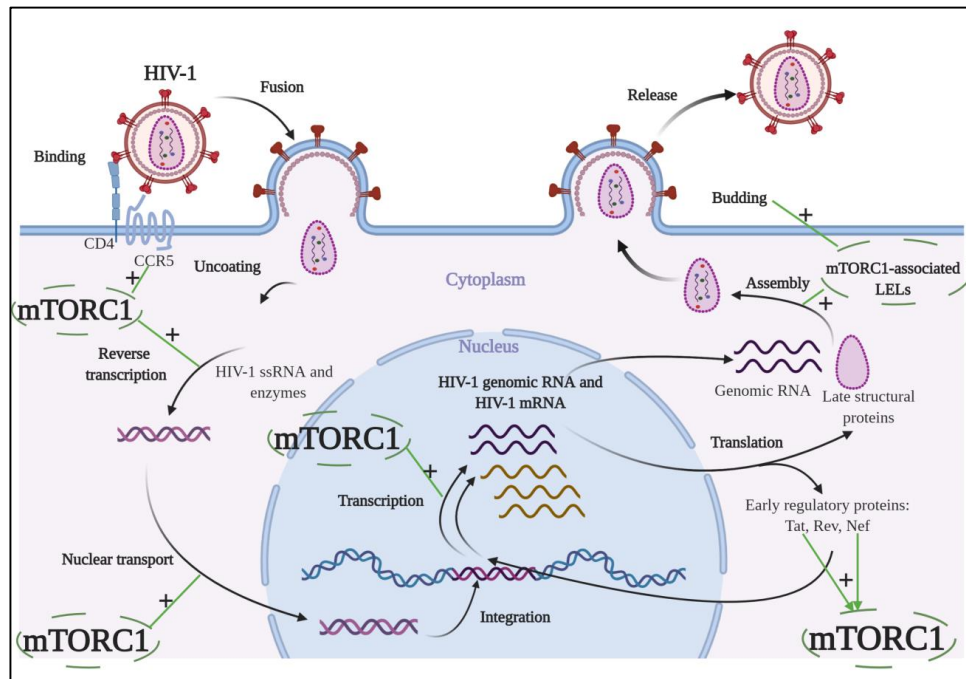


Figure 20. Regulation of mTORC1 by HIV-1. HIV-1 uses mTORC1 to promote CCR5 receptor expression to facilitate the viral entry. Reverse transcription is promoted due to the production of dNTPs pools by mTORC1, which also supports the transport of reverse transcription products. HIV-1 uses mTORC1 for efficient transcription and replication. Synthesized viral proteins can positively regulate mTORC1 activity as it is involved in many key stages of

viral infection. HIV-1 disperses mTORC1-associated LELs throughout the cytoplasm to support assembly and release processes.

5.4.2. Regulation of Autophagy by HIV-1

mTORC1 is the negative regulator of the major cellular catabolic process, autophagy (354,355). Autophagy plays a critical role in maintaining cellular homeostasis, but is also related to many pathological conditions including cancer and neurodegeneration (356). Recent studies revealed an important role of autophagy in the context of HIV-1 infection in T cells, macrophages, dendritic cells, and neuronal cells (357,358)

Autophagy can play both proviral or antiviral role, depending on the stage of HIV-1 infection and cells in contact with virus and its components (**Figure 21**). HIV-1 requires early, nondegradative autophagic events, for its replication, probably because the autophagosomal membrane provides a scaffold for virus assembly (359). The entire virus and its proteins, in particular Tat, Nef and Env, can induce autophagy in infected cells to maximize HIV-1 production. At the same time, the virus developed multiple strategies to escape degradation of newly synthesized viral proteins (357). For example, Nef can interact with BECLIN-1, an autophagy initiation protein, to inhibit autophagosome maturation in macrophages and T cells (359–362). Nef/BECLIN-1 interaction inhibits autophagy at the transcriptional level by preventing nuclear translocation of the pro-autophagic factor TFEB in an mTORC1-dependent manner (360). Nef can also block autophagosome formation by increasing the interaction between BECLIN-1 and its inhibitor BCL2, an interaction that requires E3-ubiquitin ligase PRKN (361). HIV-1 proteins can also disrupt autophagy in uninfected cells. For example, HIV-1 Tat inhibits autophagy induction in non-infected primary macrophages through AKT activation (363).

At the initial steps of infection, HIV-1 envelope proteins at the surface of the virus bind to CD4 receptors, mainly CCR5, initiating autophagy in CD4 T cells (364). This autophagic process represents an anti-HIV response of the host cell, because it selectively degrades HIV-1 Tat (365,366). Similarly, induction of autophagy in macrophages with dual inhibitors of PI3K and mTORC1 results in degradation of intracellular viral particles and reduction of viral release (348). The virus has however evolved to counteract autophagy (366). Different HIV-1 proteins participate in this inhibition in different cells and at various steps of infection. For example, Vpr tries to inhibit autophagy in T cells already at the early stages of infection to facilitate efficient viral replication (367), Vif inhibits autophagy at the late steps of viral replication in CD4+ T cells (368), Nef blocks autophagy in macrophages and in T cells (360,361), HIV-1 Tat blocks autophagy in

bystander macrophages (363), while envelope proteins inhibit autophagy through mTORC1 activation in dendritic cells, but not in macrophages and CD4⁺ T cells (343).

Regulation of autophagy was also studied in the context of HIV-1 infection in neuronal cells, as HIV-1-associated neurocognitive disorders lead to increased mortality in HIV-1 patients (369). HIV-1 Tat induces autophagy, and increases neurotoxicity (370–372). HIV-1 Tat can even trigger neuronal cell death when combined with methamphetamine (352,373,374). HIV-1 Tat also plays a role in the regulation of mitophagy, a specialized form of autophagy, which removes damaged mitochondria. Treatment with HIV-1 Tat of primary microglial cells, the key target for HIV-1 infection in central nervous system, results in accumulation of damaged mitochondria and increased expression of mitophagy signaling proteins PINK1 and PARKIN. However, even though mitophagy was induced, mitophagosomes fail to fuse with lysosomes and accumulate in the cells, thus resulting in overall defective mitophagy and subsequent neuroinflammation (375).

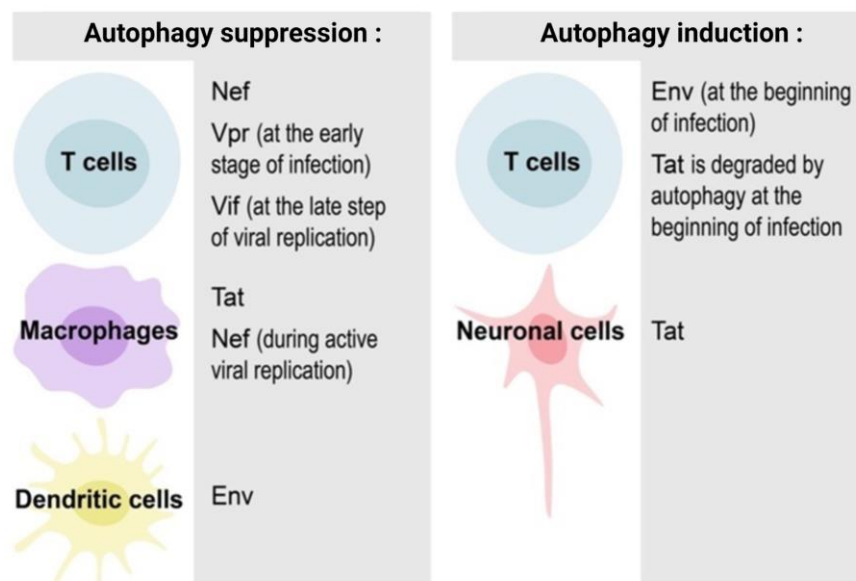


Figure 21. Implication of various HIV-1 proteins in modulation of autophagy in different cells. HIV-Nef protein suppresses autophagy, while Vpr is inhibiting autophagy at the early stage and Vif at later stage of infection in T lymphocytes. Autophagy is positively regulated by Env protein at the beginning of infection, in which Tat protein undergoes degradation. Autophagy is suppressed by Tat and Nef in macrophages, but Tat induces autophagy in neuronal cells. Env protein reduces autophagy in dendritic cells.

5.4.3. mTORC1 in HIV-1 Latency

HIV-1 latency is defined as a reversibly non-productive infection of a cell, in which an integrated viral DNA remains transcriptionally silent, even if it is replication-competent. Several mechanisms have been

associated with the latency: transcriptional interference, chromatin modifications, limited availability of transcription and elongation factors, and insufficient Tat activity (**Figure 22**) (376,377). When the access of the transcription machinery to the HIV-1 promoter is repressed due to host cellular processes e.g. epigenetic silencing, Tat expression is restricted, viral gene expression feedback from Tat is disrupted, leading to HIV-1 latency (378). Tat can remove the repression of LTR promoter by recruiting histone acetyl transferases, e.g. the (CBP)/p300 complex to the promoter region and thus enabling reactivation of latent HIV-1 (**Figure 22**) (379).

In 1995-96, a HIV-1 treatment known as highly active antiretroviral therapy (HAART) (later called combination antiretroviral therapy (cART) or antiretroviral therapy (ART)) was introduced. cART is a combination of three or more antiviral drugs which target multiple stages of viral life cycle and keep undetectable viral loads by inhibiting HIV-1 replication. cART contains HIV-1 entry inhibitors, nucleoside and non-nucleoside reverse transcriptase inhibitors, integrase and protease inhibitors. Since its introduction cART dramatically reduced deaths from HIV-1 infection and have saved millions of lives. HIV-1, however, is not eradicated by cART due to the existence of latent reservoirs, and thus patients have to take cART lifelong (380,381).

Latent reservoir is “a cell type or anatomical site in which replication-competent form of virus accumulates and persists in spite of long periods of ART-suppression viremia” (382,383). The latent reservoir can be established early after HIV-1 infection (384). HIV-1 cellular reservoirs consist of resting memory CD4+ T cells, myeloid cells, macrophages and dendritic cells which allow the virus to survive and replicate (**Figure 22**) (385). Although the existence of anatomical HIV-1 reservoirs remains debated, the lymphoid tissues (spleen, thymus, lymph nodes and gut-associated lymphoid tissues), which are the most important sites of viral replication during infection, are considered as the most prominent reservoirs.

Two opposite strategies are proposed to disrupt HIV-1 latency: “shock and kill” and “block and lock” for the sake of an efficient treatment (deep latency) (386,387). In the “shock and kill” approach, the latent HIV-1 is reactivated (“shock”) by latency reversal agents (LRAs). The re-activated cells are further “killed” either by the attack of the host immune system or by the cytotoxic effect of HIV-1 itself, while uninfected cells are protected against the viral infection by cART. Unfortunately, none of the LRAs tested so far have shown a significant effect on the viral reservoir in clinical trials (388). In addition, agents inducing global T cell activation induced severe adverse reactions, mainly through inflammation, and were abandoned after several clinical trials. “Shocking” alone seems to be insufficient to eliminate latent T cell reservoir. Effective “killing” strategies need to be developed to optimize this approach. An alternative “block and lock” strategy aims to bring the proviral HIV-1 to a deeply silenced state so that HIV-1 will not rebound even if cART is discontinued.

The first notion of the importance of mTORC1 in HIV-1 latency came from the study of Besnard *et al.* who demonstrated that both mTORC1 and mTORC2 complexes are essential for HIV-1 reactivation from latency (389). Accordingly, inhibitors which block both mTORC1 and mTORC2, pp242 and Torin1, prevent HIV-1 reactivation in primary CD4⁺ T cells from uninfected donors, when infected *ex vivo* (342,389). Rapamycin, which mostly blocks mTORC1, is a less effective inhibitor of HIV-1 reactivation. Moreover, in CD4⁺ T cells from aviremic HIV-1 positive donors under cART, HIV-1 proviral reactivation is unaffected by addition of rapamycin, even if the drug downregulates markers of toxicity related to inflammation (390). At the same time, active mTORC1 might be important for the natural HIV-1 suppression control, because mTORC1 activation was also detected in HIV-1 elite controllers, a rare (<1%) group of HIV-1-infected patients who do not take antiretroviral therapy (ART) and do not develop AIDS (391).

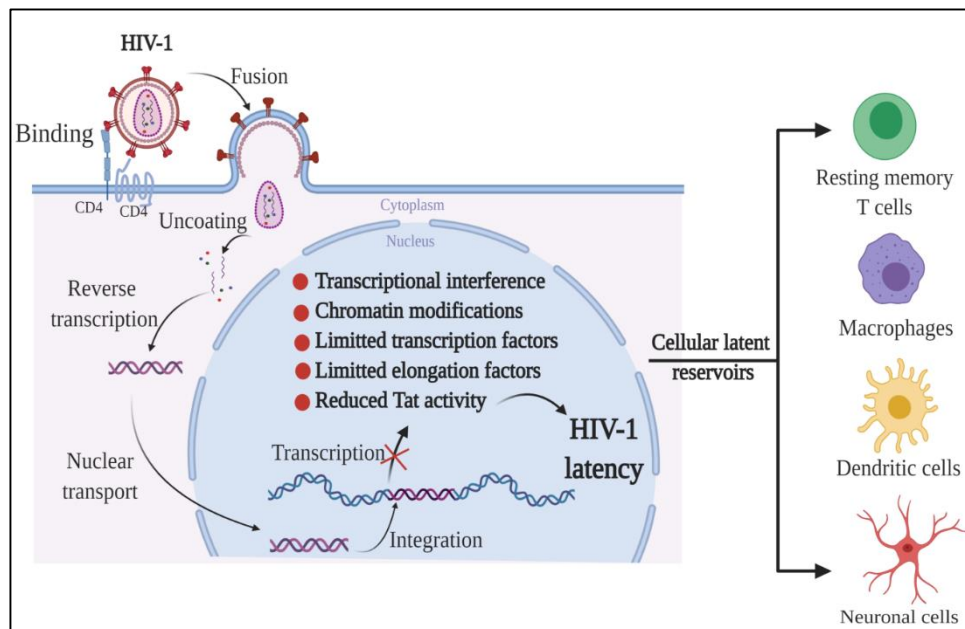


Figure 22. HIV-1 latency. Integrated proviral DNA enters a transcriptionally silent state due to the interference at transcriptional level, chromatin modifications, deficiency of transcription factors and elongation factors and reduced Tat activity. Resting memory CD4⁺ T cells, myeloid cells, macrophages, dendritic cells and neuronal cells potentially serve as reservoirs for HIV-1 latency, preventing the complete HIV-1 eradication.

The use of rapamycin and pan-inhibitors of mTORC1 is associated with multiple adverse effects; therefore, targeting mTORC1 signaling pathway and not mTOR kinase itself appears as an attractive alternative approach. Recent genome-wide CRISPR screening for host factors required for HIV-1 latency identified two upstream mTORC1 inhibitory genes – TSC1 and DEPDC5. Both TSC1 and DEPDC5 are

known negative regulators of mTORC1, but they suppress mTORC1 *via* different upstream branches: TSC1, a TSC complex member, inhibits mTORC1 and maintains HIV-1 latency by downregulation of RHEB, whereas DEPDC5, GATOR1 component, *via* suppression of RAGA (392). Therefore, targeting TSC1 and DEPDC5 might be a useful strategy in metabolic inhibition to “block and lock” the latent reservoir. In an effort to optimize the “shock and kill” approach, recent findings revealed that interaction of infected CD4+ T cells with DC could activate latent HIV-1 (393). The contact with dendritic cells also activates the PI3K-AKT-mTOR pathway in CD4+ T cells and contributes to HIV-1 purge.

CD4+ T cells express on their surface various receptors that target them to different peripheral tissues in the body. One such receptor, CCR6, directs Th17 T-helper cells to the gut. The gut-associated lymphoid tissues are considered as an important site of HIV-1 replication and a viral reservoir. The analysis of colon biopsies taken from HIV-1 patients under cART revealed that CCR6+ Th17-polarized CD4+ T cells express more mTOR and that mTOR phosphorylation is also increased in these cells (339). The use of mTOR inhibitors limited HIV-1 replication in gut-homing Th17 cells during reverse transcription and prior integration, with mTORC1/mTORC2 inhibitor INK128 being more effective than rapamycin. Thus, mTOR inhibitors could have a potential beneficial effect in decreasing HIV-1

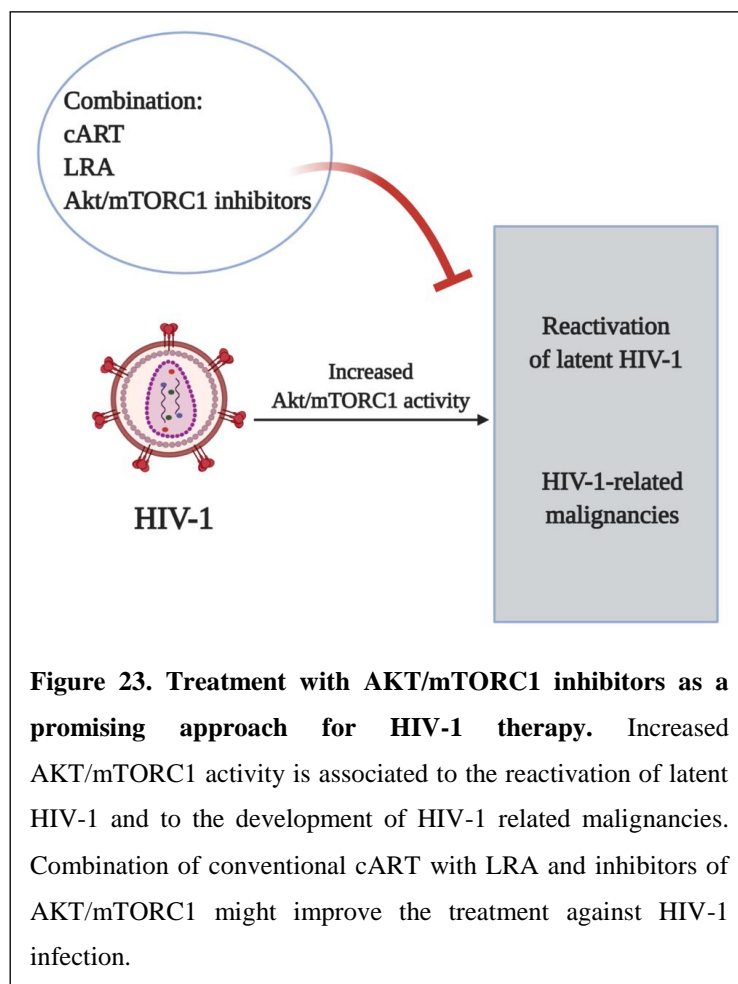




Figure 23. Treatment with AKT/mTORC1 inhibitors as a promising approach for HIV-1 therapy. Increased AKT/mTORC1 activity is associated to the reactivation of latent HIV-1 and to the development of HIV-1 related malignancies. Combination of conventional cART with LRA and inhibitors of AKT/mTORC1 might improve the treatment against HIV-1 infection.

reservoirs and restoring Th17 immunity in intestines during cART. Finally, recent mathematical models predict that persistence of a majority of HIV-1 infected cells is due to cellular proliferation rather than HIV-1 replication (394); therefore, reducing cell proliferation could decrease the size of HIV-1 reservoirs. Because mTORC1 is a major regulator of cellular proliferation, specific targeting of this pathway in HIV-1 latent cells appears as a promising approach for AIDS cure (**Figure 23**).

Review

Modulation of mTORC1 Signaling Pathway by HIV-1

Burkitkan Akbay ^{1,2}, Anna Shmakova ^{1,2} , Yegor Vassetzky ^{1,2,3}  and Svetlana Dokudovskaya ^{1,2,*}

¹ CNRS UMR 9018, Université Paris-Saclay, Institut Gustave Roussy, 114, rue Édouard Vaillant, 94805 Villejuif, France; akbayburkitkan@gmail.com (B.A.); anyashm@gmail.com (A.S.); yegor.vassetzky@cnrs.fr (Y.V.)

² LIA 1066 LFR2O French-Russian Joint Cancer Research Laboratory, 114, rue Édouard-Vaillant, 94805 Villejuif, France

³ Koltzov Institute of Developmental Biology, 26, Vavilova str., 119334 Moscow, Russia

* Correspondence: svetlana.dokudovskaya@igr.fr

Received: 25 March 2020; Accepted: 25 April 2020; Published: 28 April 2020



Abstract: Mammalian target of rapamycin complex 1 (mTORC1) is a master regulator of cellular proliferation and survival which controls cellular response to different stresses, including viral infection. HIV-1 interferes with the mTORC1 pathway at every stage of infection. At the same time, the host cells rely on the mTORC1 pathway and autophagy to fight against virus replication and transmission. In this review, we will provide the most up-to-date picture of the role of the mTORC1 pathway in the HIV-1 life cycle, latency and HIV-related diseases. We will also provide an overview of recent trends in the targeting of the mTORC1 pathway as a promising strategy for HIV-1 eradication.

Keywords: HIV-1; HIV-1 related diseases; mTORC1 pathway; autophagy

1. Introduction

Viruses generate an environment that is favorable for their successful replication and transmission during infection. In order to optimize their biosynthetic needs, viruses use the nutrient, energy and macromolecule synthesis systems of the host cells and manipulate their metabolism [1]. Host cells in turn respond to viral infection by changing their transcriptional and translational programs and employing antiviral metabolic changes [2–4]. Cellular response to various stresses, including viral infection, is under the control of the mechanistic target of rapamycin complex 1 (mTORC1), which drives proliferation and survival by the regulation of anabolic and catabolic processes. Thus, it is no wonder that viruses try to use this signaling pathway to their benefit [4].

The human immunodeficiency virus type-1 (HIV-1) is a lentivirus containing two positive-sense single strand RNAs encapsulated in a capsid formed by p24. Structural HIV-1 proteins (Gag, Pol and Env) are produced as polypeptides and subsequently processed into matrix proteins, protease, reverse transcriptase, integrase and surface proteins gp120 and gp41. HIV-1 also codes for two regulatory components: Tat (transcriptional trans-activator) and Rev (regulator of expression of virion proteins). Finally, Vpr, Vif, Nef and Vpu serve as accessory regulatory elements [5]. During viral entry, gp120 binds to the CD4 molecule of the host cell and gp41 binds to the cellular coreceptors such as CCR5 and CXCR4. After fusion with the host cell, a conical capsid around the HIV-1 genome disassembles (a process known as uncoating), and viral RNA is released into the cytoplasm where it is transcribed by a viral-encoded reverse transcriptase. Uncoating probably occurs in the cytoplasm in coordination with reverse transcription or at the nuclear envelope during nuclear import. Subsequently, viral dsDNA uses the host nuclear import machinery to move to the host cell nucleus, where it integrates into the host DNA with the help of a viral-encoded integrase. Remarkably, recent studies revealed that intact viral cores can enter to the nucleus and uncoat just before integration to their chromosomal integration sites [6]. Pro-viruses use the host RNA polymerase to synthesize mRNA, which is subsequently

translated into viral proteins. HIV-1 infects and kills cells of the immune system such as T-helper cells, macrophages and dendritic cells, leading to immunodeficiency and further increasing the incidence of opportunistic infections and cancers.

The mechanistic target of rapamycin (mTOR) is an evolutionarily-conserved, serine-threonine protein kinase that belongs to the phosphatidylinositol 3-kinase PI3K-related family. mTOR forms two different macromolecular protein complexes, mTORC1 and mTORC2, which differ in their composition, downstream targets and regulation [7]. mTORC1 is sensitive, while mTORC2 is much less responsive to an allosteric mTOR inhibitor rapamycin (Sirolimus®), an immunosuppressor, which suppresses T and B cell activation by inhibition of the cell cycle. Various analogues of rapamycin, so called rapalogues (Everolimus®, Temsirolimus®), are also frequently used in clinics for immunosuppression. In addition, a number of alternative mTOR inhibitors have been developed. These inhibitors block both mTORC1 and mTORC2 (pan-inhibitors or TOR-KIs, i.e., INK128) or act on mTOR kinase and another protein (dual inhibitors), most often targeting a network upstream of mTORC1/2 [8]. Viruses are the leading cause of infections after solid-organ transplant and during anticancer treatment; the use of mTOR inhibitors decreases the incidence of viral infection in these medical conditions [9–11].

One of the first pieces of evidence that mTORC1 was involved in HIV-1 infection came from the observation that treatment with rapamycin causes downregulation of CCR5 expression in T cells [12]. A number of studies that immediately followed confirmed that rapamycin possessed anti-HIV-1 properties both in vitro and in vivo, pointing to the mTORC1 importance during HIV-1 propagation (reviewed in [13,14]). Pan-inhibitors of mTORC1 block HIV-1 even more efficiently, interfering both with virus entry (by reducing CCR5 levels) and with basal and induced transcription, as shown in preclinical humanized mice models [15]. Our review is focused on recently discovered mechanisms of mTORC1 contribution to HIV-1 infection, latency and development of HIV-1 related diseases.

2. mTORC1, a Main Metabolic Network of the Cell

mTORC1 integrates signals from many intracellular and extracellular cues: amino acids, growth factors, energy, oxygen, DNA damage and infectious agents, including viruses. Depending on the nature of the signal, its duration, cell type and many other factors, mTORC1 will “determine” the subsequent cell fate. mTORC1 can accelerate proliferation via the phosphorylation of its key targets, p70S6 Kinase 1 (S6K1) and members of eIF4E Binding Protein family (4E-BPs), which participate in the synthesis of three major cell constituents: proteins, nucleotides and lipids [7]. Alternatively, mTORC1 can drive the cell through a catabolic process, since it controls both autophagy (via phosphorylation of ULK1 and TFEB) and protein degradation by ubiquitin-proteasome system (via phosphorylation of ERK5) [16]. In order to operate through signals and responses, mTORC1 manipulates one of the most complex signaling networks in the cell, the mTORC1 pathway.

Growth factors and cytokines activate mTORC1 through the PI3K-AKT signaling pathway [17]. Signals from these stimuli activate serine-threonine kinase AKT, leading to the phosphorylation and inactivation of the TSC2 component of tuberous sclerosis complex (TSC), an mTORC1 inhibitor. TSC inactivation results in the stimulation of RHEB GTPase, which activates mTORC1 [18] (Figure 1). AKT can also activate mTORC1 directly, in a TSC-independent way, by phosphorylating and inactivating the mTORC1 inhibitory component, PRAS40 [19]. Amino acids activate mTORC1 through a number of specific amino acid sensors. These sensors in turn stimulate GATOR1/GATOR2 complexes, containing a number of proteins, including tumor suppressors such as DEPDC5 [20]. Stimulation of GATORs further induces Rag GTPases (RagA/B and RagC/D), leading to mTORC1 activation (Figure 1) [21]. In contrast, low energy levels and hypoxic conditions result in mTORC1 inhibition via stimulation of AMP-activated protein kinase (AMPK) [22,23]. DNA damaging agents also suppress mTORC1 activity, in part by activation of the most powerful mTORC1 antagonist, p53 (Figure 1) [8,24].

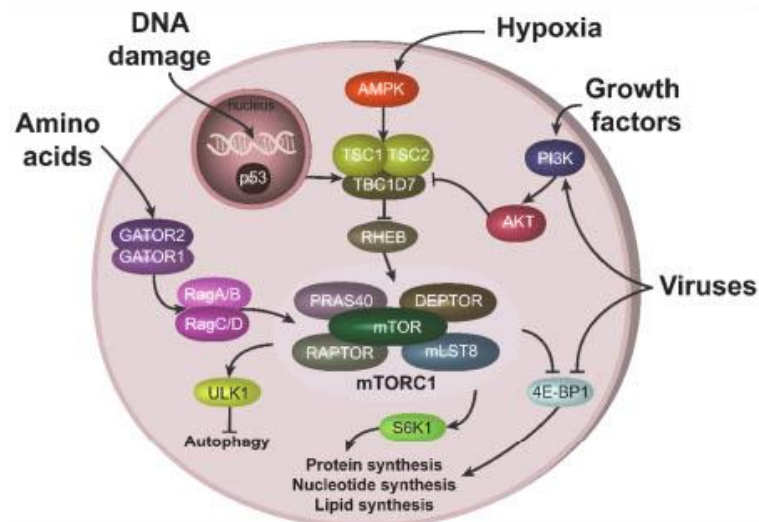


Figure 1. Upstream and downstream mTORC1 signaling. mTORC1 can be stimulated by various cues. Amino acids activate mTORC1 through GATOR1/GATOR2 complexes and RAGs. Growth factors recruits PI3K to the plasma membrane, which results in subsequent AKT activation. AKT can stimulate mTORC1 either directly through the phosphorylation of its component PRAS40, or via phosphorylation and inhibition of TSC1 from the TSC complex, which leads to RHEB and mTORC1 activations. Under hypoxic condition, mTORC1 is repressed due to TSC activation via AMPK. TSC can also be stimulated (i.e., mTORC1 repressed) via p53 pathway during DNA damage. Viruses can activate mTORC1, especially at the late stage of infection, both upstream, via PI3K/AKT, or acting on downstream mTORC1 target 4E-BP1.

Viruses can both activate and inhibit the mTORC1 pathway, depending on the type of the virus, stage on infection and cells in contact with the virus (reviewed in [4]). HIV-1 infection is generally associated with inhibition of mTORC1 in CD4 T cells at the early stage of infection and the activation of mTORC1 at the late stage (see below). This behavior is often related with the ability of mTORC1 to inhibit autophagy. Indeed, at the early state of infection, host cells try to activate autophagy and/or apoptosis to eliminate the spread of the virus; therefore, they need to suppress mTORC1 activity. In contrast, at the late state of infection, when host cells have “lost the battle”, viruses try to activate mTORC1 in order to suppress autophagy. Interestingly, viruses can target the mTORC1 pathway both upstream and downstream of mTORC1. For instance, Kaposi’s sarcoma-associated herpes virus (KSHV), related to the AIDS defining cancer of the same name, activates mTORC1 upstream through the PI3K-AKT axis [25], while herpesviruses can also act downstream of the mTORC1 node, mainly through stimulation of phosphorylation and inactivation of 4E-BP1 (Figure 1) [4].

3. mTORC1 in the Immune System

mTORC1 plays a central role in the metabolism, differentiation and effector functions of immune cells [26,27]. Here, we will only briefly describe the role of mTORC1 in B and T lymphocytes, as they are the most relevant during HIV-1 infection and in the development of HIV-1-related diseases (e.g., B-cell lymphomas). Many more details can be found in exhaustive recent reviews about the role of mTORC1 in B cells [28], T cells [29] and NK, macrophages and dendritic cells [30].

T lymphocytes in the resting state are catabolic and use autophagy to produce molecules necessary for protein synthesis and energy. After activation, T cells become anabolic and switch to glycolysis to

produce energy and various substrates for proliferation. A transition from the resting to active state requires the upregulation of metabolic pathways controlled by mTORC1.

CD4 T cells, which are the primary target of HIV-1, are effector T cells which can be differentiated into distinct effector lineages (Th1, Th2, Th17) in response to various inflammatory cytokines. mTORC1 helps this differentiation process by promoting glycolysis and lipid biosynthesis [31–34]. mTORC1-deficient T cells have an impaired ability to differentiate [31]. Similarly, T cells lacking the mTORC1 activator RHEB also fail to differentiate into Th1 and Th17 [32]. Alternatively, the deletion of mTORC1 inhibitor TSC1 results in enhanced mTORC1 activity, leading to elevated Th1 and Th17 differentiation and multiorgan inflammation in mice [35]. In addition, a deficiency in the leucine transporter LAT1 [36] and glutamine transporter ASCT2 [37] impairs Th1 and Th17 differentiation in a mTORC1-dependent manner.

CD8 T cells have the ability to kill cancer cells or virally-infected cells (effector cytotoxic T cells), and maintain long-term memory (memory T cells). Increased mTORC1 activity is beneficial for the generation of effector CD8 T cells. In contrast, active mTORC1 downregulates memory T cell formation, resulting in a decreased response to secondary immunization [38–40].

Tregs are immune cells that negatively control both cytotoxic effector CD8 T cells and effector CD4 T cells. mTORC1 activity has negative effects on Treg generation [41], while it is positively correlated with the inhibitory functions of Tregs towards other T cells [42]. Active mTORC1 promotes the conversion of Tregs to effector-like T cells and further impairs Treg stability and function [35,43,44]. Toll-like receptor signals, which promote Treg proliferation, increase mTORC1 signaling, glycolysis and expression of glucose transporter Glut1 [43].

B cells undergo multiple steps of maturation before becoming plasma cells and acquiring antibody-secreting capacity, which is critical for defense against recurrent infections. mTORC1 is highly active at the earliest stages of B cell development, and its activity reduces during B cell maturation [45–47]. Homozygous deletion of mTORC1 component RAPTOR prior to lineage specification results in a total lack of B cell maturation [45,46].

Thus, mTORC1 senses both intra- and extra- cellular cues, but also the immune microenvironment, in order to influence the differentiation and maturation of immune cells.

4. HIV-1 Modulates mTORC1 Activity

HIV-1 infection, similarly to other viral infections, generally increases mTORC1 activity both in productively-infected and bystander host cells, thus promoting successful viral integration and replication [48–51] (graphical abstract). Moreover, mTORC1 activity is required for optimal synthesis of viral proteins, e.g., Gag [52]. mTORC1 is also activated following HIV-1 infection in peripheral blood mononuclear cells and in several model cell lines such as Jurkat cells, HeLa and HEK293 [48,49,52,53]. Nevertheless, HIV-1 is not able to sustain mTORC1 activity in conditions of nutrient deprivation or upon pharmacological inhibition of mTORC1.

When amino acids are present, GTP-loaded RagA and RagB induce mTORC1 translocation from the cytoplasm to late endosomes/lysosomes (LEs) [7]. Interestingly, Gag can also be found at late endosomes during HIV-1 egress [54]. Nutrient starvation or oxidative stress result in the accumulation of a pool of mTOR-associated LEs at a juxtanuclear position. HIV-1 is capable of redistributing perinuclear clusters of mTORC1-associated LEs [52]. This HIV-1 activity depends on the RagA and RagB, which can interact with Vif and Gag, although nothing is known about the molecular mechanisms of these interactions. Depletion of RagA and RagB reduces virus production and results in the accumulation of viral particles at the plasma membrane [52].

Although HIV-1 does not enter or replicate in neurons, its infection of brain glia cells can induce neurotoxicity and inflammation. mTORC1 activation is involved in the interaction between the proinflammatory extracellular matrix protein osteopontin in the brain and HIV-1 Env proteins, which stimulates neurite growth [55].

mTORC1 activity can be modulated both by the entire HIV-1 and by its different proteins (Figure 2). For example, mTORC1 can be activated by treatment with HIV-1 Nef, Env or Tat proteins, resulting in efficient viral replication and the generation of new virions [48,49,51,56]. In contrast, combined treatment of neuronal cells with HIV-1 Tat and methamphetamine (a stimulant drug which increases exposure to HIV-1) results in the inhibition of mTORC1 activity [57]. As Tat is present in high concentrations in patient blood serum [58,59], and because of Tat's ability to enter almost any cell in the human body due to its cell penetration domain [60], this viral transactivator can probably remotely regulate mTORC1 activity in many noninfected cells.

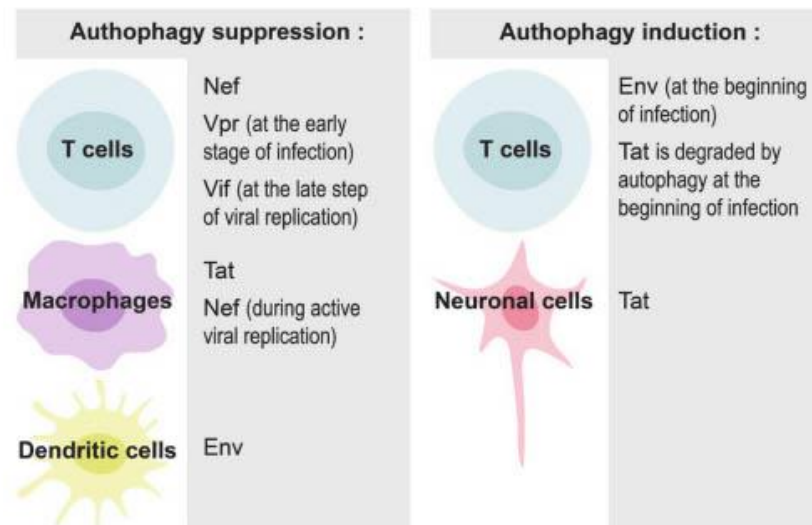


Figure 2. Implication of various HIV-1 proteins in the modulation of autophagy in different cells.

5. Regulation of Autophagy by HIV-1

mTORC1 is the negative regulator of the major cellular catabolic process, i.e., autophagy [61,62]. Autophagy plays a critical role in maintaining cellular homeostasis, but is also related to many pathological conditions including cancer and neurodegeneration [63]. Recent studies revealed an important role of autophagy in the context of HIV-1 infection in T cells, macrophages, dendritic cells and neuronal cells [64,65] (graphical abstract, Figure 2).

Autophagy can play both pro- or anti-viral roles, depending on the stage of HIV-1 infection and the cells in contact with virus and its components. HIV-1 requires early, nondegradative autophagic events for its replication, probably because the autophagosomal membrane provides a scaffold for virus assembly [66]. The entire virus and its proteins, in particular Tat, Nef and Env, can induce autophagy in infected cells to maximize HIV-1 production. At the same time, the virus has developed multiple strategies to escape the degradation of newly-synthesized viral proteins [64]. For example, Nef can interact with BECLIN-1, an autophagy initiation protein, to inhibit autophagosome maturation in macrophages and T cells [66–69]. Nef/BECLIN-1 interaction inhibits autophagy at the transcriptional level by preventing nuclear translocation of the pro-autophagic factor TFEB in an mTORC1-dependent manner [67]. Nef can also block autophagosome formation by increasing the interaction between BECLIN-1 and its inhibitor BCL2, an interaction that requires E3-ubiquitin ligase PRKN [68]. HIV-1 proteins can also disrupt autophagy in uninfected cells. For example, HIV-1 Tat inhibits autophagy induction in noninfected primary macrophages through AKT activation [50].

At the initial steps of infection, HIV-1 envelope proteins at the surface of the virus bind to CD4 receptors, mainly CCR5, initiating autophagy in CD4 T cells [70]. This autophagic process represents an anti-HIV response of the host cell, because it selectively degrades HIV-1 Tat [71,72]. Similarly, the induction of autophagy in macrophages with dual inhibitors of PI3K and mTORC1 results in the degradation of intracellular viral particles and the reduction of viral release [73]. The virus has, however, evolved to counteract autophagy [72]. Different HIV-1 proteins participate in this inhibition in different cells and at various steps of infection. For example, Vpr tries to inhibit autophagy in T cells already at the early stages of infection to facilitate efficient viral replication [74]; Vif inhibits autophagy at the late steps of viral replication in CD4+ T cells [75]; Nef blocks autophagy in macrophages and in T cells [67,68]; HIV-1 Tat blocks autophagy in bystander macrophages [50], while envelope proteins inhibit autophagy through mTORC1 activation in dendritic cells, but not in macrophages and CD4+ T cells [56].

The regulation of autophagy was also studied in the context of HIV-1 infection in neuronal cells, as HIV-1-associated neurocognitive disorders lead to increased mortality in HIV-1 patients [76]. HIV-1 Tat induces autophagy and increases neurotoxicity [77–79]. HIV-1 Tat can even trigger neuronal cell death when combined with methamphetamine [57,80,81]. HIV-1 Tat also plays a role in the regulation of mitophagy, a specialized form of autophagy, which removes damaged mitochondria. Treatment with HIV-1 Tat of primary microglial cells, the key target for HIV-1 infection in the central nervous system, results in the accumulation of damaged mitochondria and increased expression of mitophagy-signaling proteins PINK1 and PARKIN. However, even though mitophagy was induced, mitophagosomes fail to fuse with lysosomes and accumulate in the cells, resulting in overall defective mitophagy and subsequent neuroinflammation [82].

6. mTORC1 in HIV-1 Latency

Combination antiretroviral therapy (cART), which efficiently controls viral replication, remarkably improves the quality of life of HIV-1-positive individuals, although this therapy is not available to all people living with HIV-1, particularly in countries with limited resources. In addition, elevated risks of chronic inflammation [83], asynchronous muscle aging [84], the increased occurrence of various cancers [85,86] and developing HIV-1 drug resistance [87,88] create an urgent need to find a cure rather than to control of HIV-1 replication.

One of the major problems preventing the complete eradication of HIV-1 is the existence of latent reservoirs, “a cell type or anatomical site in which replication-competent form of virus accumulates and persists in spite of long periods of ART-suppression viremia” [89,90]. HIV-1 cellular reservoirs consist of resting memory CD4+ T cells, myeloid cells, macrophages and dendritic cells (DC) which allow the virus to survive and replicate [91]. Although the existence of anatomical HIV-1 reservoirs remains debated, the lymphoid tissues (spleen, thymus, lymph nodes and gut-associated lymphoid tissues), which are the most important sites of viral replication during infection, are considered the most prominent reservoirs.

Two opposite strategies are proposed to disrupt HIV-1 latency: “shock and kill” and “block and lock” (deep latency) [92,93]. In the “shock and kill” approach, the latent HIV-1 is reactivated (“shock”) by latency reversal agents (LRAs). The re-activated cells are further “killed” either by the attack of the host immune system or by the cytotoxic effect of HIV-1 itself, while uninfected cells are protected against viral infection by cART. Unfortunately, none of the LRAs tested so far have shown a significant effect on the viral reservoir in clinical trials [94]. In addition, agents inducing global T cell activation induced severe adverse reactions, mainly through inflammation, and were abandoned after several clinical trials. “Shocking” alone seems to be insufficient to eliminate latent T-cell reservoir. Effective “killing” strategies need to be developed to optimize this approach. An alternative “block and lock” strategy aims to bring the proviral HIV-1 to a deeply silenced state so that HIV-1 will not rebound, even if cART is discontinued. Recent findings demonstrate that manipulation of the mTORC1 pathway is important for the efficient development of both strategies.

The first notion of the importance of mTORC1 in HIV-1 latency came from the study by Besnard et al., who demonstrated that both mTORC1 and mTORC2 complexes are essential for HIV-1 reactivation from latency [95]. Accordingly, inhibitors which block both mTORC1 and mTORC2, pp242 and Torin1, prevent HIV-1 reactivation in primary CD4+ T cells from uninfected donors when infected *ex vivo* [49, 95]. Rapamycin, which mostly blocks mTORC1, is a less effective inhibitor of HIV-1 reactivation. Moreover, in CD4+ T cells from aviremic HIV-1-positive donors under cART, HIV-1 proviral reactivation is unaffected by the addition of rapamycin, even if the drug downregulates markers of toxicity related to inflammation [96]. At the same time, active mTORC1 might be important for natural HIV-1 suppression control, because mTORC1 activation was also detected in HIV-1 elite controllers, a rare (<1%) group of HIV-1-infected patients who do not take antiretroviral therapy (ART) and do not develop AIDS [97].

The use of rapamycin and pan-inhibitors of mTORC1 is associated with multiple adverse effects; therefore, targeting the mTORC1 signaling pathway and not mTOR kinase itself appears to be an attractive alternative approach. Recent genome-wide CRISPR screening for the host factors required for HIV-1 latency identified two upstream mTORC1 inhibitory genes: TSC1 and DEPDC5; both are known negative regulators of mTORC1, but they suppress mTORC1 via different upstream branches. TSC1, a TSC complex member, inhibits mTORC1 and maintains HIV-1 latency by downregulation of RHEB, whereas DEPDC5, GATOR1 component, does so via suppression of RagA [98]. Therefore, targeting of TSC1 and DEPDC5 might be a useful strategy in metabolic inhibition to “block and lock” the latent reservoir.

In an effort to optimize the “shock and kill” approach, recent findings revealed that interaction of infected CD4+ T cells with DC could activate latent HIV-1 [99]. The contact with dendritic cells also activates the PI3K-Akt-mTOR pathway in CD4+ T cells and contributes to HIV-1 purge.

CD4+ T cells express on their surface various receptors that target them to different peripheral tissues in the body. One such receptor, CCR6, directs Th17 T-helper cells to the gut. The gut-associated lymphoid tissues are considered an important site of HIV-1 replication and a viral reservoir. The analysis of colon biopsies taken from HIV-1 patients under cART revealed that CCR6+ Th17-polarized CD4+ T cells express more mTOR, and that mTOR phosphorylation is also increased in these cells [53]. The use of mTOR inhibitors limited HIV-1 replication in gut-homing Th17 cells during reverse transcription and prior integration, with mTORC1/mTORC2 inhibitor INK128 being more effective than rapamycin. Thus, mTOR inhibitors could have a potential beneficial effect in decreasing HIV-1 reservoirs and restoring Th17 immunity in intestines during cART.

Finally, recent mathematical models predict that the persistence of a majority of HIV-1 infected cells is due to cellular proliferation rather than HIV-1 replication [100]; therefore, reducing cell proliferation could decrease the size of the HIV-1 reservoir. mTORC1 is a major regulator of cellular proliferation, and the specific targeting of this pathway in HIV-1 latent cells appears to be a promising approach by which to cure AIDS.

7. mTORC1 Pathway in HIV-1-Related Diseases

HIV-1-infected individuals have an elevated risk of developing of so-called AIDS-defining cancers: Kaposi's sarcoma, B-cell lymphomas and cervical cancer [101] (Figure 3). Current antiretroviral therapy efficiently suppresses HIV-1, but high incidence of HIV-1-associated malignancies persists, although this has sharply declined in developed countries since the introduction of cART [86]. Thus, it is important to understand the mechanisms of the development of HIV-1-related diseases, even in patients under cART.

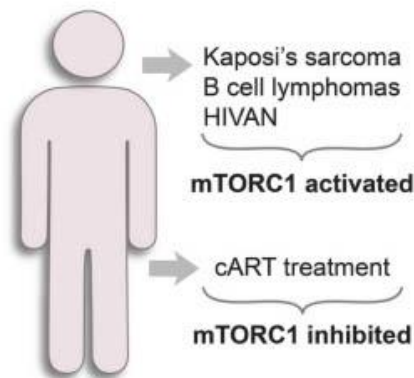


Figure 3. mTORC1 status in HIV-1-related diseases.

Kaposi's sarcoma is caused by a herpesvirus (KSHV or HHV8). Ectopic expression of the viral proteins K1 and vGPCR in primary human umbilical vein endothelial cells (HUEVC) was shown to activate PI3K-AKT-mTOR pathway, which plays a central role in Kaposi's sarcomagenesis [51,102–104]. KSHV infection selectively upregulates mTORC1 signaling in primary human endothelial cells (LECs), but not in blood endothelial cells [105]. KSHV infection of LECs makes these cells dependent on the mTORC1 pathway for their survival, because the treatment of these cells with rapamycin triggers apoptosis. Interestingly, primary effusion lymphoma, a tumor of B cell origin linked to KSHV infection, was strongly inhibited by rapamycin both in vitro and in mouse models [106].

Although HIV-1 does not infect B lymphocytes, the most frequent AIDS-related lymphomas (ARLs) are always of B cell origin [107]. One of the reasons for these malignancies might be the genomic instability and chromatin remodeling in B cells caused by the entire HIV-1 and HIV-1 Tat protein, which is present in the blood of HIV-1-infected individuals and can penetrate different cells, including B cells [59,108]. Another possibility might involve the mTORC1 pathway, because studies in cell lines, primary cultures and tissue samples from patients have shown that mTORC1 is hyperactivated in diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Hodgkin lymphoma (HL), Burkitt lymphoma (BL) and anaplastic large cell lymphoma [109–114]. In order to inhibit mTORC1, rapalogues were used in clinical trials in MCL, DLBCL, HL and indolent lymphoma, but failed to provide substantial benefits for patients [115–119]. This failure is not specific to B-cell malignancies, but also concerns many other cancers. The main reason for rapalogue inefficiency is related to the activation of alternative proliferation pathways and genetic and functional intratumoral heterogeneity of mTORC1 activity [120]. Recent advances in the development of alternative mTOR inhibitors (pan-inhibitors and dual inhibitors) and the results of combination therapies with DNA damaging anticancer drugs should bring an essential breakthrough in the treatment of these cancers [8]. Indeed, the simultaneous inhibition of Akt with Nelfinavir and MK-2206 and mTORC1 with rapamycin showed a synergistic effect in suppressing DLBCL [113,121].

Similarly, the combination of mTORC1 inhibitors with different anti-HIV-1 drugs showed significant effects in suppressing viral replication, even rendering the antiviral drug-resistant strains sensitive to medication in T cells, PBMCs and in a humanized mouse model [15,48,122,123].

One of the frequent complications occurring typically in young adults of African ancestry with advanced HIV-1 is a HIV-associated nephropathy (HIVAN). The role of mTORC1 in HIVAN was addressed both in HIVAN murine models and upon analysis of tissue samples obtained from renal cortical sections of HIVAN patients [124–128]. Both HIVAN mice and HIVAN patients exhibit the activation of the mTORC1 pathway, as shown by elevated phosphorylation of mTOR itself and its substrates p70S6K1 and 4EBP1. Treatment with rapamycin not only attenuated the HIVAN phenotype,

but also partially inhibited tubular cell protein synthesis, specific miRNA expression and renal lesions [124–126]. In addition, inhibition of the mTORC1 pathway in HIVAN mice resulted in the downregulation of renal tissue p53 expression, and provided protection against p53-mediated oxidative kidney cell injury [127].

Besides the suppression of HIV-1 replication, cART drugs have several additional pharmacological activities that interfere with the mTORC1 pathway. A canonical cART is a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) with one of the following drugs: HIV-protease inhibitors (PIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs) or HIV-integrase strand transfer inhibitors (INSTIs) [129]. Other FDA-approved classes of antiretroviral drugs are fusion inhibitors, CCR5 antagonists, CD4 post-attachment inhibitors [129]. PIs act via different mechanisms, mainly associated with the inhibition of cellular proteasome machinery (Hsp90 inhibition, ER stress). This leads to decreased PI3K-AKT signaling and downstream mTORC1 downregulation [130–132] (Figure 3). CCR5 antagonists also disrupt the PI3K-AKT-mTORC1 pathway [133,134]. In contrast, the side action of NRTIs can implicate active mTORC1 signaling in the host cell, although a direct link between them has not yet been established. The administration of NRTIs may cause severe mitochondrial toxicity [135], related to their moderate affinity to host DNA polymerases, especially the mitochondrial polymerase γ [136]. Mitochondrial toxicity can lead to the inhibition of late stages of autophagy mediated by active mTORC1 signaling, as shown for zidovudine (NRTI) in myocytes [137]. Another study demonstrated that stavudine (NRTI) could activate mTORC1 signaling in a mouse model of peripheral neuropathic pain [138]. Treatment with rapamycin attenuates persistent neuropathic pain in mice. Additionally, several cART drugs, being substrates of the CYP450 enzyme family, can modify the metabolism of mTORC1 inhibitors when used concomitantly, leading to altered mTORC1 axis regulation in patients [139].

8. Concluding Remarks

As a central integrator of various stimuli from intra- and extra- cellular environments, mTORC1 plays a vital role both in physiological and pathological conditions, including viral infections and cancers. In the immune system, mTORC1 senses cues from the immune microenvironment and influences the differentiation and maturation of immune cells. During HIV-1 infection, the mTORC1 pathway can be modulated not only by the entire virus, but also by its proteins. A combination of anti-HIV drugs with inhibitors of the mTORC1 pathway might be an efficient measure to cure, rather than control, HIV-1 replication.

9. Outstanding Questions

What are the molecular mechanisms of the interaction of HIV-1 and its separate proteins with the components of the mTORC1 pathway?

Are there ways to efficiently and specifically control mTORC1 suppression (and autophagy activation) in host cells?

Will a new generation of mTORC1 inhibitors provide additional benefits in overcoming of HIV-1 latency?

To what extent is the mTORC1 pathway involved in the appearance and persistence of HIV-related malignancies in patients under cART?

Funding: This research was funded by French agencies INSERM (Enviburkitt) and ANRS. B.A. was funded by PhD exchange program between Kazakhstan and France.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Eisenreich, W.; Rudel, T.; Heesemann, J.; Goebel, W. How Viral and Intracellular Bacterial Pathogens Reprogram the Metabolism of Host Cells to Allow Their Intracellular Replication. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 42. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Mehrbod, P.; Ande, S.R.; Alizadeh, J.; Rahimizadeh, S.; Shariati, A.; Malek, H.; Hashemi, M.; Glover, K.K.M.; Sher, A.A.; Coombs, K.M.; et al. The roles of apoptosis, autophagy and unfolded protein response in arbovirus, influenza virus, and HIV infections. *Virulence* **2019**, *10*, 376–413. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Ahmad, L.; Mostowy, S.; Sancho-Shimizu, V. Autophagy-Virus Interplay: From Cell Biology to Human Disease. *Front. Cell Dev. Biol.* **2018**, *6*, 155. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Le Sage, V.; Cinti, A.; Amorim, R.; Moulard, A. Adapting the Stress Response: Viral Subversion of the mTOR Signaling Pathway. *Viruses* **2016**, *8*, 152. [\[CrossRef\]](#)
5. Votteler, J.; Schubert, U. Human Immunodeficiency Viruses: Molecular Biology. In *Encyclopedia of Virology*; Elsevier: Amsterdam, The Netherlands, 2008; pp. 517–525.
6. Burdick, R.C.; Li, C.; Munshi, M.H.; Rawson, J.M.O.; Nagashima, K.; Hu, W.S.; Pathak, V.K. HIV-1 uncoats in the nucleus near sites of integration. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 5486–5493. [\[CrossRef\]](#)
7. Saxton, R.A.; Sabatini, D.M. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **2017**, *169*, 361–371. [\[CrossRef\]](#)
8. Ma, Y.; Vassetzky, Y.; Dokudovskaya, S. mTORC1 pathway in DNA damage response. *Biochim. Biophys. Acta—Mol. Cell Res.* **2018**, *1865*, 1293–1311. [\[CrossRef\]](#)
9. Cervera, C.; Cofan, F.; Hernandez, C.; Soy, D.; Marcos, M.A.; Sandemonte, G.; Bodro, M.; Moreno, A.; Diekmann, F.; Campistol, J.M.; et al. Effect of mammalian target of rapamycin inhibitors on cytomegalovirus infection in kidney transplant recipients receiving polyclonal antilymphocyte globulins: A propensity score-matching analysis. *Transpl. Int.* **2016**, *29*, 1216–1225. [\[CrossRef\]](#)
10. Piñana, J.L.; Perez-Pitarch, A.; Guglieri-Lopez, B.; Giménez, E.; Hernandez-Boluda, J.C.; Terol, M.J.; Ferriols-Lisart, R.; Solano, C.; Navarro, D. Sirolimus exposure and the occurrence of cytomegalovirus DNAemia after allogeneic hematopoietic stem cell transplantation. *Am. J. Transplant.* **2018**, *18*, 2885–2894. [\[CrossRef\]](#)
11. Mannick, J.B.; Morris, M.; Hockey, H.-U.P.; Roma, G.; Beibel, M.; Kulmatycki, K.; Watkins, M.; Shavlakadze, T.; Zhou, W.; Quinn, D.; et al. TORC1 inhibition enhances immune function and reduces infections in the elderly. *Sci. Transl. Med.* **2018**, *10*, eaag1564. [\[CrossRef\]](#)
12. Heredia, A.; Amoroso, A.; Davis, C.; Le, N.; Reardon, E.; Dominique, J.K.; Klingebiel, E.; Gallo, R.C.; Redfield, R.R. Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV -chemokines: An approach to suppress R5 strains of HIV-1. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10411–10416. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Donia, M.; McCubrey, J.A.; Bendtzen, K.; Nicoletti, F. Potential use of rapamycin in HIV infection. *Br. J. Clin. Pharmacol.* **2010**, *70*, 784–793. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Nicoletti, F.; Fagone, P.; Meroni, P.; McCubrey, J.; Bendtzen, K. mTOR as a multifunctional therapeutic target in HIV infection. *Drug Discov. Today* **2011**, *16*, 715–721. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Heredia, A.; Le, N.; Gartenhaus, R.B.; Sausville, E.; Medina-Moreno, S.; Zapata, J.C.; Davis, C.; Gallo, R.C.; Redfield, R.R. Targeting of mTOR catalytic site inhibits multiple steps of the HIV-1 lifecycle and suppresses HIV-1 viremia in humanized mice. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 9412–9417. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Kocaturk, N.M.; Gozuacik, D. Crosstalk Between Mammalian Autophagy and the Ubiquitin-Proteasome System. *Front. Cell Dev. Biol.* **2018**, *6*, 128. [\[CrossRef\]](#)
17. Manning, B.D.; Toker, A. AKT/PKB Signaling: Navigating the Network. *Cell* **2017**, *169*, 381–405. [\[CrossRef\]](#)
18. Dibble, C.C.; Elis, W.; Menon, S.; Qin, W.; Klekota, J.; Asara, J.M.; Finan, P.M.; Kwiatkowski, D.J.; Murphy, L.O.; Manning, B.D. TBC1D7 Is a Third Subunit of the TSC1-TSC2 Complex Upstream of mTORC1. *Mol. Cell* **2012**, *47*, 535–546. [\[CrossRef\]](#)
19. Vander Haar, E.; Lee, S.; Bandhakavi, S.; Griffin, T.J.; Kim, D.-H. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat. Cell Biol.* **2007**, *9*, 316–323. [\[CrossRef\]](#)
20. Dokudovskaya, S.; Rout, M.P. SEA you later all-GATOR—a dynamic regulator of the TORC1 stress response pathway. *J. Cell Sci.* **2015**, *128*, 2219–2228. [\[CrossRef\]](#) [\[PubMed\]](#)

21. Wolfson, R.L.; Sabatini, D.M. The Dawn of the Age of Amino Acid Sensors for the mTORC1 Pathway. *Cell Metab.* **2017**, *26*, 301–309. [\[CrossRef\]](#)
22. Howell, J.J.; Hellberg, K.; Turner, M.; Talbot, G.; Kolar, M.J.; Ross, D.S.; Hoxhaj, G.; Saghatelian, A.; Shaw, R.J.; Manning, B.D. Metformin Inhibits Hepatic mTORC1 Signaling via Dose-Dependent Mechanisms Involving AMPK and the TSC Complex. *Cell Metab.* **2017**, *25*, 463–471. [\[CrossRef\]](#)
23. Brugarolas, J.; Lei, K.; Hurley, R.L.; Manning, B.D.; Reiling, J.H.; Hafen, E.; Witters, L.A.; Ellisen, L.W.; Kaelin, W.G. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev.* **2004**, *18*, 2893–2904. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Awasthi, P.; Foiani, M.; Kumar, A. ATM and ATR signaling at a glance. *J. Cell Sci.* **2016**, *129*, 1285. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Montaner, S.; Sodhi, A.; Pece, S.; Mesri, E.A.; Gutkind, J.S. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor promotes endothelial cell survival through the activation of Akt/protein kinase B. *Cancer Res.* **2001**, *61*, 2641–2648.
26. Powell, J.D.; Pollizzi, K.N.; Heikamp, E.B.; Horton, M.R. Regulation of Immune Responses by mTOR. *Annu. Rev. Immunol.* **2012**, *30*, 39–68. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Linke, M.; Fritsch, S.D.; Sukhbaatar, N.; Hengstschläger, M.; Weichhart, T. mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett.* **2017**, *591*, 3089–3103. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Iwata, T.N.; Ramirez-Komo, J.A.; Park, H.; Iritani, B.M. Title: Control of B Lymphocyte Development and Functions by the mTOR Signaling Pathways. *Cytokine Growth Factor Rev.* **2017**, *35*, 47–62. [\[CrossRef\]](#)
29. Liu, C.; Chapman, N.M.; Karmaus, P.W.E.; Zeng, H.; Chi, H. mTOR and metabolic regulation of conventional and regulatory T cells. *J. Leukoc. Biol.* **2015**, *97*, 837–847. [\[CrossRef\]](#)
30. Weichhart, T.; Hengstschläger, M.; Linke, M. Regulation of innate immune cell function by mTOR. *Nat. Rev. Immunol.* **2015**, *15*, 599–614. [\[CrossRef\]](#)
31. Delgoffe, G.M.; Kole, T.P.; Zheng, Y.; Zarek, P.E.; Matthews, K.L.; Xiao, B.; Worley, P.F.; Kozma, S.C.; Powell, J.D. The mTOR Kinase Differentially Regulates Effector and Regulatory T Cell Lineage Commitment. *Immunity* **2009**, *30*, 832–844. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Delgoffe, G.M.; Pollizzi, K.N.; Waickman, A.T.; Heikamp, E.; Meyers, D.J.; Horton, M.R.; Xiao, B.; Worley, P.F.; Powell, J.D. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat. Immunol.* **2011**, *12*, 295–303. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Kurebayashi, Y.; Nagai, S.; Ikejiri, A.; Ohtani, M.; Ichiyama, K.; Baba, Y.; Yamada, T.; Egami, S.; Hoshii, T.; Hirao, A.; et al. PI3K-Akt-mTORC1-S6K1/2 Axis Controls Th17 Differentiation by Regulating Gfi1 Expression and Nuclear Translocation of ROR γ . *Cell Rep.* **2012**, *1*, 360–373. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Yang, K.; Shrestha, S.; Zeng, H.; Karmaus, P.W.E.; Neale, G.; Vogel, P.; Guertin, D.A.; Lamb, R.F.; Chi, H. T Cell Exit from Quiescence and Differentiation into Th2 Cells Depend on Raptor-mTORC1-Mediated Metabolic Reprogramming. *Immunity* **2013**, *39*, 1043–1056. [\[CrossRef\]](#)
35. Park, Y.; Jin, H.-S.; Lopez, J.; Elly, C.; Kim, G.; Murai, M.; Kronenberg, M.; Liu, Y.-C. TSC1 regulates the balance between effector and regulatory T cells. *J. Clin. Investig.* **2013**, *123*, 5165–5178. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Sinclair, L.V.; Rolf, J.; Emslie, E.; Shi, Y.-B.; Taylor, P.M.; Cantrell, D.A. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat. Immunol.* **2013**, *14*, 500–508. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Nakaya, M.; Xiao, Y.; Zhou, X.; Chang, J.-H.; Chang, M.; Cheng, X.; Blonska, M.; Lin, X.; Sun, S.-C. Inflammatory T Cell Responses Rely on Amino Acid Transporter ASCT2 Facilitation of Glutamine Uptake and mTORC1 Kinase Activation. *Immunity* **2014**, *40*, 692–705. [\[CrossRef\]](#)
38. Araki, K.; Turner, A.P.; Shaffer, V.O.; Gangappa, S.; Keller, S.A.; Bachmann, M.F.; Larsen, C.P.; Ahmed, R. mTOR regulates memory CD8 T-cell differentiation. *Nature* **2009**, *460*, 108–112. [\[CrossRef\]](#)
39. Pearce, E.L.; Walsh, M.C.; Cajas, P.J.; Harms, G.M.; Shen, H.; Wang, L.-S.; Jones, R.G.; Choi, Y. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* **2009**, *460*, 103–107. [\[CrossRef\]](#)
40. Rao, R.R.; Li, Q.; Odunsi, K.; Shrikant, P.A. The mTOR Kinase Determines Effector versus Memory CD8 + T Cell Fate by Regulating the Expression of Transcription Factors T-bet and Eomesodermin. *Immunity* **2010**, *32*, 67–78. [\[CrossRef\]](#)
41. Zeng, H.; Yang, K.; Cloer, C.; Neale, G.; Vogel, P.; Chi, H. mTORC1 couples immune signals and metabolic programming to establish Treg-cell function. *Nature* **2013**, *499*, 485–490. [\[CrossRef\]](#)

42. De Rosa, V.; Galgani, M.; Porcellini, A.; Colamatteo, A.; Santopaolo, M.; Zuchegna, C.; Romano, A.; De Simone, S.; Procaccini, C.; La Rocca, C.; et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. *Nat. Immunol.* **2015**, *16*, 1174–1184. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Gerriets, V.A.; Kishton, R.J.; Johnson, M.O.; Cohen, S.; Siska, P.J.; Nichols, A.G.; Warmoes, M.O.; de Cubas, A.A.; MacIver, N.J.; Locasale, J.W.; et al. Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat. Immunol.* **2016**, *17*, 1459–1466. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Wei, J.; Long, L.; Yang, K.; Guy, C.; Shrestha, S.; Chen, Z.; Wu, C.; Vogel, P.; Neale, G.; Green, D.R.; et al. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. *Nat. Immunol.* **2016**, *17*, 277–285. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Iwata, T.N.; Ramírez, J.A.; Tsang, M.; Park, H.; Margineantu, D.H.; Hockenbery, D.M.; Iritani, B.M. Conditional Disruption of Raptor Reveals an Essential Role for mTORC1 in B Cell Development, Survival, and Metabolism. *J. Immunol.* **2016**, *197*, 2250–2260. [\[CrossRef\]](#)
46. Raybuck, A.L.; Cho, S.H.; Li, J.; Rogers, M.C.; Lee, K.; Williams, C.L.; Shlomchik, M.; Thomas, J.W.; Chen, J.; Williams, J.V.; et al. B Cell-Intrinsic mTORC1 Promotes Germinal Center-Defining Transcription Factor Gene Expression, Somatic Hypermutation, and Memory B Cell Generation in Humoral Immunity. *J. Immunol.* **2018**, *200*, 2627–2639. [\[CrossRef\]](#)
47. Zhang, S.; Pruitt, M.; Tran, D.; Du Bois, W.; Zhang, K.; Patel, R.; Hoover, S.; Simpson, R.M.; Simmons, J.; Gary, J.; et al. B Cell-Specific Deficiencies in mTOR Limit Humoral Immune Responses. *J. Immunol.* **2013**, *191*, 1692–1703. [\[CrossRef\]](#)
48. Zhang, H.S.; Zhang, Z.-G.; Zhou, Z.; Du, G.-Y.; Li, H.; Yu, X.-Y.; Huang, Y.-H. PKM2-mediated inhibition of autophagy facilitates Tat's inducing HIV-1 transactivation. *Arch. Biochem. Biophys.* **2017**, *625–626*, 17–23. [\[CrossRef\]](#)
49. Kumar, B.; Arora, S.; Ahmed, S.; Banerjee, A.C. Hyperactivation of mammalian target of rapamycin complex 1 by HIV-1 is necessary for virion production and latent viral reactivation. *FASEB J.* **2017**, *31*, 180–191. [\[CrossRef\]](#)
50. Van Grol, J.; Subauste, C.; Andrade, R.M.; Fujinaga, K.; Nelson, J.; Subauste, C.S. HIV-1 inhibits autophagy in bystander macrophage/monocytic cells through Src-Akt and STAT3. *PLoS ONE* **2010**, *5*, e11733. [\[CrossRef\]](#)
51. Xue, M.; Yao, S.; Hu, M.; Li, W.; Hao, T.; Zhou, F.; Zhu, X.; Lu, H.; Qin, D.; Yan, Q.; et al. HIV-1 Nef and KSHV oncogene K1 synergistically promote angiogenesis by inducing cellular miR-718 to regulate the PTEN/AKT/mTOR signaling pathway. *Nucleic Acids Res.* **2014**, *42*, 9862–9879. [\[CrossRef\]](#)
52. Cinti, A.; Le Sage, V.; Milev, M.P.; Valiente-Echeverría, F.; Crossie, C.; Miron, M.-J.; Panté, N.; Olivier, M.; Moulant, A.J. HIV-1 enhances mTORC1 activity and repositions lysosomes to the periphery by co-opting Rag GTPases. *Sci. Rep.* **2017**, *7*, 5515. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Planas, D.; Routy, J.; Ancuta, P.; Planas, D.; Zhang, Y.; Monteiro, P.; Goulet, J.; Gosselin, A. HIV-1 selectively targets gut-homing mechanisms Find the latest version: HIV-1 selectively targets gut-homing mechanisms. *JCI Insight* **2017**, *2*, e93230. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Molle, D.; Segura-Morales, C.; Camus, G.; Berlioz-Torrent, C.; Kjems, J.; Basyuk, E.; Bertrand, E. Endosomal trafficking of HIV-1 gag and genomic RNAs regulates viral egress. *J. Biol. Chem.* **2009**, *284*, 19727–19743. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Calvez, M.; Hseeh, G.; Benzer, S.; Brown, A.M. Osteopontin counters human immunodeficiency virus type 1-induced impairment of neurite growth through mammalian target of rapamycin and beta-integrin signaling pathways. *J. Neurovirol.* **2019**, *25*, 384–396. [\[CrossRef\]](#)
56. Blanchet, F.P.; Moris, A.; Nikolic, D.S.; Lehmann, M.; Cardinaud, S.; Stalder, R.; Garcia, E.; Dinkins, C.; Leuba, F.; Wu, L.; et al. Human immunodeficiency virus-1 inhibition of immunoamphisomes in dendritic cells impairs early innate and adaptive immune responses. *Immunity* **2010**, *32*, 654–669. [\[CrossRef\]](#)
57. Li, J.; Wang, W.; Tong, P.; Leung, C.-K.; Yang, G.; Li, Z.; Li, N.; Sun, X.; Han, Y.; Lu, C.; et al. Autophagy Induction by HIV-Tat and Methamphetamine in Primary Midbrain Neuronal Cells of Tree Shrews via the mTOR Signaling and ATG5/ATG7 Pathway. *Front. Neurosci.* **2018**, *12*, 921. [\[CrossRef\]](#)
58. Poggi, A.; Carosio, R.; Fenoglio, D.; Brenci, S.; Murdaca, G.; Setti, M.; Indiveri, E.; Scabini, S.; Ferrero, E.; Zocchi, M.R. Migration of V delta 1 and V delta 2 T cells in response to CXCR3 and CXCR4 ligands in healthy donors and HIV-1-infected patients: Competition by HIV-1 Tat. *Blood* **2004**, *103*, 2205–2213. [\[CrossRef\]](#)

59. Germini, D.; Tsfasman, T.; Klibi, M.; El-Amine, R.; Pichugin, A.; Iarovaia, O.V.; Bilhou-Nabera, C.; Subra, E.; Bou Saada, Y.; Sukhanova, A.; et al. HIV Tat induces a prolonged MYC relocalization next to IGH in circulating B-cells. *Leukemia* **2017**, *31*, 2515–2522. [\[CrossRef\]](#)
60. Kurneva, M.A.; Sheval, E.V.; Musinova, Y.R.; Vassetzky, Y.S. Tat basic domain: A “Swiss army knife” of HIV-1 Tat? *Rev. Med. Virol.* **2019**, *29*, e2031. [\[CrossRef\]](#)
61. Lahiri, V.; Hawkins, W.D.; Klionsky, D.J. Watch What You (Self-) Eat: Autophagic Mechanisms that Modulate Metabolism. *Cell Metab.* **2019**, *29*, 803–826. [\[CrossRef\]](#)
62. Dossou, A.S.; Basu, A. The Emerging Roles of mTORC1 in Macromanaging Autophagy. *Cancers (Basel)* **2019**, *11*, 1422. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Van Beek, N.; Klionsky, D.J.; Reggiori, F. Genetic aberrations in macroautophagy genes leading to diseases. *Biochim. Biophys. Acta—Mol. Cell Res.* **2018**, *1865*, 803–816. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Nardacci, R.; Ciccosanti, E.; Marsella, C.; Ippolito, G.; Piacentini, M.; Fimia, G.M. Role of autophagy in HIV infection and pathogenesis. *J. Intern. Med.* **2017**, *281*, 422–432. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Liu, Z.; Xiao, Y.; Torresilla, C.; Rassart, E.; Barbeau, B. Implication of Different HIV-1 Genes in the Modulation of Autophagy. *Viruses* **2017**, *9*, 389. [\[CrossRef\]](#)
66. Kyei, G.B.; Dinkins, C.; Davis, A.S.; Roberts, E.; Singh, S.B.; Dong, C.; Wu, L.; Kominami, E.; Ueno, T.; Yamamoto, A.; et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J. Cell Biol.* **2009**, *186*, 255–268. [\[CrossRef\]](#)
67. Campbell, G.R.; Rawat, P.; Bruckman, R.S.; Spector, S.A. Human Immunodeficiency Virus Type 1 Nef Inhibits Autophagy through Transcription Factor EB Sequestration. *PLoS Pathog.* **2015**, *11*, e1005018. [\[CrossRef\]](#)
68. Castro-Gonzalez, S.; Shi, Y.; Colomer-Lluch, M.; Song, Y.; Mowery, K.; Almodovar, S.; Bansal, A.; Kirchhoff, F.; Sparrer, K.; Liang, C.; et al. HIV-1 Nef counteracts autophagy restriction by enhancing the association between BECN1 and its inhibitor BCL2 in a PRKN-dependent manner. *Autophagy* **2020**, *26*, 1–25. [\[CrossRef\]](#)
69. Chang, C.; Young, L.N.; Morris, K.L.; von Bülow, S.; Schöneberg, J.; Yamamoto-Imoto, H.; Oe, Y.; Yamamoto, K.; Nakamura, S.; Stjepanovic, G.; et al. Bidirectional Control of Autophagy by BECN1 BAR Domain Dynamics. *Mol. Cell* **2019**, *73*, 339–353. [\[CrossRef\]](#)
70. Espert, L.; Denizot, M.; Grimaldi, M.; Robert-Hebmann, V.; Gay, B.; Varbanov, M.; Codogno, P.; Biard-Piechaczyk, M. Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *J. Clin. Investig.* **2006**, *116*, 2161–2172. [\[CrossRef\]](#)
71. Espert, L.; Varbanov, M.; Robert-Hebmann, V.; Sagnier, S.; Robbins, I.; Sanchez, F.; Lafont, V.; Biard-Piechaczyk, M. Differential role of autophagy in CD4 T cells and macrophages during X4 and R5 HIV-1 infection. *PLoS ONE* **2009**, *4*, e5787. [\[CrossRef\]](#)
72. Sagnier, S.; Daussey, C.E.; Borel, S.; Robert-Hebmann, V.; Faure, M.; Blanchet, F.P.; Beaumelle, B.; Biard-Piechaczyk, M.; Espert, L. Autophagy Restricts HIV-1 Infection by Selectively Degrading Tat in CD4 + T Lymphocytes. *J. Virol.* **2015**, *89*, 615–625. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Campbell, G.R.; Bruckman, R.S.; Herns, S.D.; Joshi, S.; Durden, D.L.; Spector, S.A. Induction of autophagy by PI3K/MTOR and PI3K/MTOR/BRD4 inhibitors suppresses HIV-1 replication. *J. Biol. Chem.* **2018**, *293*, 5808–5820. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Alfaisal, J.; Machado, A.; Galais, M.; Robert-Hebmann, V.; Arnauné-Pelloquin, L.; Espert, L.; Biard-Piechaczyk, M. HIV-1 Vpr inhibits autophagy during the early steps of infection of CD4 T cells. *Biol. Cell* **2019**, *111*, 308–318. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Borel, S.; Robert-Hebmann, V.; Alfaisal, J.; Jain, A.; Faure, M.; Espert, L.; Chaloin, L.; Paillart, J.-C.; Johansen, T.; Biard-Piechaczyk, M. HIV-1 viral infectivity factor interacts with microtubule-associated protein light chain 3 and inhibits autophagy. *AIDS* **2015**, *29*, 275–286. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Yakasai, A.M. Impact of Symptomatic HIV-Related Neurocognitive Disorders in Survival of HIV-Infected Individuals: A Systematic Review and Meta-Analyses. *J. Neuroinfect. Dis.* **2015**, *06*, 1–7. [\[CrossRef\]](#)
77. Fields, J.; Dumaop, W.; Elueteri, S.; Campos, S.; Serger, E.; Trejo, M.; Kosberg, K.; Adame, A.; Spencer, B.; Rockenstein, E.; et al. HIV-1 Tat Alters Neuronal Autophagy by Modulating Autophagosome Fusion to the Lysosome: Implications for HIV-Associated Neurocognitive Disorders. *J. Neurosci.* **2015**, *35*, 1921–1938. [\[CrossRef\]](#)
78. Bruno, A.P.; De Simone, E.L.; Iorio, V.; De Marco, M.; Khalili, K.; Sariyer, I.K.; Capunzo, M.; Nori, S.L.; Rosati, A. HIV-1 Tat protein induces glial cell autophagy through enhancement of BAG3 protein levels. *Cell Cycle* **2014**, *13*, 3640–3644. [\[CrossRef\]](#)

79. Wu, X.; Dong, H.; Ye, X.; Zhong, L.; Cao, T.; Xu, Q.; Wang, J.; Zhang, Y.; Xu, J.; Wang, W.; et al. HIV-1 Tat increases BAG3 via NF- κ B signaling to induce autophagy during HIV-associated neurocognitive disorder. *Cell Cycle* **2018**, *17*, 1614–1623. [\[CrossRef\]](#)
80. Zeng, X.F.; Li, Q.; Li, J.; Wong, N.; Li, Z.; Huang, J.; Yang, G.; Sham, P.C.; Li, S.B.; Lu, G. HIV-1 Tat and methamphetamine co-induced oxidative cellular injury is mitigated by N-acetylcysteine amide (NACA) through rectifying mTOR signaling. *Toxicol. Lett.* **2018**, *299*, 159–171. [\[CrossRef\]](#)
81. Qi, L.; Gang, L.; Hang, K.W.; Ling, C.H.; Xiaofeng, Z.; Zhen, L.; David Wai, Y.; Sang, P.W. Programmed neuronal cell death induced by HIV-1 tat and methamphetamine. *Microsc. Res. Tech.* **2011**, *74*, 1139–1144. [\[CrossRef\]](#)
82. Thangaraj, A.; Periyasamy, P.; Liao, K.; Bendi, V.S.; Callen, S.; Pendyala, G.; Buch, S. HIV-1 TAT-mediated microglial activation: Role of mitochondrial dysfunction and defective mitophagy. *Autophagy* **2018**, *14*, 1596–1619. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Sereti, I.; Krebs, S.J.; Phanuphak, N.; Fletcher, J.L.; Slike, B.; Pinyakorn, S.; O'Connell, R.J.; Rupert, A.; Chomont, N.; Valcourt, V.; et al. Persistent, Albeit Reduced, Chronic Inflammation in Persons Starting Antiretroviral Therapy in Acute HIV Infection. *Clin. Infect. Dis.* **2017**, *64*, 124–131. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Tran, T.; Guardigni, V.; Pencina, K.M.; Amato, A.A.; Floyd, M.; Brawley, B.; Mozeleski, B.; McKinnon, J.; Woodbury, E.; Heckel, E.; et al. Atypical Skeletal Muscle Profiles in Human Immunodeficiency Virus-Infected Asymptomatic Middle-Aged Adults. *Clin. Infect. Dis.* **2018**, *66*, 1918–1927. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Thrift, A.P.; Chiao, E.Y. Are Non-HIV Malignancies Increased in the HIV-Infected Population? *Curr. Infect. Dis. Rep.* **2018**, *20*, 22. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Shmakova, A.; Germini, D.; Vassetzky, Y. HIV-1, HAART and cancer: A complex relationship. *Int. J. Cancer* **2020**, *146*, 2666–2679. [\[CrossRef\]](#) [\[PubMed\]](#)
87. Hamers, R.L.; Rinke de Wit, T.F.; Holmes, C.B. HIV drug resistance in low-income and middle-income countries. *Lancet HIV* **2018**, *5*, e588–e596. [\[CrossRef\]](#)
88. Chimukangara, B.; Lessells, R.J.; Rhee, S.Y.; Giandhari, J.; Kharsany, A.B.M.; Naidoo, K.; Lewis, L.; Cawood, C.; Khanyile, D.; Ayalew, K.A.; et al. Trends in Pre-treatment HIV-1 Drug Resistance in Antiretroviral Therapy-naïve Adults in South Africa, 2000–2016: A Pooled Sequence Analysis. *EClinicalMedicine* **2019**, *9*, 26–34. [\[CrossRef\]](#)
89. Blankson, J.N.; Persaud, D.; Siliciano, R.F. The Challenge of Viral Reservoirs in HIV-1 Infection. *Annu. Rev. Med.* **2002**, *53*, 557–593. [\[CrossRef\]](#)
90. Eisele, E.; Siliciano, R.F. Redefining the Viral Reservoirs that Prevent HIV-1 Eradication. *Immunity* **2012**, *37*, 377–388. [\[CrossRef\]](#)
91. Vanhamel, J.; Bruggemans, A.; Debyser, Z. Establishment of latent HIV-1 reservoirs: What do we really know? *J. Virus Erad.* **2019**, *5*, 3–9.
92. Abner, E.; Jordan, A. HIV “shock and kill” therapy: In need of revision. *Antivir. Res.* **2019**, *166*, 19–34. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Elsheikh, M.M.; Tang, Y.; Li, D.; Jiang, G. Deep latency: A new insight into a functional HIV cure. *EBioMedicine* **2019**, *45*, 624–629. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Spivak, A.M.; Planellas, V. Novel Latency Reversal Agents for HIV-1 Cure. *Annu. Rev. Med.* **2018**, *69*, 421–436. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Besnard, E.; Hakre, S.; Kampmann, M.; Lim, H.W.; Hosmane, N.N.; Martin, A.; Bassik, M.C.; Verschueren, E.; Battivelli, E.; Chan, J.; et al. The mTOR Complex Controls HIV Latency. *Cell Host Microbe* **2016**, *20*, 785–797. [\[CrossRef\]](#)
96. Martin, A.R.; Pollack, R.A.; Capoferri, A.; Ambinder, R.F.; Durand, C.M.; Siliciano, R.F. Rapamycin-mediated mTOR inhibition uncouples HIV-1 latency reversal from cytokine-associated toxicity. *J. Clin. Invest.* **2017**, *127*, 651–656. [\[CrossRef\]](#)
97. Chowdhury, F.Z.; Ouyang, Z.; Buzon, M.; Walker, B.D.; Lichterfeld, M.; Yu, X.G. Metabolic pathway activation distinguishes transcriptional signatures of CD8⁺ T cells from HIV-1 elite controllers. *AIDS* **2018**, *32*, 2669–2677. [\[CrossRef\]](#)
98. Jin, S.; Liao, Q.; Chen, J.; Zhang, L.; He, Q.; Zhu, H.; Zhang, X.; Xu, J. TSC1 and DEPDC5 regulate HIV-1 latency through the mTOR signaling pathway. *Emerg. Microbes Infect.* **2018**, *7*, 1–11. [\[CrossRef\]](#)

99. Van Montfort, T.; van der Sluis, R.; Darcis, G.; Beaty, D.; Groen, K.; Pasternak, A.O.; Pollakis, G.; Vink, M.; Westerhout, E.M.; Hamdi, M.; et al. Dendritic cells potently purge latent HIV-1 beyond TCR-stimulation, activating the PI3K-Akt-mTOR pathway. *EBioMedicine* **2019**, *42*, 97–108. [\[CrossRef\]](#)
100. Reeves, D.B.; Duke, E.R.; Wagner, T.A.; Palmer, S.E.; Spivak, A.M.; Schiffer, J.T. A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation. *Nat. Commun.* **2018**, *9*, 4811. [\[CrossRef\]](#)
101. Shiels, M.S.; Engels, E.A. Evolving epidemiology of HIV-associated malignancies. *Curr. Opin. HIV AIDS* **2017**, *12*, 6–11. [\[CrossRef\]](#)
102. Tomlinson, C.C.; Damania, B. The K1 Protein of Kaposi's Sarcoma-Associated Herpesvirus Activates the Akt Signaling Pathway. *J. Virol.* **2004**, *78*, 1918–1927. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Montaner, S. Akt/TSC/mTOR activation by the KSHV G protein-coupled receptor: Emerging insights into the molecular oncogenesis and treatment of Kaposi's sarcoma. *Cdl Cycle* **2007**, *6*, 438–443. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Kerr, D.A.; Busarla, S.V.P.; Gimbel, D.C.; Sohani, A.R.; Nazarian, R.M. mTOR, VEGF, PDGFR, and c-kit signaling pathway activation in Kaposi sarcoma. *Hum. Pathol.* **2017**, *65*, 157–165. [\[CrossRef\]](#) [\[PubMed\]](#)
105. Chang, H.H.; Ganem, D. A Unique Herpesviral Transcriptional Program in KSHV-Infected Lymphatic Endothelial Cells Leads to mTORC1 Activation and Rapamycin Sensitivity. *Cdl Host Microbe* **2013**, *13*, 429–440. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Sin, S.-H.; Roy, D.; Wang, L.; Staudt, M.R.; Fakhari, E.D.; Patel, D.D.; Henry, D.; Harrington, W.J.; Damania, B.A.; Dittmer, D.P. Rapamycin is efficacious against primary effusion lymphoma (PEL) cell lines in vivo by inhibiting autocrine signaling. *Blood* **2007**, *109*, 2165–2173. [\[CrossRef\]](#) [\[PubMed\]](#)
107. Re, A.; Cattaneo, C.; Rossi, G. HIV and lymphoma: From epidemiology to clinical management. *Mediterr. J. Hematol. Infect. Dis.* **2019**, *11*, e2019004. [\[CrossRef\]](#)
108. Wang, X.; Duan, Z.; Yu, G.; Fan, M.; Scharff, M.D. Human Immunodeficiency Virus Tat Protein Aids V Region Somatic Hypermutation in Human B Cells. *MBio* **2018**, *9*, e02315–e02317. [\[CrossRef\]](#)
109. El-Salem, M.; Raghunath, P.N.; Marzec, M.; Liu, X.; Kasprzycka, M.; Robertson, E.; Wasik, M.A. Activation of mTORC1 signaling pathway in AIDS-related lymphomas. *Am. J. Pathol.* **2009**, *175*, 817–824. [\[CrossRef\]](#)
110. Sebestyén, A.; Sticz, T.B.; Márk, Á.; Hajdu, M.; Timár, B.; Nemes, K.; Nagy, N.; Váradi, Z.; Koppert, L. Activity and complexes of mTOR in diffuse large B-cell lymphomas—A tissue microarray study. *Mod. Pathol.* **2012**, *25*, 1623–1628. [\[CrossRef\]](#)
111. Ágnes Márk, M.H.; Zsófia, V.; Sticz, T.B.; Nagy, N.; Csomor, J.; Berczi, L.; Varga, V.; Csóka, M.; Sebestyén, L.K.A. Characteristic mTOR activity in Hodgkin-lymphomas offers a potential therapeutic target in high risk disease—A combined tissue microarray, in vitro and in vivo study. *BMC Cancer* **2013**, *13*, 250.
112. Browne, S.H.; Diaz-Perez, J.A.; Preziosi, M.; King, C.C.; Jones, G.A.; Jain, S.; Sun, X.; Reid, E.G.; Vandenberg, S.; Wang, H.Y. mTOR activity in AIDS-related diffuse large B-cell lymphoma. *PLoS ONE* **2017**, *12*, e0170771. [\[CrossRef\]](#) [\[PubMed\]](#)
113. Col, J.D.; Zancai, P.; Terrin, L.; Guidoboni, M.; Ponzoni, M.; Pavan, A.; Spina, M.; Bergamin, S.; Rizzo, S.; Tirelli, U.; et al. Distinct functional significance of Akt and mTOR constitutive activation in mantle cell lymphoma. *Blood* **2008**, *111*, 5142–5151. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Sekihara, K.; Saitoh, K.; Han, L.; Ciurea, S.; Yamamoto, S.; Kikkawa, M.; Kazuno, S.; Taka, H.; Kaga, N.; Arai, H.; et al. Targeting mantle cell lymphoma metabolism and survival through simultaneous blockade of mTOR and nuclear transporter exportin-1. *Oncotarget* **2017**, *8*, 34552–34564. [\[CrossRef\]](#) [\[PubMed\]](#)
115. Hess, G.; Herbrecht, R.; Romaguera, J.; Verhoef, G.; Crump, M.; Gisselbrecht, C.; Laurell, A.; Offner, F.; Straß, A.; Berkenblit, A.; et al. Phase III Study to Evaluate Temsirolimus Compared With Investigator's Choice Therapy for the Treatment of Relapsed or Refractory Mantle Cell Lymphoma. *J. Clin. Oncol.* **2009**, *27*, 3822–3829. [\[CrossRef\]](#) [\[PubMed\]](#)
116. Witzig, T.E.; Reeder, C.B.; LaPlant, B.R.; Gupta, M.; Johnston, P.B.; Micallef, I.N.; Porrata, L.F.; Ansell, S.M.; Colgan, J.P.; Jacobsen, E.D.; et al. A phase II trial of the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. *Leukemia* **2011**, *25*, 341–347. [\[CrossRef\]](#)
117. Johnston, P.B.; LaPlant, B.; McPhail, E.; Habermann, T.M.; Inwards, D.J.; Micallef, I.N.; Colgan, J.P.; Nowakowski, G.S.; Ansell, S.M.; Witzig, T.E. Everolimus combined with R-CHOP-21 for new, untreated, diffuse large B-cell lymphoma (NCCTG 1085 [Alliance]): Safety and efficacy results of a phase 1 and feasibility trial. *Lancet Haematol.* **2016**, *3*, e309–e316. [\[CrossRef\]](#)

118. Bennari, N.N.; LaPlant, B.R.; Ansell, S.M.; Habermann, T.M.; Irwands, D.J.; Micallef, I.N.; Johnston, P.B.; Porrata, L.E.; Colgan, J.P.; Markovic, S.N.; et al. Efficacy of the oral mTORC1 inhibitor everolimus in relapsed or refractory indolent lymphoma. *Am. J. Hematol.* **2017**, *92*, 448–453. [\[CrossRef\]](#)
119. Johnston, P.B.; Pinter-Brown, L.C.; Warsi, G.; White, K.; Ramchandren, R. Phase 2 study of everolimus for relapsed or refractory classical Hodgkin lymphoma. *Exp. Hematol. Oncol.* **2018**, *7*, 12. [\[CrossRef\]](#)
120. Faes, S.; Demartines, N.; Dormond, O. Resistance to mTORC1 Inhibitors in Cancer Therapy: From Kinase Mutations to Intratumoral Heterogeneity of Kinase Activity. *Oxid. Med. Cdl. Longev.* **2017**, *2017*, 1726078. [\[CrossRef\]](#)
121. Petrich, A.M.; Leshchenko, V.; Kuo, P.-Y.; Xia, B.; Thirukonda, V.K.; Ulahannan, N.; Gordon, S.; Fazzari, M.J.; Ye, B.H.; Sparano, J.A.; et al. Akt Inhibitors MK-2206 and Nelfinavir Overcome mTOR Inhibitor Resistance in Diffuse Large B-cell Lymphoma. *Clin. Cancer Res.* **2012**, *18*, 2534–2544. [\[CrossRef\]](#)
122. Heredia, A.; Gilliam, B.; Latinovic, O.; Le, N.; Bamba, D.; DeVico, A.; Melikyan, G.B.; Gallo, R.C.; Redfield, R.R. Rapamycin reduces CCR5 density levels on CD4 T cells, and this effect results in potentiation of enfuvirtide (T-20) against R5 strains of human immunodeficiency virus type 1 in vitro. *Antimicrob. Agents Chemother.* **2007**, *51*, 2489–2496. [\[CrossRef\]](#) [\[PubMed\]](#)
123. Heredia, A.; Latinovic, O.; Gallo, R.C.; Melikyan, G.; Reitz, M.; Le, N.; Redfield, R.R. Reduction of CCR5 with low-dose rapamycin enhances the antiviral activity of vicriviroc against both sensitive and drug-resistant HIV-1. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 20476–20481. [\[CrossRef\]](#) [\[PubMed\]](#)
124. Kumar, D.; Konkimalla, S.; Yadav, A.; Sataranatarajan, K.; Kasinath, B.S.; Chander, P.N.; Singhal, P.C. HIV-Associated Nephropathy. *Am. J. Pathol.* **2010**, *177*, 813–821. [\[CrossRef\]](#) [\[PubMed\]](#)
125. Rehman, S.; Husain, M.; Yadav, A.; Kasinath, B.S.; Malhotra, A.; Singhal, P.C. HIV-1 Promotes Renal Tubular Epithelial Cell Protein Synthesis: Role of mTOR Pathway. *PLoS ONE* **2012**, *7*, e30071. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Cheng, K.; Rai, P.; Plagov, A.; Lan, X.; Mathieson, P.W.; Saleem, M.A.; Husain, M.; Malhotra, A.; Singhal, P.C. Rapamycin-induced modulation of miRNA expression is associated with amelioration of HIV-associated nephropathy (HIVAN). *Exp. Cdl Res.* **2013**, *319*, 2073–2080. [\[CrossRef\]](#)
127. Rai, P.; Plagov, A.; Lan, X.; Chandel, N.; Singh, T.; Lederman, R.; Ayasolla, K.R.; Mathieson, P.W.; Saleem, M.A.; Husain, M.; et al. mTOR plays a critical role in p53-induced oxidative kidney cell injury in HIVAN. *Am. J. Physiol. Physiol.* **2013**, *305*, F343–F354. [\[CrossRef\]](#)
128. Rai, P.; Lederman, R.; Haque, S.; Rehman, S.; Kumar, V.; Sataranatarajan, K.; Malhotra, A.; Kasinath, B.S.; Singhal, P.C. Renin angiotensin system modulates mTOR pathway through AT2R in HIVAN. *Exp. Mol. Pathol.* **2014**, *96*, 431–437. [\[CrossRef\]](#)
129. Department of Health and Human Services. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. Available online: <https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf> (accessed on 10 April 2020).
130. Shim, J.S.; Rao, R.; Beebe, K.; Neckers, L.; Han, I.; Nahta, R.; Liu, J.O. Selective Inhibition of HER2-Positive Breast Cancer Cells by the HIV Protease Inhibitor Nelfinavir. *JNCI J. Natl. Cancer Inst.* **2012**, *104*, 1576–1590. [\[CrossRef\]](#)
131. Srirangam, A.; Mitra, R.; Wang, M.; Gorski, J.C.; Badve, S.; Baldrige, L.; Hamilton, J.; Kishimoto, H.; Hawes, J.; Li, L.; et al. Effects of HIV protease inhibitor ritonavir on Akt-regulated cell proliferation in breast cancer. *Clin. Cancer Res.* **2006**, *12*, 1883–1896. [\[CrossRef\]](#)
132. Goda, J.; Pachpor, T.; Basu, T.; Chopra, S.; Gota, V. Targeting the AKT pathway: Repositioning HIV protease inhibitors as radiosensitizers. *Indian J. Med. Res.* **2016**, *143*, 145. [\[CrossRef\]](#)
133. Aldinucci, D.; Casagrande, N. Inhibition of the CCL5/CCR5 axis against the progression of gastric cancer. *Int. J. Mol. Sci.* **2018**, *19*, 1477. [\[CrossRef\]](#) [\[PubMed\]](#)
134. Gao, D.; Rahbar, R.; Fish, E.N. CCL5 activation of CCR5 regulates cell metabolism to enhance proliferation of breast cancer cells. *Open Bid.* **2016**, *6*, 160122. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Young, M.J. Off-target effects of drugs that disrupt human mitochondrial DNA maintenance. *Front. Mol. Biosci.* **2017**, *4*, 74. [\[CrossRef\]](#) [\[PubMed\]](#)
136. Margolis, A.M.; Heverling, H.; Pham, P.A.; Stolbach, A. A Review of the Toxicity of HIV Medications. *J. Med. Toxicol.* **2014**, *10*, 26–39. [\[CrossRef\]](#)

- 137. Lin, H.; Stankov, M.V.; Hegermann, J.; Budida, R.; Panayotova-Dimitrova, D.; Schmidt, R.E.; Behrensa, G.M.N. Zidovudine-mediated autophagy inhibition enhances mitochondrial toxicity in muscle cells. *Antimicrob. Agents Chemother.* **2019**, *63*, e01443-18. [[CrossRef](#)]
- 138. Cheng, H.; Wu, L.Y. Influence of phosphatidylinositol-3-kinase/protein kinase b-mammalian target of rapamycin signaling pathway on the neuropathic pain complicated by nucleoside reverse transcriptase inhibitors for the treatment of HIV infection. *Chin. Med. J. (Engl.)* **2018**, *131*, 1849–1856. [[CrossRef](#)]
- 139. Hardy, W.D. *Fundamentals of HIV Medicine 2019*; Oxford University Press: Oxford, UK, 2019; ISBN 9780190942519.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Objectives of the thesis

Aggressive B cell lymphomas are the main cause of death in HIV-1 infected individuals, although B cells are not targeted by virus. Even with the introduction of cART the incidence of B cell lymphomas is remaining high in HIV-1 infected patients. The exact mechanisms of the development of these lymphomas are not known. Previous studies of our team revealed that HIV-1 Tat can penetrate B cells, where it can induce ROS production, DNA damage and increase the chances of the Myc/IGH oncogenic translocation, a hallmark of Burkitt lymphoma. In addition, in many immune cells HIV-1 and its proteins Tat, Env and Nef can regulate AKT/mTORC1 pathway, a central integrator of many intra and extracellular signals including viral infection and DNA damage. However, no studies have examined the regulation of AKT/mTORC1 pathway by Tat in B cells.

Considering the importance of AKT/mTORC1 signaling pathway in oncogenesis and combining the results of our team's previous works on Tat, we have come up with the hypothesis that HIV-1 Tat might produce oncogenic effects on B cells by modulating AKT/mTORC1 signaling pathway and regulating expression of genes, involved in lymphomagenesis. Among a number of candidates, we choose to follow the expression of *AICDA* not only because its expression is associated with mTORC1 pathway, but also because we have recently found that Tat induces aberrant AICDA expression in B cells.

Major part of my thesis focused on the regulation of AKT/mTORC1 pathway by Tat in B cells. With our collaborators we have generated doxycycline-inducible Tat-expressing RPMI 8866 cells on which most of my thesis experiments were conducted. Detailed procedure describing the construction of this cell line is published in the *Russian Journal of Developmental Biology*. We next studied the regulation of AKT/mTORC1 pathway and autophagy by Tat in doxycycline-inducible Tat-expressing RPMI 8866 cells as well as in immortalized RPMI 8866 B cell lines and primary B cells from healthy donors, by treating them with purified recombinant Tat protein. We have also studied the DNA damage and ROS production in doxycycline-inducible Tat-expressing RPMI 8866 cells. Finally, we analyzed the AICDA gene expression in the presence and in the absence of its transcriptional activators and repressors depending on the activation of mTORC1 pathway. All the results obtained are described in detail in our article published in the Special Issue "mTOR Signaling network in Cell Biology and Disease" in the *Journal of International Molecular Sciences*.

Materials and Methods

1. Isolation of the peripheral blood mononuclear cells

In order to isolate PBMC from the blood samples of healthy donors, the density gradient centrifugation technique using Pancoll (PAN Biotech, Aidenbach, Germany, lymphocyte separation medium) was applied. Whole blood samples were obtained from the “Etablissement Français du Sang”, Hôpital Saint-Louis, Paris, France. In agreement with French law, the study was registered as a non-interventional study at the Agence Nationale de Sécurité du Médicament (2016-A01316-45) and informed consent was obtained from all subjects.

The blood was first diluted by the same volume of the sterile PBS and poured slowly along the walls of the tubes on top of the 12 m Pancoll solution in 50 ml falcon tubes. After centrifugation for 30 minutes at 630 rcf at room temperature (low acceleration, without brake), the white rings obtained in the center of the tubes containing the PBMCs were collected in tubes containing sterile PBS and washed three times with sterile PBS in order to get rid of the remaining Pancoll. The resulting PBMCs were incubated in the RPMI 1640-GlutaMAX medium (#61870-010, Gibco) supplemented with the 10% fetal bovine serum (#10270-106, Gibco) and 1% penicillin/streptomycin (#15140-122, Gibco) overnight at 37°C and with 5% CO₂ in the incubator, to get rid of debris, dead cells, and to allow the macrophages to adhere to the surface of the culture flask.

2. Purification of B lymphocytes

Following the overnight culture of PMBCs, B cells were purified by negative sorting using MagniSort B cells enrichment kit II (Invitrogen, Thermo Fisher Scientific). Briefly, all PBMC cells except B cells were stained with a cocktail of biotinylated antibodies CD2, CD14, CD16, CD36, CD43 and CD235a (glycophorin A). These cells were then magnetically labeled with anti-Biotin “Anti-Biotin MicroBeads”. Magnetically marked non-B cells were removed by negative sorting. Pure B cells were obtained by removing the magnetically marked non-B cells through a magnetic column. The purified B cells were kept in culture in the RPMI 1640-GlutaMAX medium supplemented with the 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C, 5% CO₂, for 24 hours before conducting any experiments.

3. Cell lines

Lymphoblastoid B cell line (RPMI 8866) was cultured in in the RPMI 1640-GlutaMAX medium supplemented with the 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C, 5% CO₂.

Detailed procedure on the generation of Tat-expressing cells on the basis of RPMI 8866 is thoroughly described in the Gorbacheva et al paper (see Results). Briefly, Tat-encoding gene was inserted into pSBtet-GP plasmid (#60495, Addgene). Transfection of the RPMI 8866 was performed using the Neon electroporator (Invitrogen, United States) according to the manufacturer's recommendations with the simultaneous delivery of SB100x transposase. After electroporation, cells were incubated in the medium containing 1 µg/mL of puromycin for 48 h. Transfected cells were separated using the FACSaria SORP cell sorter (BD Biosciences) and cultured in RPMI 1640-Gluta- Max medium supplemented with the 10% tetracycline-free fetal bovine serum and 1% penicillin/streptomycin for 1 month. Following 1 month of culture, cells were used for the experiments.

4. Tat treatment and expression induction

Primary B cells and RPMI 8866 cells were treated with purified recombinant Tat protein produced by ABL. We obtained Tat through the NIH-AIDS research and Reagent program. According to the manufacturer, the purity of Tat is >95%. It is purified by the affinity chromatography with heparin and by reverse-phase HPLC high-performance liquid chromatography in order to remove the endotoxins. Tat was used at a concentration of 250 ng/ml in all experiments. To induce Tat expression, doxycycline-inducible Tat-expressing cells were treated with doxycycline (#3447, Sigma-Aldrich) at a final concentration of 1 µg/µl for different time points starting from 6, 24, and 48 hours. After treatment, cells were kept in the culture for the above mentioned time lengths without any interruptions.

5. Inhibition of AKT/mTORC1 pathway by MK-2206 and Rapamycin treatments

To inhibit mTORC1 activity, rapamycin (#R-0395, Sigma-Aldrich) was added to culture medium at a final concentration of 200 nM for 6, 24, and 48 hours. Cells were treated either only with rapamycin or in combination with doxycycline treatment to induce the Tat expression simultaneously inhibiting mTORC1.

MK-2206 (#S1078, Selleckchem) is a highly selective inhibitor of AKT. MK-2206 inhibits phosphorylation of both AKT threonine 308 and serine 473. MK-2206 also inhibits AKT-mediated phosphorylation of downstream signaling molecules like TSC2, PRAS40 and ribosomal S6 proteins. To inhibit the AKT activation, cells were treated with MK-2206 at a final concentration of 0.5 µM for 6, 24, and 48 hours. Cells were treated either only with MK-2206 or in combination with doxycycline treatment to induce the Tat expression simultaneously inhibiting AKT signaling.

6. Western Blot

After appropriate treatments, cell suspension was centrifuged at 2000 g for 5 minutes at 4°C. The cells were collected and washed twice with ice-cold PBS. Cell pellet was resuspended in the ice-cold lysis buffer (150 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 7.5, and 0.5% Nonidet P-40) containing protease inhibitors (#04693159001, Roche) and phosphatase inhibitors (#04906837001, Roche) and left on the ice for 30 minutes, followed by a sonication for 15 seconds with 5 seconds “on” and 3 seconds “off” regulation at 30% amplitude. Samples were centrifuged at 16000g for 10 minutes at 4°C. The supernatant was collected in a new tube for the protein quantification. Protein concentration was quantified using the BCA protein assay kit (#23227, Thermo Fisher Scientific) according to the manufacturer’s protocol. Samples were resuspended in the LDS buffer (#NP0007, Thermo) and run on 4–12% Bis-Tris gel (#NP0323, Thermo Fisher Scientific) in the MOPS-SDS buffer (#NP0001, Thermo Fisher Scientific) for 30 minutes at 80v and for 1 hour at 120V. Proteins were transferred onto the PVDF membrane (IPVH00010, Millipore) for 2 hours at 90V at 4°C. Membrane was blocked in 5% milk at room temperature for 1 h. Blocked membrane was incubated with primary antibodies overnight. Nonspecific bindings were washed three times with a washing buffer (0.05 M Tris, 0.15 M NaCl, pH 7.6) containing 0.1% Tween 20. Membrane was then incubated with anti-mouse or anti-rabbit secondary peroxidase-conjugated antibodies for 2 hours at room temperature. The membrane was washed three times, incubated with the SuperSignal West Pico chemiluminescent substrate (#34580, Thermo Fisher Scientific) for 1 min and then visualised using the ImageQuant LAS 4000 Mini system (GE Healthcare). Western blot bands on the images were quantified by ImageJ. The intensities were then normalized to the corresponding GAPDH band intensity used as loading control. Relative band intensities were presented as fold change.

The following antibodies were used: HIV-1 Tat antibody (#sc-65912, Santa Cruz Biotechnology); AKT (#9272), phospho-AKT (Ser473) (#9271), p70 S6 Kinase (#9202), phospho-p70 S6 Kinase (Thr389) (#9206), 4E-BP1 (#9452), phospho-4E-BP1 (Thr37/46) (#2855), phospho-Chk1 (Ser345) (#234), phospho-Chk2 (Thr 68) (#26611), GAPDH (#2118) antibodies were from Cell Signaling Technology; phospho-Histone H2A.X (Ser139) antibody (#AB_2793161, Active Motif); anti-mouse (#315035003) and anti-rabbit (#111035144) peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch.

7. Monitoring ROS production

To monitor the ROS production, Tat expression was induced for 6 hours either in the presence or in the absence of Tempol (#10-2471, Focus Biomolecules) at a final concentration of 80 µM. In response to Tat induction, ROS level was detected using the General Oxidative Stress Indicator CM-H2DCFDA (C6827, Thermo Fisher Scientific) according to the manufacturer’s guidelines. Briefly, after 6 hours of treatment cells

were collected, washed once with PBS, resuspended in PBS containing a 1 μ M of CM-H2DCFDA and incubated at 37°C for 15 minutes. Cells were further washed once with PBS and resuspended in PBS. Fluorescence emission spectra of oxidized CM-H2DCFDA at 488 nm was detected by BD Accuri C6 Plus flow cytometer. All the experiments were conducted avoiding the light exposure. Staining intensity is represented as the mean fluorescence in arbitrary units.

8. qRT-PCR

Total RNA was extracted using the NucleoSpin® RNA II kit according to the manufacturer's recommendations (#740955.250, Macherey-Nagel). Total RNA (300 ng) was primed in a 20 μ l cDNA Synthesis Master Mix (#M1661, Thermo Scientific) in order to obtain complementary DNA (cDNA). Then, the PCR was carried out on 1 μ l of the cDNA using the PowerUp SYBR Green Master mix (#A25742, Appliedbiosystems) containing the Taq polymerase, the nucleotides as well as the fluorescent marker SYBR Green I, for 1 μ l of the cDNA, 10 μ l of the PowerUp SYBR Green Master mix was mixed with 1 μ l of the 10 μ M sense primer, 1 μ l of the 10 μ M anti-sense primer and 7 μ l of sterile water to obtain a final volume of 20 μ l. The PCR reactions were carried out on the StepOne Plus thermal cycler (Life Technologies) according to the following program: one step at 95°C for 10 minutes, followed by 40 cycles of amplification including one step at 95°C for 15 seconds and one step at 60°C for 1 minute. The primers used in the qRT-PCR experiments are listed in (Table 2). Expression of target genes was analyzed with the $2^{-\Delta\Delta C_t}$ method by normalizing against GAPDH and the expression was compared between different groups. For quantification, expression levels were set to 1 in controls.

Table 2. List of primers used for qRT-PCR

Gene Name	Forward primer (5'-3')	Reverse primer (5'-3')
PAX5	ACAGCTCTTTTCCTTCCCCTC	GGGAAGTTGGGCTAGGTCTT
AICDA	TCTTGATGAACCGGAGGAAG	AGCCGTTCTTATTGCGAAGA
BATF	GCAAGGAGATCAAGCAGCTC	GAGCTGACATGAGGTTGGTG
C/EBPb	TTTTGTCCAAACCAACCGCA	TGCATCAACTTCGAAACCGG
C-Myb	CATTTGATCCGCATCCCCTG	TCAAAAGTTCAGTGCTGGCC
E2A	ATGGGGCATTGTTGGGAC	TCCTGTCTACGTCACGATGG
E2F1	GGTCCCTGAGCTGTTCTTCT	CCACTCACCTCTCCCATCTC
E2F2	CTCCTGGGTGAGCTGAAGAA	AAGGAGGCTTACATGGTGCT
E2F3	GGTGGGGTCAAGACAGATGA	ACCAAGTCCAGTGTGTGTGA
E2F4	ACAGTGGTGAGCTCAGTTCA	GAGGTAGAAGGGTTGGGTCC
E2F5	CGGCGTTCTGGATCTCAAAG	TTACAGCCAGCACCTACACC
E2F6	TGTTCCAGCTCCCAGAGAAG	TCTTCTTCCTCAGGGCCTTC
E2F7	CGTCTTTCAGTGTCCCTTGC	TATTGATCCAAGGCCAGGCA
E2F8	GGAGGTGAGACGGTCTTCAA	TGGGAAGGGTGCAGAATTCT
HOXC4	TCCTCTCCCTCCCACTGTTA	AAGCCAGACCATCACACCTT
SMAD3	CTCTGGGTGCTTGGAACATA	ATCCAAATGCAGCCAAACGT
SMAD4	ACAAGTCAGCCTGCCAGTAT	GGTGCAGTCCTACTTCCAGT
SP1	GAGCAAAACCAGCAGACACA	ACTGTTGGTGTCCGGATGAT
SP3	TGCCTTGGACGTGGATAGC	GCCCTATCTTGCTGCAGGTA
STAT6	AAGAGCACAGGTTAGGGCAT	TAACCACATGTCCAGACCCC
Tat	CTAGACTAGAGCCCTGGAAGCA	TGAGGAGGTCTTCGTCGCT

9. DNA damage detection with immunofluorescence

One of the most common techniques used in the detection of the double-strand break is the immunofluorescence labeling of phosphorylated histone H2A variant X following DNA damage (γ H2AX), particularly double strand breaks.

In order to see the presence of DNA damage following Tat expression for 6 and 24 hours of doxycycline treatment, cells seeded on 15 x 15 mm coverslips coated with Poly-L-lysine for 20 minutes at 37°C and 5% CO₂ in order for the cells to adhere to the surface of the culture flask. After adhesion, cells were rinsed with 0.3X PBS for one minute, then fixed with 4% paraformaldehyde (Euromedex) in 0.3X PBS for 10 minutes at room temperature, followed by 3 washes in PBS for 5 minutes. Cells were permeabilized with 2% Triton X-100 (Sigma-Aldrich # T8787) and washed three times in PBS for 5 minutes. Non-specific sites were blocked with 0.5% of bovine serum albumin (Euromedex) in PBS for 40 minutes at room temperature. Cells on the coverslips were incubated with the primary antibody against γ H2AX (phospho-Histone H2A.X Ser139, Active Motif, 1:500 dilution) for 2 hours at room temperature, followed by 3 washes with PBS and by incubation with the secondary antibody conjugated to Alexa Fluor-488 (Life Technologies,

1: 200) for one hour at room temperature. Cells were washed 3 times with PBS, and labeled with Vectashield mounting medium.

Immunofluorescent cells were visualized by a confocal microscope, TCS SP8 (Leica Microsystems) with a 63X oil immersion objective Z stacks were acquired using a frame size of 1024×1024 and $0.5 \mu\text{m}$ in z, with sequential multitrack scanning using excitation / emission laser wavelength: 488/519 nm, green fluorescence. Image analysis was performed using ImageJ software.

10. Statistical analyses

One-way Anova test with Bonferroni's post-test was used to compare between groups. Student t-test with two-tail distribution was used to compare dual samples. All tests were conducted on Graphpad Prism 5 software (Graphpad software).

Results

1. Generation of B cell lines providing inducible and constant Tat expression

Cell lines expressing the Tat protein are the most convenient tool for studying the cellular effects of this protein. Although the lines of cultured T-cells (Jurkat) (69) and macrophages (66) expressing the Tat protein have been described and have found broad use, no analogues among the B cell lines exist so far. Even though B-cells are a more physiological system to study the effect of Tat, it is costly, time consuming and the result may vary from donor to donor. Moreover, non-activated naive B-cells from blood rapidly undergo apoptosis *ex vivo*, which limits the time span of the experiments. Therefore, we decided to create the new line of the cultured RPMI 8866 B-cells with constant and inducible expression of the Tat protein without a tag. The comparison of these lines can make it possible to differentiate between the effects of the viral protein and compensatory responses of the cell.

In a collective effort with our colleagues from Moscow, we generated doxycycline-inducible RPMI8866 based B cell line stably expressing untagged Tat protein. In my thesis work I used this cell line to study and compare the short- term effects produced by inducible expression and the long-term effects produced by constitutive expression of Tat. Although during my thesis I mostly studied the effect of Tat on AKT/mTORC1 pathway, it is clear that we can use this system to describe other protein's effects on the cell as well as the possible compensatory responses of the cell to the presence of the viral protein.

MECHANISMS OF CELL PROLIFERATION AND DIFFERENTIATION

Production of Stable Cell Lines on the Basis of the Cultured RPMI 8866 B-Cells with Constant and Inducible Expression of the Human Immunodeficiency Virus Tat Protein

M. A. Gorbacheva^a, M. A. Tikhomirova^b, D. M. Potashnikova^{c, d}, B. Akbay^e,
E. V. Sheval^{b, c}, and Y. R. Musinova^{a, b, *}

^aKoltsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, 119334 Russia

^bBelozersky Institute of Physicochemical Biology, Moscow State University, Moscow, 119992 Russia

^cBiological Faculty, Moscow State University, Moscow, 119992 Russia

^dAtherothrombosis Laboratory, Evdokimov Moscow State University of Medicine and Dentistry, Moscow, 109240 Russia

^eUMR 8126, Université Paris-Sud Paris-Saclay, CNRS, Institut Gustave Roussy, Villejuif, France

*e-mail: musinova@genebee.msu.ru

Received April 3, 2019; revised April 26, 2019; accepted May 6, 2019

Abstract—Highly-efficient antiretroviral therapy allows controlling human immunodeficiency virus (HIV) and preventing the development of immunodeficiency. However, the patients who receive therapy may develop different complications, including B-cell lymphomas. One of oncogenesis' mechanisms in HIV-infected patients is associated with the activity of the viral Tat protein, which is able to penetrate into B-cells. In order to study the effect of the Tat protein on B-cells, a report is given on the production and characterization of the cell lines based on the cultured RPMI 8866 B-cell line demonstrating constant and inducible expression of the Tat protein.

Keywords: human immunodeficiency virus, Tat protein, B-cells, oncogenesis

DOI: 10.1134/S1062360419050060

INTRODUCTION

Infection with human immunodeficiency virus (HIV) leads to the development of acquired immunodeficiency syndrome. Antiretroviral therapy (ART), which has been developed and introduced into clinical practice in recent years, allows for the suppression of the viral infection's development. However, although they protect from the development of immunodeficiency, antiretroviral drugs do not prevent the development of various HIV-associated diseases. Specifically, the cancer detection rate becomes increased (Hernández-Ramírez et al., 2017). The frequency of developing several types of B-cell lymphomas, namely, Hodgkin's lymphoma, diffuse large B-cell lymphoma, and Burkitt's lymphoma, is particularly increased. This effect is apparently not directly associated with viral infection, since HIV does not infect B-cells.

The mechanisms of HIV-associated lymphomas' development are poorly understood (Musinova et al., 2016). One of them involves the HIV-encoded protein Tat, which is not only expressed in the infected T-cells but is also able to exit from the cells, accumulating in blood plasma, and to be further internalized by B-cells (Debaisieux et al., 2012). The role for the Tat protein in lymphoma development in HIV-infected patients is

indicated by the results of the study of transgenic mice in which the expression of Tat protein triggered the development of lymphomas and other tumors (Vogel et al., 1991; Corallini et al., 1993; Kundu et al., 1999; Altavilla et al., 1999). However, the precise mechanism of Tat protein-induced oncogenesis remains unknown.

Tat is a small protein (~12 kDa) that regulates viral expression and controls many processes taking place in the host cell nucleus as well (Kurnaeva et al., 2019). In particular, the Tat protein is capable of binding with a broad number of genes and changing their expression (Marban et al., 2011; Reeder et al., 2015), thus changing various processes in the cell. It is known that an extracellular Tat protein (i.e., the protein that has left the HIV-infected T-cell) may enhance proliferation of the germinal center B-cells (Lefevre et al., 1999). Suppression of the DNA recombination system by the Tat protein may also promote lymphogenesis (Chipitsyna et al., 2004; Sun et al., 2006; Brégnard et al., 2014).

The Tat protein may alter the 3D organization of the nuclei of B-cells circulating in the blood, thus resulting in the *MYC* gene coming close to the immunoglobulin's heavy chain gene locus (Germini et al., 2017). It was suggested that this may increase the prob-

ability of the rearrangement involving these two loci, which is characteristic of Burkitt's lymphoma. It has also been demonstrated that the Tat protein can increase the expression of the *AICDA* gene encoding the activation-induced cytidine deaminase which is capable of introducing double-stranded DNA breaks in the course of immunoglobulin gene maturation (Sall et al., 2019).

Cell lines expressing the Tat protein are the most convenient tool for studying the cellular effects of this protein. Although the lines of cultured T-cells (Jurkat) (Reeder et al., 2015) and macrophages (Carvalho et al., 2017) expressing the Tat protein have been described and have found broad use, no analogues among the B-cell lines exist so far. The present work describes the new lines of the cultured RPMI 8866 B-cells with constant and inducible expression of the Tat protein. The comparison of these lines can make it possible to differentiate between the effects of the viral protein and compensatory responses of the cell.

MATERIALS AND METHODS

The RPMI 8866 immortalized B-cell culture (Sigma) was grown at 37°C in the RPMI 1640-Gluta-Max medium (#61870-010, Gibco) with the addition of 10% embryonic serum (#SV30160.03, HyClone), sodium pyruvate (#F023 PanEko), and antibiotics (#LS15240062, Gibco).

The Tat-encoding gene was inserted into two types of plasmids, namely, pSBbi-GP and pSBtet-GP (#60511 and #60495, respectively, both Addgene) (Kowarz et al., 2015). Transformation of cells using these plasmids should lead to stable (in the case of pSBbi-GP) or induced with doxycycline (in the case of pSBtet-GP) expression of the Tat protein. The pSBtet-GP construct contains the firefly luciferase gene (*luc*) under the control of the *tight TRE* gene promoter. This construct allows one to obtain mRNA encoding the EGFP fluorescent protein, rTetR repressor protein, and the selection marker protein puromycin (Fig. 1a). This vector allows producing enough GFP quantities to perform microscopy and FACS. The bidirectional expression construct pSBbi-GP contains EFl α promoter and the synthetic promoter RPBSA, which are fused in the opposite direction to each other. Firefly luciferase gene and puromycin-resistance gene (for subsequent selection) are located under the control of the EFl α promoter (Fig. 1b). This vector provides for the constitutive production of a large amount of the recombinant protein. The opposite localization of the promoters allows for avoiding the negative effects of EFl α promoter and dramatically increases the strength of the RPBSA promoter due to the enhancer elements of the EFl α promoter. Both constructs contain two *Sfi*I restriction endonuclease recognition sites that made it possible to insert the *Tat* gene sequence in the right orientation. *E. coli* strain JM110 was used for cloning. PCR product con-

taining the full-size *Tat* protein-encoding gene, which was amplified from the EGFP-Tat vector (Musinova et al., 2015) using the primer pair Tat-SfiI_for-5'-ATATGGCCTCTGAGGCCACCATGGAGCCAG-TAGATCCTAGAC-3' and Tat-SfiI_rev-5'-ATATGGCCTGACAGGCCCTATTCTTCGGGCCTGT-CG-3' was inserted into the *Sfi*I restriction sites of both the pSBbi-GP and pSBtet-GP plasmids. Primers used for amplification contained the *Sfi*I recognition site (Fig. 1c). The presence of the insertion encoding the *Tat* sequence in the pSBbi-GP and pSBtet-GP plasmids after cloning was verified by sequencing.

Transfection of the cultured RPMI 8866 human B-cell line was performed using the Neon electroporator (Invitrogen, United States) according to the manufacturer's recommendations with the simultaneous delivery of SB100x transposase, which is necessary to transfer the target gene sequence from the plasmid to the host cell genome (Kowarz et al., 2015) into the cells. The obtained cell lines showed stable expression of the *Tat* gene (RPMI^{Tat}) and inducible expression in the presence of doxycycline (RPMI^{Tat-ind}) with extremely low background expression.

After electroporation, cells were incubated in the medium containing 1 μ g/mL of puromycin for 48 h. Fluorescent cells were then separated using the FACSaria SORP cell sorter (BD Biosciences). EGFP was excited with a 488-nm laser, and fluorescence detection was carried out in the FITC channel using 505LP + 515/20BP filters. Cell sorting was performed using 85 μ m nozzles with the corresponding pressure settings. The obtained cells were cultured for 1 month and then frozen.

RNA was isolated using the RNeasy mini kit (Qiagen #74104) according to the manufacturer's instructions. RNA concentration was measured using the Nano-Photometer (Implen). Reverse transcription was carried out using the iScript Advanced kit (BioRad); 1 μ g of total RNA was used for the first cDNA strand synthesis. A real-time polymerase chain reaction was performed in the CFX96 Real-Time System (BioRad) using the Real-time PCR with SYBR Green I kit (Syntol). The total reaction volume was 25 μ L with 2.5 μ L of 10 \times PCR buffer with SYBR-Green I, 2.5 mM MgCl₂, 2.5 mM dNTPs, 0.5 μ L of *Taq* polymerase, 10 pmol of each primer, and 5 μ L of cDNA. Amplification conditions were as follows: denaturation 95°C for 3 min and 40 cycles, including 95°C for 10 s, 62°C for 15 s, and 72°C for 30 s each. *Tat* mRNA content was assessed using the following primers TAT1F 5'-CTAGACTAGAGCCCTGGAAGCA-3' and TAT1R 5'-TGAGGAGGTCTTCGTCGCT-3'. Each sample was performed in triplicate. Normalization was carried out using three reference genes: UBC_forward 5'-GTCGCAGCCGGGATTGGGT-3' and UBC_reverse 5'-GTCAGACAGGGTGCGCCCAT-3'; YWHAZ_forward 5'-ACTTTTGGTACATTGTG-GCTCAA-3' and YWHAZ_reverse 5'-CCGCCAG-

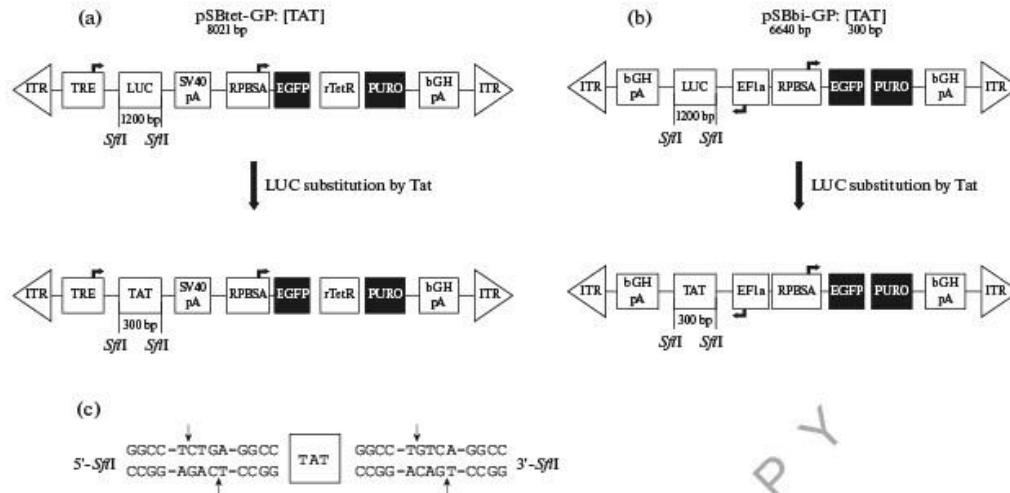


Fig. 1. Schematic depiction of the fused constructs containing the Tat sequence obtained on the basis of the (a) pSBtet-GP and (b) pSBbi-GP vectors. The Tat-coding sequence was inserted into (c) *SfiI* sites.

GACAAACCAGTAT-3', and HPRT_forward 5'-TGTAATGACCACTCAACAGGGGACA-3' and HPRT_reverse 5'-TCCAACACTTCGTGGGGTCCT-3' (Gladkikh et al. 2010). Relative cDNA quantity was calculated according to the Δ -C(t) method:

$$Q = 2^{C(t)_{\min} - C(t)_n}$$

where $C(t)_n$ is mean $C(t)$ value for each sample and $C(t)_{\min}$ is minimum mean value for the entire sampling. Normalization of the Tat expression level was performed using the geometric mean value for three reference genes as the normalization factor NF (Vandesompele et al., 2002), which was calculated according to the following formula:

$$NF_n = \sqrt[3]{HKG1 \cdot HKG2 \cdot HKG3}$$

where HKG is relative mean value for the reference gene cDNA. Normalized Tat quantity was calculated according to the following formula:

$$Q_{\text{normalized}} = Q/NF.$$

Statistical significance of the samples was calculated using the pairwise *t* test in GraphPad Prism (GraphPad Software).

To assess functional activity of the Tat protein, simultaneous flow cytometry analysis of the expression of Tat-EGFP and the rapidly maturing TurboRFP protein under the control of the viral LTR promoter was carried out in the FACSAria SORP cell sorter (BD Biosciences). TurboRFP was excited with a 561-nm laser, and fluorescence detection was carried out in the PE channel using 585/20BP filter.

To perform Western-blot, cells were pelleted by centrifugation, washed with PBS, and resuspended in the lysis buffer (150 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 7.5, and 0.5% Nonidet P-40) containing protease (#04693159001, Roche) and phosphatase inhibitors (#04906837001, Roche). Lysates were incubated on ice for 30 min and sonicated for 15 s. Protein concentration was determined using the BCA protein assay kit (#23227, Thermo) according to the manufacturer's protocol. Samples prepared using the LDS buffer (#NP0007, Thermo) were separated in 4–12% Bis-Tris gel (#NP0323, Thermo) in the MOPS-SDS buffer (#NP0001, Thermo). Proteins were transferred onto the PVDF membrane; nonspecific binding was blocked in 5% milk at room temperature for 1 h. The following antibodies were used (incubation at 4°C overnight): antibodies against HIV-1 Tat (#sc-65912, Santa Cruz Biotechnology), antibodies against GFP (#11814460001, Roche), antibodies against GAPDH (#2118, Cell signaling), and antibodies against β -actin (#A1987, Sigma). For protein detection, peroxidase-conjugated antibodies (#315035003 and #111035144, both Jackson ImmunoResearch) were used. After incubation with secondary antibodies, the membrane was incubated with the SuperSignal West Pico chemiluminescent substrate (#34580, Thermo Scientific) for 1 min and then imaged using the ImageQuant LAS 4000 Mini system (GE Healthcare).

RESULTS AND DISCUSSION

The principal purpose of the present work was to obtain cell lines expressing Tat without tags. Cell

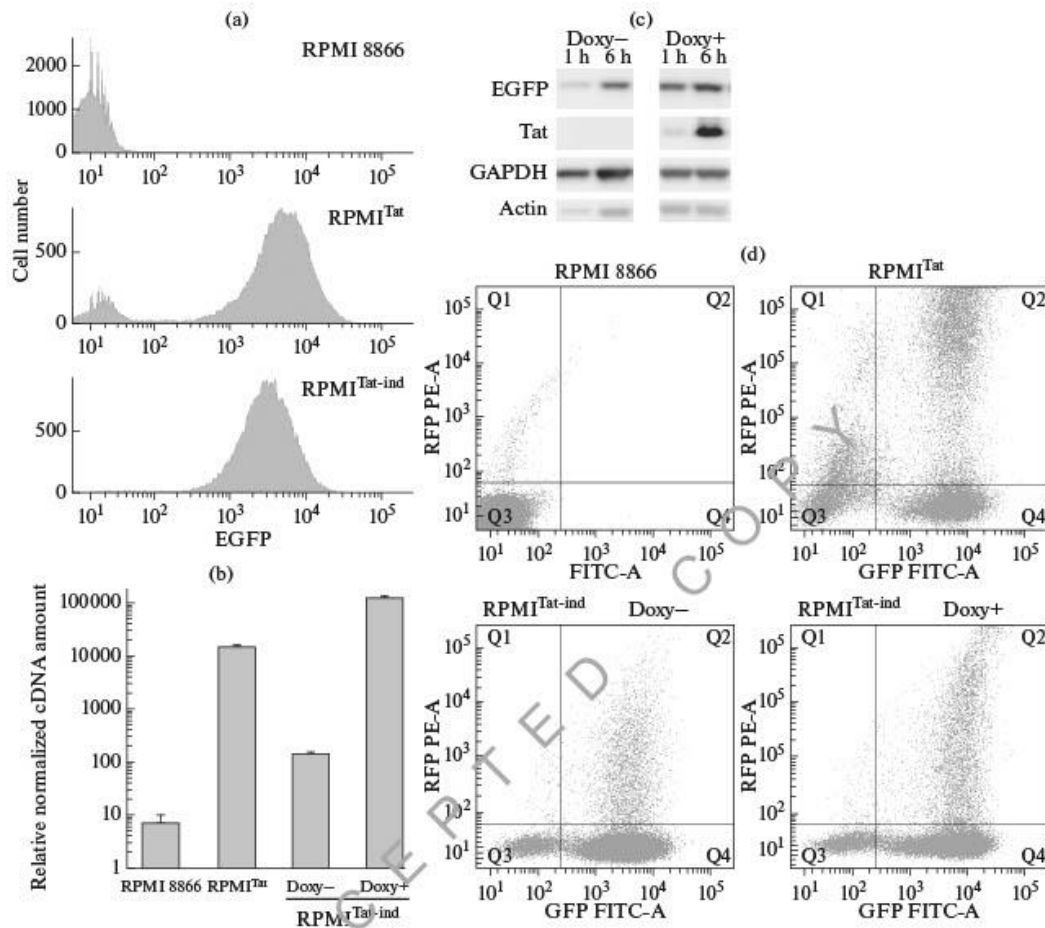


Fig. 2. Assessment of Tat protein expression in the obtained cell lines. (a) Obtained cell lines (RPMI^{Tat} and RPMI^{Tat-ind}) express EGFP unlike the original cell line (RPMI 8866) (flow cytometry). EGFP expression indicates that the target nucleotide sequence was integrated into the genome, which allows us to calculate, although indirectly, the portion of cells expressing Tat. In both obtained lines, a number of nonfluorescent cells was present. (b) Assessment of Tat mRNA content by qRT-PCR. Data were normalized for three reference genes (YWHAZ, UBC, and HPRT) and are present as mean value \pm standard deviation ($n = 3$). (c) Induction of Tat-protein expression in RPMI^{Tat-ind} cells (Western blot). In the cells without doxycycline treatment (left), Tat expression is not detected; in the cells after doxycycline treatment (right), the protein is detected as early as 6 h after the start of treatment. (d) Tat protein produced in the RPMI^{Tat} and RPMI^{Tat-ind} cell lines activates the expression of TurboRFP controlled by virus promoter (LTR), which indicated that it is capable of promoter transactivation. The data were obtained by flow cytometry.

selection and further monitoring of the cell cultures is significantly facilitated when cells express a fluorescent protein. For this reason, we used vectors that allow the Tat protein to be coexpressed with the green fluorescent protein (EGFP). To obtain stable cell lines, we chose the Sleeping beauty transposon system, which makes it possible to produce stable cell lines in which the target gene is integrated into the host

cell genome (Kowarz et al., 2015). Integration is catalyzed by the SB100x transposase, which is randomly distributed along the genome and is predominantly not associated with active genes.

Two plasmid constructs for the expression of the Tat protein gene were obtained on the basis of pSBbi-GP and pSBtet-GP vectors (Kowarz et al., 2015). The pSBbi-GP vector allows cloning genes under the con-

trol of the Efl α promoter to obtain their stable expression. The pSBtet-GP allows cloning genes under control of the inducible *tight TRE* gene promoter. This construct allows one to produce mRNA encoding the EGFP fluorescent protein and the repressor protein of tetracycline antibiotic (rTetR). Both vectors enable puromycin selection of transfectants, and contain two *SfiI* restriction endonuclease recognition sites for cloning. As a result, we obtained two plasmids providing constitutive and inducible expression of the Tat protein respectively (Fig. 1).

The RPMI 8866 line cultured B-cells were transfected with the obtained plasmids by electroporation. Tat-protein producing cells were grown in the medium with puromycin, and fluorescent cells were separated in the cell sorter using the FACS technique. As a result, we obtained cell cultures demonstrating stable expression of the Tat protein (RPMI^{Tat} line) and inducible expression of the Tat protein (RPMI^{Tat-ind} line) (Fig. 2a). It is important to note that, in the course of the experiments using the obtained cell lines, we observed the presence of nonfluorescent cells in the cell culture, with their percentage increasing with time, which indirectly indicates that cells progressively lose the integrated genes.

The level of Tat expression in the obtained cells was assessed by qRT-PCR (Fig. 2b). Induction with doxycyclin leads to a multifold increase in the Tat protein expression level in RPMI^{Tat-ind} cells, with the postinduction expression level exceeding the expression level observed in RPMI^{Tat} cells. Protein expression in RPMI^{Tat-ind} cells was detected by Western-blot. The expression of Tat protein could not be detected in the control cells; however, after the induction of expression with doxycyclin, Tat protein could be clearly detected (Fig. 2c).

To test the functional activity of the expressed protein, cells were additionally transfected with the plasmid encoding the rapidly maturing red protein TurboRFP under the control of the viral promoter (LTR). TurboRFP expression could not be detected in the control RPMI 8866 cells (Fig. 2d). In contrast, among the RPMI^{Tat} cells, cell population coexpressing EGFP and TurboRFP could be clearly distinguished. In the RPMI^{Tat-ind} cells with coexpression of EGFP and TurboRFP, i.e., in those cells in which transactivation activity of the Tat protein was observed. However, TurboRFP expression level was very low. Induction of expression with doxycyclin led to an increase in the portion of fluorescent cells (from 8.4 to 13.0%) as well as to an increase in the intensity of fluorescence (Median TurboRFP fluorescence for Q2 was 556 f.u. (CV 122.4%) before induction and 1437 f.u. (CV 138.7%) after induction).

To summarize, we have obtained cell lines with stable and inducible expression of the Tat protein in the course of the present work. To facilitate cell selection and subsequent monitoring of the cell cultures' state,

cells coexpressed Tat and fluorescent protein (EGFP). To avoid the possible effects of Tat protein fusion with EGFP, EGFP was expressed separately in the obtained plasmids and not as a fusion with the virus protein. The assessment of the functional activity of the Tat protein has demonstrated that it is capable of transactivating the viral LTR promoter, which makes it possible to use the obtained cells in further studies. These cells may be used to analyze the effects of the Tat protein on B-cells in vitro. Similar models based on T-cells (Reeder et al., 2015) and macrophages (Carvalho et al., 2017), i.e., on the cells normally infected by HIV, are currently available. The obtained cultures may serve as analogous models that are specially designed to study the effects of Tat on B-cells, which may be advantageous in understanding the mechanisms of development of B-cell lymphomas in HIV-infected patients. The comparison of the short-term effects produced by inducible expression and the long-term effects produced by constitutive expression of Tat will allow us to describe not just the protein's effects on the cell but also the possible compensatory responses of the cell to the presence of the viral protein. At present, B-lymphocyte responses to Tat protein are unknown, although they may appear to be advantageous therapeutic targets in preventing the development of B-cell lymphomas.

ACKNOWLEDGMENTS

We are grateful to Eric Kowarz for kindly providing the pSBbi-GP and pSBtet-GP plasmids (Addgene plasmids #60511 and #60495) and to Zsuzsanna Izsvak for kindly providing the SB100X plasmid (Addgene plasmid #34879).

FUNDING

The work was supported by the Russian Science Foundation (grant no. 17-75-20199).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interests. No experimentation involving animals was part of the present work.

REFERENCES

- Altavilla, G., Trabanelli, C., Merlin, M., et al., Morphological, histochemical, immunohistochemical, and ultrastructural characterization of tumors and dysplastic and non-neoplastic lesions arising in BK virus/tat transgenic mice, *Am. J. Pathol.*, 1999, vol. 154, no. 4, pp. 1231–1244.
- Altavilla, G., Caputo, C., Trabanelli, C., et al., Prevalence of liver tumours in HIV-1 tat-transgenic mice treated with urethane, *Eur. J. Cancer*, 2004, vol. 40, no. 2, pp. 275–283.
- Brégnard, C., Benkirane, M., and Laguet, N., DNA damage repair machinery and hiv escape from innate immune sensing, *Front. Microbiol.*, 2014, vol. 5, p. 176.

- Carvalho, Loreto, Lopez, et al., HIV-Tat regulates macrophage gene expression in the context of neuroAIDS, *PLoS One*, 2017, vol. 12, no. 6.
- Chipitsyna, G., Dorota, S., Siddiqui, K., et al., HIV-1 Tat increases cell survival in response to cisplatin by stimulating *Rad51* gene expression, *Oncogene*, 2004, vol. 23, no. 15, pp. 2664–2671.
- Corallini, A., Altavilla, G., Pozzi, L., et al., Systemic expression of HIV-1 Tat gene in transgenic mice induces endothelial proliferation and tumors of different histotypes, *Cancer Res.*, 1993, vol. 53, no. 22, pp. 5569–5575.
- Debaisieux, S., Rayne, F., Yezid, H., et al., The ins and outs of HIV-1 Tat, *Traffic*, 1993, vol. 13, no. 3, pp. 355–363.
- Germini, D., Tsfasman, T., Klibi, M., et al., HIV Tat induces a prolonged myc relocalization next to IGH in circulating B-cells, *Leukemia*, 2017.
- Gladkikh, A., Potashnikova, D., Korneva, E., et al., Cyclin D1 expression in B-cell lymphomas, *Exp. Hematol.*, 2010, vol. 38, no. 11, pp. 1047–1057.
- Hernández-Ramírez, R., Shiels, M., Dubrow, R., et al., Cancer risk in HIV-infected people in the USA from 1996 to 2012: a population-based, registry-linkage study, *Lancet. HIV*, 2017, vol. 4, no. 11, pp. 495–504.
- Kowarz, E., Löscher, D., and Marschalek, R., Optimized sleeping beauty transposons rapidly generate stable transgenic cell lines, *Biotechnol. J.*, 2015, vol. 10, no. 4, pp. 647–653.
- Kundu, R., Sangiorgi, L., Wu, Y., et al., Expression of the human immunodeficiency virus-*tat* gene in lymphoid tissues of transgenic mice is associated with B-cell lymphoma, *Blood*, 1999, vol. 94, no. 1, pp. 275–282.
- Kurnaeva, M., Sheval, E., Musinova, Y., et al., Tat basic domain: a 'Swiss army knife' of HIV-1 Tat?, *Rev. Med. Virol.*, 2019.
- Lefevre, E.A., Krzysiek, R., Lóret, E.P., et al., Cutting edge: HIV-1 Tat protein differentially modulates the B cell response of naive, memory, and germinal center B cells, *J. Immunol.*, 1999, vol. 163, no. 3, pp. 1119–1122.
- Marban, C., Su, T., Ferrari, R., et al., Genome-wide binding map of the HIV-1 Tat protein to the human genome, *PLoS One*, 2011, vol. 6, p. 26894.
- Musinova, Y.R. and Sheval, E.V., The accumulation of the basic domain of HIV-1 Tat protein in the nuclei and the nucleoli is different from the accumulation of full-length Tat proteins, *Biopolym. Cell*, 2015, vol. 31, no. 2, pp. 154–158.
- Musinova, Y.R., Sheval, E.V., Dib, C., et al., Functional roles of HIV-1 Tat protein in the nucleus, *Cell. Mol. Life Sci.*, 2016, vol. 73, no. 3, pp. 589–601.
- Reeder, J.E., Kwak, Y.T., McNamara, R.P., et al., HIV Tat controls RNA polymerase II and the epigenetic landscape to transcriptionally reprogram target immune cells, *Elife*, 2015, vol. 4.
- Sall, F.B., El Amine, R., Markozashvili, D., et al., HIV-1 Tat protein induces aberrant activation of AICDA in human B-lymphocytes from peripheral blood, *J. Cell Physiol.*, 2019.
- Sun, Y., Huang, Y.C., Xu, Q.Z., et al., HIV-1 Tat depresses DNA-PK(CS) expression and DNA repair, and sensitizes cells to ionizing radiation, *Int. J. Radiat. Oncol. Biol. Phys.*, 2006, vol. 65, pp. 842–850.
- Vandesompele, J., De Preter, K., Pattyn, F., et al., Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes, *Genome Biol.*, 2002, vol. 3.
- Vogel, J., Hinrichs, S.H., Napolitano, L.A., et al., Liver cancer in transgenic mice carrying the human immunodeficiency virus *tat* gene, *Cancer Res.*, 1991, vol. 51, pp. 6686–6690.

Translated by E. Martynova

2. HIV-1 Tat activates AKT/mTORC1 pathway and AICDA expression by downregulating its transcriptional inhibitors in B cells

The main target of HIV-1 is T cells, macrophages, and dendritic cells which express receptors and coreceptors allowing HIV-1 to infect them directly. Yet, lymphomas developed in HIV-1 patients are always originated from B cells. Moreover, the main cause of death in HIV-1 patients is not the viral infection itself, but is the HIV-associated malignancies including aggressive B cell lymphomas (95,96). Introduction of cART reduced certain HIV-associated malignancies, but the incidence of B cell lymphomas is still high (99).

The exact mechanisms of the development of B cell lymphomas in HIV-1 patients are remaining unclear. It has been hypothesized that the secreted or transmitted viral proteins of HIV-1 might be responsible for the oncogenic transformation (106,107). Previous studies of our team showed that Tat penetrates B cells, induces ROS production and DNA damage, brings oncogene *Myc* and *IGH* loci to the close proximity which presumably leads to their oncogenic translocation, a hallmark of Burkitt lymphoma (73,112).

AKT/mTORC1 pathway is hyperactivated in ARLs, indicating that it might be one of the major contributors to lymphoma development in HIV-1 patients (106,114,116–118,395,396). HIV-1 and its proteins Tat, Env and Nef can regulate AKT/mTORC1 pathway in T cells, macrophages, dendritic cells and peripheral blood mononuclear cells. Strikingly, however, no studies have been conducted to examine the regulation of AKT/mTORC1 by Tat in B cells, though the majority of lymphomas in HIV-1 patients are of B cell origin.

Therefore, considering the importance of AKT/mTORC1 signaling pathway and combining the results of our team's previous works, we speculated that Tat might produce oncogenic effects by penetrating into B cells, inducing ROS production, leading to DNA damage, and further modulating AKT/mTORC1 pathway leading to the regulation of gene expressions, among which *AICDA* gene is of importance as it is involved in lymphomagenesis (397,398) and its expression is associated with mTORC1 pathway (322,323). In addition our team has recently identified that Tat induces aberrant *AICDA* expression in B cells (399).

This chapter describes the data of the main part of my thesis project, where I studied: 1) Expression kinetics of Tat protein in doxycycline-inducible Tat-expressing RPMI 8866 at mRNA and protein level; 2) DNA damage induction by Tat; 3) Regulation of AKT/mTORC1 by Tat in RPMI 8866 cells, primary B cells from healthy donors by treating them with exogenous recombinant Tat protein as well as doxycycline-inducible Tat-expressing RPMI 8866 cells by inducing Tat expression, ROS production and its relation with AKT/mTORC1 regulation by using Tempol, a ROS scavenger; 4) *AICDA* gene expression with its regulators, *C-Myb* and *E2F8*, and its dependency on mTORC1 signaling pathway using rapamycin in combination with Tat induction; 5) Regulation of *AICDA*, *C-Myb*, *E2F8* expression in response to AKT inhibition using specific inhibitor MK-2206).

Article

HIV-1 Tat Activates Akt/mTORC1 Pathway and AICDA Expression by Downregulating Its Transcriptional Inhibitors in B Cells

Burkitkan Akbay ^{1,2}, Diego Germini ¹, Amangeldy K. Bissenbaev ^{2,3}, Yana R. Musinova ^{4,5}, Evgeny V. Sheval ⁵, Yegor Vassetzky ^{1,4} and Svetlana Dokudovskaya ^{1,*}

¹ CNRS UMR9018, Institut Gustave Roussy, Université Paris-Saclay, 94805 Villejuif, France; akbayburkitkan@gmail.com (B.A.); germinidiego@gmail.com (D.G.); Yegor.VASSETZKY@gustaveroussy.fr (Y.V.)

² Department of Molecular Biology and Genetics, Faculty of Biology and Biotechnology, al-Farabi Kazakh National University, Almaty 050040, Kazakhstan; Amangeldy.Bissenbaev@kaznu.kz

³ Scientific Research Institute of Biology and Biotechnology Problems, al-Farabi Kazakh National University, Almaty 050040, Kazakhstan

⁴ Koltzov Institute of Developmental Biology of Russian Academy of Sciences, 119991 Moscow, Russia; musinova.yana@gmail.com

⁵ Belozersky Institute of Physicochemical Biology, Moscow State University, 119899 Moscow, Russia; esheval@gmail.com

* Correspondence: svetlana.dokudovskaya@igr.fr

Citation: Akbay, B.; Germini, D.; Bissenbaev, A.K.; Musinova, Y.R.; Sheval, E.V.; Vassetzky, Y.; Dokudovskaya, S. HIV-1 Tat Activates Akt/mTORC1 Pathway and AICDA Expression by Downregulating Its Transcriptional Inhibitors in B Cells. *Int. J. Mol. Sci.* **2021**, *22*, 1588. <https://doi.org/10.3390/ijms22041588>

Academic Editor: Elena A.

Goncharova

Received: 21 December 2020

Accepted: 1 February 2021

Published: 4 February 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Abstract: HIV-1 infects T cells, but the most frequent AIDS-related lymphomas are of B-cell origin. Molecular mechanisms of HIV-1-induced oncogenic transformation of B cells remain largely unknown. HIV-1 Tat protein may participate in this process by penetrating and regulating gene expression in B cells. Both immune and cancer cells can reprogram communications between extracellular signals and intracellular signaling pathways via the Akt/mTORC1 pathway, which plays a key role in the cellular response to various stimuli including viral infection. Here, we investigated the role of HIV-1 Tat on the modulation of the Akt/mTORC1 pathway in B cells. We found that HIV-1 Tat activated the Akt/mTORC1 signaling pathway; this leads to aberrant activation of activation-induced cytidine deaminase (AICDA) due to inhibition of the AICDA transcriptional repressors *c-Myb* and *E2F8*. These perturbations may ultimately lead to an increased genomic instability and proliferation that might cause B cell malignancies.

Keywords: HIV-1; Tat; Akt/mTORC1 pathway; AICDA; B cells

1. Introduction

HIV-1 trans-activator of transcription (Tat) is one of the key viral regulatory proteins essential for HIV-1 replication, establishment of infection and virus reactivation [1,2]. Tat is a small protein of 86–102 amino acids (depending on the viral strain) with a predicted molecular weight of 14–16 kDa. HIV-1 predominantly infects T cells and macrophages, and Tat is expressed in productively infected cells. Tat can be released from infected cells into blood, where it can penetrate many cell types due to its protein transduction domain [3–6]. Therefore, Tat may affect both infected and non-infected cells including B cells, which may participate in the oncogenesis of HIV-associated malignancies [7,8].

HIV infection is associated with an elevated risk of developing AIDS-defining cancers, including a number of B-cell lymphomas [9,10]. Even though a current antiretroviral therapy is quite efficient, a high incidence of HIV-1-associated malignancies persists and the mechanism of development of these diseases is largely unclear [11]. Tat, which persists in the blood of HIV-1 patients even under the antiretroviral therapy [12], can penetrate uninfected cells and modulate host gene expression, having direct or indirect oncogenic

effects on B cells [13,14]. We have recently demonstrated that Tat induces oxidative stress and DNA damage in peripheral blood lymphocytes [15], and induces repositioning of the *MYC* locus on chromosome 8 in the close proximity to the *IGH* locus on the chromosome 14, thereby increasing the chances of the oncogenic translocation t(8;14) characteristic of Burkitt lymphomas [12]. Deregulation of the Akt/mTORC1 pathway could be the major contributor to the development of AIDS-related lymphomas (ARLs), as various studies have shown the hyper-activation of this signaling pathway in different subgroups of ARLs [13,16–21].

The mTORC1 pathway integrates signals from many intracellular and extracellular cues: growth factors, amino acids, energy, oxygen, DNA damage and infectious agents, including viruses [22,23]. The Akt/mTORC1 axis is activated in response to growth factors, cytokines, insulin and DNA damage [23,24]. mTORC1 phosphorylates many targets, among them eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and p70S6 kinase 1 (S6K1), promoting the synthesis of three major cell constituents: proteins, nucleotides and lipids [25–29].

mTORC1 plays a central role in the metabolism, differentiation and effector functions of immune cells [30,31] and is essential for the development of B cells as well as for the antibody production [32–34]. mTORC1 inactivation leads to decreased expression of the activation-induced cytidine deaminase (*AICDA*), which impairs the generation of high-affinity antibodies [34,35]. The physiological function of *AICDA* is to promote the somatic hypermutation and class-switch recombination (CSR) of the immunoglobulin (Ig) loci. Aberrant expression of *AICDA* and its involvement in lymphomagenesis have been observed in different subtypes of B-cell lymphomas [36,37]. Our recent results demonstrate that HIV-1 Tat can induce aberrant expression of *AICDA* in peripheral blood B cells from HIV-infected individuals [38].

We hypothesized that Tat could modulate the Akt/mTORC1 pathway in B cells, and here, we demonstrated that in B cells, HIV-1 Tat not only induced reactive oxygen species (ROS) production and DNA damage, but also activates the Akt/mTORC1 pathway, resulting in *AICDA* overexpression via mTORC1-dependent inhibition of *AICDA* repressors *c-Myb* and *E2F8*.

2. Results

2.1. HIV-1 Tat Activates the AKT/mTORC1 Pathway in B Cells as a Response to ROS-Induced DNA Damage

To study the function of HIV-1 Tat in B cells, we recently generated an inducible model system using the immortalized lymphoblastoid B cell line (RPMI-8866), where Tat expression can be induced by doxycycline [39]. Tat expression initiates after 6 h of treatment with 1 µg/mL doxycycline and peaks at 24 h both at the mRNA (Figure 1A) and protein level (Figure 1B).

We and others have recently shown that HIV-1 Tat induces oxidative stress and DNA damage in different cell types [15,40,41]. In order to verify if HIV-1 Tat activates the DNA damage in our inducible model, we checked the levels of different DNA damage markers. We found that DNA damage was induced 6 h after HIV-1 Tat expression induction, as demonstrated by the increased level of γ H2AX (Figure 1C,D) and phosphorylation of CHK1 (p-CHK1 Ser345) and CHK2 (p-CHK2 Thr68) (Figure 1C,E,F). We confirmed this by immunofluorescence staining of γ H2AX (Figure 1G). We found that $42.7 \pm 1.2\%$ of cells with induced Tat expression exhibited γ H2AX staining compared to $15.8 \pm 3\%$ of non-induced control cells (Figure 1H). The DNA damage is then rapidly repaired by the DNA damage response system (DDR), as previously demonstrated [12,15].

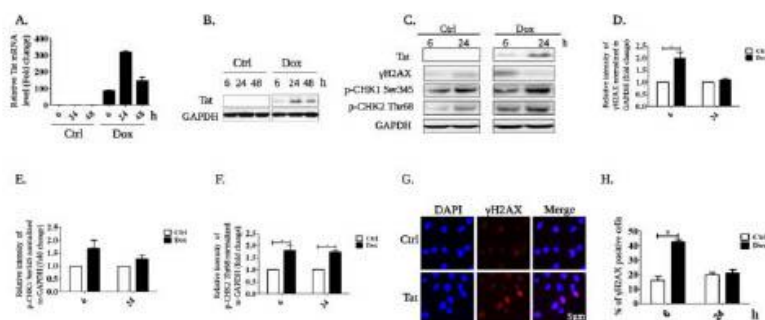


Figure 1. HIV-1 Tat induces DNA damage in B cells. (A) Doxycycline-inducible Tat-expressing RPMI-8866 cells were treated (Dox) or not (Ctrl) with 1 $\mu\text{g/mL}$ doxycycline for 6, 24 and 48 h. Tat expression was analyzed using qRT-PCR and quantified by the $2^{-\Delta\Delta\text{ct}}$ method comparing the expression of the target gene in cells treated with Dox with the untreated control (set as 1) after normalization with *GAPDH* gene expression. (B) Doxycycline-inducible Tat-expressing RPMI-8866 cells were treated as in (A) for 6 and 24 h. Tat expression was identified with an appropriate antibody. (C) RPMI-8866 cells were treated as in (A) and analyzed 6 and 24 h after induction. Tat, $\gamma\text{-H2AX}$ and p-CHK2 Thr68 were detected by Western blotting with appropriate antibodies. (D) Intensities of $\gamma\text{-H2AX}$, (E) p-CHK1 Ser345 and (F) p-CHK2 Thr68 bands were quantified with ImageJ software in Dox-treated cells compared to the untreated control (set as 1) after normalization with *GAPDH* band intensities, which was used as the loading control. (G) $\gamma\text{-H2AX}$ was immuno-stained in doxycycline-inducible Tat-expressing RPMI-8866 cells treated as in (A) for 6 h. Nuclei stained with DAPI are in blue, and $\gamma\text{-H2AX}$ staining is shown in red. (H) Images from D were analyzed using the ImageJ software to evaluate the percentage of cells with the $\gamma\text{-H2AX}$ staining. All data represent the results of three independent experiments and are expressed as the mean \pm SEM. The statistical significance was calculated vs. untreated controls; * $0.01 < p < 0.5$, ** $0.001 < p < 0.01$.

Activation of the Akt/mTORC1 pathway is one of the consequences of the DDR. In order to determine if Tat treatment affects the Akt/mTORC1 pathway, we used three cell models: primary B cells from healthy donors, immortalized lymphoblastoid cell lines (LCLs, RPMI-8866) and the doxycycline-inducible Tat-expressing RPMI-8866 cell line. Primary B cells and LCLs were treated with 250 ng/mL Tat for 6, 24 and 48 h (B cells) by mimicking the penetration of secreted Tat protein from infected cells, and Akt phosphorylation at serine 473 and the phosphorylation 4E-BP1 (p-4E-BP1 Thr 37/46), a marker of mTORC1 activity, were analyzed. We found that exogenous Tat treatment significantly increased the phosphorylation of Akt Ser473 and 4E-BP1 Thr37/46 starting from 24 h, indicating that Tat activates the Akt/mTORC1 signaling pathway (Figures 2A,B and S1A–D). Activation of Akt and mTORC1 was also confirmed in doxycycline-inducible Tat-expressing RPMI-8866 cells, where phosphorylation of Akt Ser473, 4E-BP1 Thr37/46 and P70S6K Thr389 was increased in response to Tat induction starting 6 h after Dox treatment (Figures 2C, and S1E–G). Altogether, these results demonstrate that Tat activates the Akt/mTORC1 pathway in B cells.

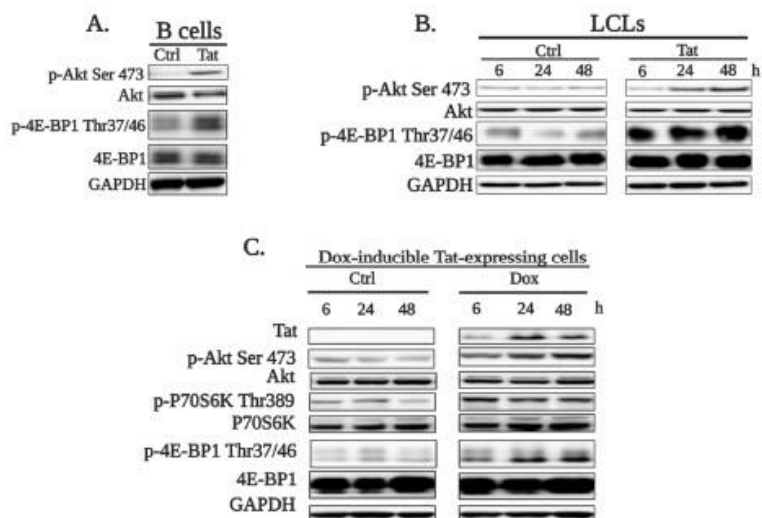


Figure 2. HIV-1 Tat activates the Akt/mTORC1 pathway in B cells. (A) Primary B cells purified from the blood of healthy donors were left untreated (Ctrl) or treated (Tat) with 250 ng/mL Tat for 48 h. (B) Immortalized lymphoblastoid cell lines (LCLs) were treated with 250 ng/mL Tat for 6, 24 and 48 h. (C) Tat expression was induced (Dox) in doxycycline-inducible Tat-expressing RPMI-8866 cells by treating with 1 µg/mL doxycycline for 6, 24 and 48 h. Tat, Akt and its phosphorylated form (p-Akt Ser473), P70S6K and its phosphorylated form (p-P70S6K Thr389) and 4E-BP1 as well as its phosphorylated form (p-4E-BP1 Thr 37/46) were detected by Western blotting with specific antibodies. GAPDH was used as the loading control. Representative images of three independent experiments are shown. The image quantification and statistical analysis are shown in Figure S1.

It is known that HIV-1 Tat induces reactive oxygen species (ROS) production and DNA damage within several hours after penetration in various cell types, including B cells [15,40,41]. ROS production can activate the Akt/mTORC1 pathway. Therefore, we checked if it is the case in our model. Tat expression was induced in the presence or absence of 80 µM Tempol, a ROS scavenger, and ROS level was measured 6 h after induction. ROS levels were three times higher in HIV-1 Tat-expressing cells compared to the untreated cells. Treatment with Tempol completely abolished HIV-1 Tat-induced ROS production (Figure 3A). Consistently, increased ROS levels were associated with increased γH2AX staining 6 h after Tat induction. Consistently, Tempol treatment decreased γH2AX levels (Figure 3B). Akt/mTORC1 pathway activation was also prevented by Tempol (Figure 3B). Interestingly, DNA damage was decreased at 24 and 48 h in all conditions regardless of the treatments, as indicated by a significant reduction of γH2AX level. The Akt/mTORC1 pathway, however, remained activated up to 48 h after Tat induction. These results suggest that once activated in response to DNA damage caused by ROS, Akt/mTORC1 pathway activity is maintained by Tat, even if the DNA damage signal decreases.

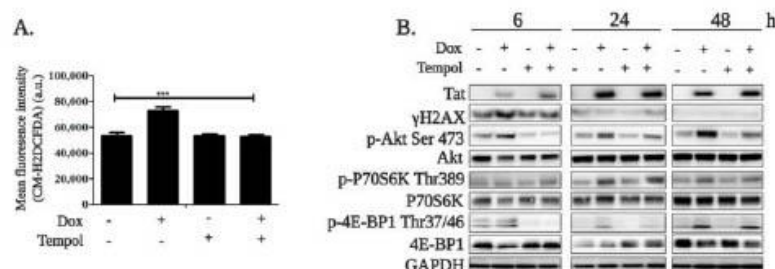


Figure 3. Activation of the Akt/mTORC1 axis is dependent on the DNA damage induced by reactive oxygen species (ROS) production. (A) Tat expression was induced in the RPMI-8866 cells by treating with 1 µg/mL doxycycline for 6 h (Dox) in the presence or absence of 80 µM Tempol. ROS production was measured by flow cytometry after CM-H2DCFDA staining as described in the Materials and Methods. Staining intensity is represented as the mean fluorescence in arbitrary units. (B) Tat and phosphorylated and non-phosphorylated forms of Akt, P70S6K and 4E-BP1 were analyzed in Tat-expressing cells, in cells treated with Tempol at a final concentration. The results of three independent experiments are expressed as the mean ± SEM. The statistical significance was calculated vs. untreated controls; *** $p < 0.001$.

2.2. Tat Induces AICDA Expression by Downregulating *c-Myb* and *E2F8* in an mTORC1-Dependent Manner

We have previously demonstrated that *AICDA* transcription was induced in B cells from healthy donors incubated with HIV-1 Tat and in B cells from HIV-patients, yet how this activation occurs was not clear [38]. mTORC1 positively regulates *AICDA* expression [42]; therefore, we hypothesized that HIV-1 Tat-induced *AICDA* transcription in B cells is dependent on the mTORC1 pathway. To test this hypothesis, we analyzed *AICDA* expression after induction of Tat expression in the absence or presence of the mTORC1 inhibitor rapamycin. *AICDA* expression was upregulated 1.7-fold and 2-fold 24 and 48 h after Tat induction, respectively (Figure 4A). mTORC1 inhibition by rapamycin robustly downregulated *AICDA* expression even in the presence of Tat, demonstrating the dependency of Tat-derived *AICDA* upregulation from the mTORC1 pathway (Figure 4A).

To elucidate how mTORC1 regulates *AICDA* in Tat-expressing B cells, we analyzed the expression of known transcriptional regulators of *AICDA* (10 activators and 2 repressors) in the presence or absence of Tat and rapamycin. The expression of all transcriptional activators was essentially non-affected in all conditions tested (Figure S2), while both repressors, *c-Myb* and *E2F8*, were found to be strongly inhibited by HIV-1 Tat with or without rapamycin (Figure 4B–C). In the absence of Tat, treatment with rapamycin strongly induced *c-Myb* expression, but did not significantly affect *E2F8*. These results demonstrate that Tat upregulates *AICDA* expression by downregulating the expression of its repressors *c-Myb* and *E2F8* in an mTORC1-dependent manner (Figure 5).

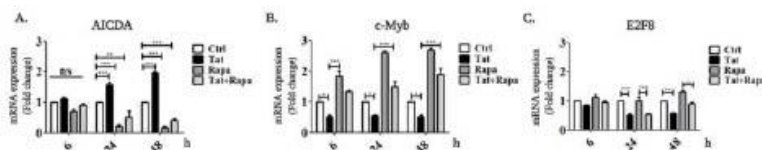


Figure 4. Tat induces activation-induced cytidine deaminase (*AICDA*) overexpression by inhibiting its repressors in an mTORC1-dependent way. Tat expression was induced in the RPMI-8866 cells by addition of 1 µg/mL doxycycline for 6, 24 and 48 h, followed by cells being treated with 200 nM of rapamycin (Tat+Rapa) or left untreated (Tat). RPMI-8866 cells not expressing Tat were

also treated with 200 nM rapamycin (Rapa) for the indicated times or left untreated (Ctrl). *AICDA* (A), *c-Myb* (B) and *E2F8* (C) mRNA expression levels were analyzed at different time points by qRT-PCR and quantified using the $2^{-\Delta\Delta Ct}$ method comparing the expression of the target gene in Tat-induced, rapamycin-treated and Tat-induced + rapamycin-treated cells with the untreated control (set as 1) after normalization with *GAPDH* gene expression. Results are obtained from three independent experiments. The statistical analyses were carried out by the one-way ANOVA test. All data are expressed as the mean \pm SEM. The statistical significance was calculated between groups; *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$.

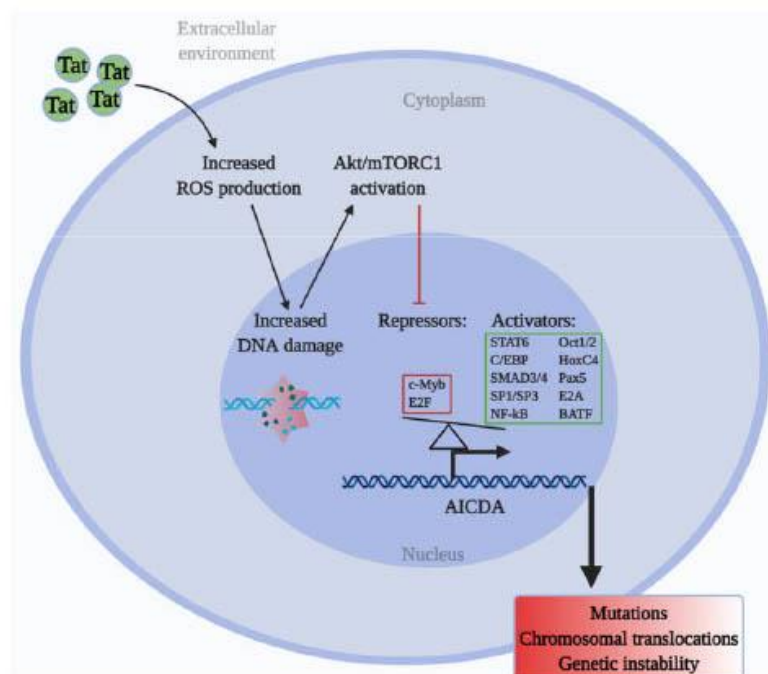


Figure 5. Oncogenic effects of HIV-Tat in B cells. Tat increases the ROS production [15], leading to the induction of DNA damage and activation of the Akt/mTORC1 pathway. This results in the increased expression of *AICDA* due to the mTORC1-dependent inhibition of its transcriptional repressors. Deregulated expression of *AICDA* increases the possibility of increased mutations and chromosomal translocations causing genetic instability in B cells, leading to the emergence of B-cell malignancies.

3. Discussion

The Akt/mTORC1 pathway is important for the successful entry, integration and replication of HIV-1 in peripheral blood lymphocytes, memory T cells and dendritic cells [43–48]. Akt/mTORC1 activity can also be modulated by individual HIV-1 proteins. For example, mTORC1 can be activated by treatment with HIV-1 Nef and Env in HEK293T, endothelial and dendritic cells [43,49]. Activation of Akt/mTORC1 by Tat has been documented in HeLa, Jurkat and peripheral blood mononuclear cells [45,48]. To the best of our knowledge, however, no studies have been conducted to examine the regulation of mTORC1 by HIV-Tat in B cells, even though they are the main part of the immune system.

Here, we demonstrated that, in agreement with previous studies conducted on other cell models [15,40,41], Tat induced ROS production and DNA damage in B cells, which resulted in hyper-activation of the Akt/mTORC1 pathway. The Akt/mTORC1 pathway is

often upregulated in AIDS-related B-cell lymphomas [13,16–21]. Tat is constantly present in detectable concentrations in patients' blood serum [12,50] and can potentially enter any cell in a human body due to its cell penetration domain [51]. Tat's presence leads to transient ROS activation and DNA damage, which in the long term may induce chromosomal aberrations typical of lymphomas [15]. In addition, Tat can probably remotely regulate mTORC1 activity in many non-infected cells.

Our group previously described that Tat could remodel the nuclear architecture, leading to MYC-IGH loci proximity [12] and *AICDA* overexpression in B cells from healthy donors and from HIV-infected patients [38]. Thus, Tat can predispose to the MYC-IGH t(8;14) chromosomal translocation, a hallmark of Burkitt lymphoma. At the same time, the mechanisms leading to *AICDA* activation are unexplained. *AICDA* expression is significantly elevated in PBMCs of HIV-infected individuals who later developed AIDS-associated lymphomas, especially in those who developed Burkitt's lymphoma [52]. Elevated *AICDA* expression is also found in lymph node tissue samples from lymphoma patients, and it is suggested to be used as a marker of unfavorable outcomes in diffuse large B-cell lymphoma patients treated with chemotherapy [53]. HIV proteins could also contribute to the development of lymphomas in HIV-infected patients. Indeed, HIV-Nef significantly promotes *AICDA* expression along with *MYC* expression in Burkitt lymphoma [54].

An increasing number of studies have demonstrated a positive relation between mTORC1 activity and *AICDA* expression. Deletion of the mTORC1 component Raptor in B lymphocytes results in decreased *AICDA* expression, which was associated with the loss of *BCL6*, a key transcriptional regulator involved in germinal center fate [35]. Moreover, inhibition of mTORC1 by rapamycin results in the decreased *AICDA* protein level, leading to decreased antibody class-switching [55]. mTORC1 is found to be highly activated and *AICDA*, its transcriptional activator *PAX5*, and *BCL6* are overexpressed in a population of atypical memory B cells in patients with active lupus [56]. Therefore, it is possible that mTORC1 regulates *AICDA* expression either through *BCL6* or through direct transcriptional regulators of *AICDA*. Consistently, we have found that mTORC1 positively regulated *AICDA* expression in B cells. Even though we did not observe the overexpression of *PAX5*, we found that mTORC1 promotes *AICDA* expression by inhibiting its two major repressors, *c-Myb* and *E2F8*.

Our data suggest that extracellular Tat penetrates B cells and induces ROS production, leading to DNA damage within a short time. Increased DNA damage is further sensed by the Akt/mTORC1 pathway. Activated mTORC1 inhibits the repressors of *AICDA*, *c-Myb* and *E2F8*, leading to the disruption of the balance between the transcriptional activators and repressors of *AICDA*, which ultimately results in the overexpression of *AICDA*. Deregulated *AICDA* expression may increase the possibilities of mutations and chromosomal translocations underlying B-cell malignancies.

4. Materials and Methods

4.1. Cell Lines and Blood Samples

The lymphoblastoid cell line RPMI-8866 and its Tat-expressing variant [39] were cultured at 37 °C in RPMI 1640-GlutaMAX medium (#61870-010, Gibco, Bourgoïn-Jallieu, France) supplemented with 10% fetal bovine serum (#10270-106, Gibco, France) and 1% penicillin/streptomycin (#15140-122, Gibco, France).

Peripheral blood mononuclear cells were isolated from whole blood by Pancoll (PAN Biotech, Germany, lymphocyte separation medium). B cells were then purified using the MagniSort B cells enrichment kit II (Thermo Fisher Scientific, Bourgoïn-Jallieu, France). Purified B cells were then kept at 37 °C in the culture medium described above.

4.2. Treatments

Doxycycline (#3447, Sigma-Aldrich, Saint Louis, MO, USA) at a final concentration of 1 µg/mL was added to the culture medium to induce Tat expression in RPMI-8866 cells. After treatment, cells were kept in the Dox-containing medium for 6, 24 and 48 h. To monitor ROS production, Tat expression was induced in the RPMI-8866 cells by treating with 1 µg/mL doxycycline for 6 h (Dox) in the presence or absence of Tempol (#10-2471, Focus Biomolecules, Plymouth Meeting, PA, USA) at a final concentration of 80 µM. Rapamycin (#R-0395, Sigma-Aldrich, Saint Louis, MO, USA) was added to culture medium at a final concentration of 200 nM for 6, 24 and 48 h. Purified and functionally active Tat protein was produced by Advance Bioscience Laboratories (ABL, Rockville, MD, USA) and obtained through the NIH AIDS Research and Reference Reagent Program, USA. According to the manufacturer, the Tat protein was >95% pure after purification by heparin affinity chromatography and reverse-phase HPLC with removal of endotoxins. Tat was added to culture medium at final concentration of 250 ng/mL. Cells were kept in the Tat-containing medium for 6, 24 and 48 h.

4.3. Western Blotting

Cells were collected by centrifugation, washed once with ice-cold 1× PBS and resuspended in the lysis buffer (150 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 7.5 and 0.5% Nonidet P-40) containing protease inhibitors (#04693159001, Roche, Neuilly sur Seine, France) and phosphatase inhibitors (#04906837001, Roche, France). Cell lysates were kept on ice for 30 min and sonicated for 15 s—5 s on, 3 s off—at 30% amplitude. Protein concentration was quantified using the BCA protein assay kit (#23227, Thermo Fisher Scientific, France) according to the manufacturer's protocol. Samples were prepared using LDS buffer (#NP0007, Thermo Fisher Scientific, France) and run on 4–12% Bis-Tris gel (#NP0323, Thermo Fisher Scientific, France) in MOPS-SDS buffer (#NP0001, Thermo Fisher Scientific, France) for 30 min at 80 V and for 1 h at 120 V. Separated proteins were transferred onto the PVDF membrane (IPVH00010, Millipore, France) for 2 h at 90 V at 4 °C. Membrane was blocked in 5% milk at room temperature for 1 h. Blocked membrane was incubated with primary antibodies overnight. Nonspecific bindings were washed three times with a washing buffer (0.05 M Tris, 0.15 M NaCl, pH 7.6) containing 0.1% Tween 20. Membrane was then incubated with anti-mouse or anti-rabbit secondary peroxidase-conjugated antibodies for 2 h at room temperature. The membrane was washed three times, incubated with the SuperSignal West Pico chemiluminescent substrate (#34580, Thermo Fisher Scientific, France) for 1 min and then visualized using the ImageQuant LAS 4000 Mini system (GE Healthcare, Vélizy-Villacoublay, France).

Western blot bands on the images obtained from ImageQuant LAS 4000 Mini system were quantified by ImageJ and normalized to the corresponding GAPDH band intensity used as the loading control. Relative band intensities were presented as fold change.

The following antibodies were used: HIV-1 Tat antibody (#sc-65912, Santa Cruz Biotechnology, Germany); Akt (#9272), phospho-Akt (Ser473) (#9271), p70 S6 Kinase (#9202), phospho-p70 S6 Kinase (Thr389) (#9206), 4E-BP1 (#9452), phospho-4E-BP1 (Thr37/46) (#2855), phospho-Chk1 (Ser345) (#234), phospho-Chk2 (Thr 68) (#26611) and GAPDH (#2118) antibodies were from Cell Signaling Technology, Boston, MA, USA; phospho-Histone H2A.X (Ser139) antibody (#AB_2793161, Active Motif, Belgium); anti-mouse (#315035003) and anti-rabbit (#111035144) peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch, UK.

4.4. ROS Production Analysis

To monitor the ROS production, Tat expression was induced either in the presence or in the absence of Tempol. ROS level was detected using the General Oxidative Stress Indicator CM-H2DCFDA (C6827, Thermo Fisher Scientific, France) according to the manufacturer's guidelines. Briefly, cells were collected, washed once with PBS, resuspended

in PBS containing 1 μ M of CM-H2DCFDA and incubated at 37 °C for 15 min. Cells were further washed once with PBS and resuspended in PBS. Fluorescence emission spectra of oxidized CM-H2DCFDA at 488 nm were detected by a BD Accuri C6 Plus flow cytometer.

4.5. qRT-PCR

Total RNA was extracted using the NucleoSpin® RNA II kit according to the manufacturer's recommendations (#740955.250, Macherey-Nagel, Oensingen, Switzerland). Total RNA (300 ng) was primed in a 20 μ L cDNA Synthesis Master Mix (#M1661, Thermo Fisher Scientific, France). The obtained cDNA was amplified using specific primers and the PowerUp SYBR Green Master mix (#A25742, Applied Biosystems, Bourgoin-Jallieu, France). Expression of target genes was analyzed with the $2^{-\Delta\Delta Ct}$ method by normalizing against *GAPDH* and the expression was compared between different groups. For quantification, expression levels were set to 1 in controls. The primers used for RT-qPCR were designed in Primer3web v. 4.1.0 and are listed in Table S1.

4.6. Immunofluorescence

Cells were seeded on Poly-L-lysine-coated 15 \times 15 mm coverslips (20 min, 37 °C and 5% CO₂), rinsed with 0.3 \times PBS and fixed with 4% paraformaldehyde (Euromedex, Souffelweyersheim, France) in 0.3 \times PBS for 10 min at room temperature. After washing three times with 1 \times PBS, coverslips were incubated with 0.5% BSA (Euromedex, France) in 1 \times PBS for 40 min at room temperature. Then, cells on the coverslips were incubated with the primary antibody against γ H2AX (1:500 dilution) for 2 h at room temperature, then washed with PBS 3 times for 5 min and incubated with the secondary antibody conjugated to anti-rabbit Alexa Fluor-488 (Life Technologies, France 1:200 dilution) for 1 h at room temperature. Nuclei were counterstained with DAPI diluted into the Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). Coverslips were mounted on glass slides and further analyzed by a confocal microscope. For visual effect, the Alexa Fluor-488 staining in Figure 1G is represented in red.

4.7. Microscope Image Acquisition and Analysis

Immunofluorescence images for γ H2AX staining were acquired using a TCS SP8 confocal microscope (Leica Microsystems, Berlin, Germany) with a 63 \times oil immersion objective. Z-stacks were obtained using a frame size of 1024 \times 1024 and 0.5 μ m z-steps, with sequential multitrack scanning using 488 nm laser wavelengths. Images were analyzed by ImageJ software.

4.8. Statistical Analyses

One-way ANOVA test with Bonferroni's post-test was used to compare between groups. The Student t-test with two-tailed distribution was used to compare pairs of samples. All tests were conducted on Graphpad Prism v. 5 software (Graphpad software Inc., La Jolla, CA, USA).

Supplementary Materials: The following are available online at www.mdpi.com/1422-0067/22/4/1588/s1.

Author Contributions: S.D., Y.V., D.G., and B.A. designed the study; B.A. carried out the experiments; S.D., Y.V., D.G., and B.A. analyzed the data; A.K.B., Y.R.M., and E.V.S. contributed materials; S.D., Y.V., D.G., and B.A. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ligue Nationale Contre le Cancer and the IDB RAS Government basic research program 0088-2021-0007. B.A. was funded by a Campus France PhD exchange program.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Whole blood samples were obtained from healthy donors of different sex and age from the “Etablissement Français du Sang”, Hôpital Saint-Louis, Paris, France. In agreement with French law, the study was registered as a non-interventional study at the Agence Nationale de Sécurité du Médicament (2016-A01316-45) and informed consent was obtained from all subjects.

Data Availability Statement: Not Applicable.

Acknowledgments: We are grateful to the NIH AIDS Research and Reference Reagent Program, USA, for providing purified HIV-1 Tat.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Dayton, A.G.S.; Craig, A.; Rosen, W.C.G.; Haseltine, W.A. The Trans-Activator Gene of the Human T Cell Lymphotropic Virus Type III Is Required for Replication. *Cell* **1986**, *44*, 941–947, doi:10.1016/0092-8674(86)90017-6.
- Hauber, J.; Perkins, A.; Heimer, E.P.; Cullen, B.R. Trans-Activation of Human Immunodeficiency Virus Gene Expression Is Mediated by Nuclear Events. *Proc. Nat. Acad. Sci. USA* **1987**, *84*, 6364–6368, doi:10.1073/pnas.84.18.6364.
- Chang, H.C.; Samaniego, F.; Nair, B.C.; Buonaguro, L.; Ensoli, B. HIV-1 Tat Protein Exits from Cells via a Leaderless Secretory Pathway and Binds to Extracellular Matrix-Associated Heparan Sulfate Proteoglycans through Its Basic Region. *Aids* **1997**, *11*, 1421–1431, doi:10.1097/00002030-199712000-00006.
- Choi, J.Y.; Hightower, G.K.; Wong, J.K.; Heaton, R.; Woods, S.; Grant, I.; Marcotte, T.D.; Ellis, R.J.; Letendre, S.L.; Collier, A.C.; et al. Genetic Features of Cerebrospinal Fluid-Derived Subtype B HIV-1 Tat. *J. Neurovirol.* **2012**, *18*, 81–90, doi:10.1007/s13365-011-0059-9.
- Westendorp, M.O.; Frank, R.; Ochsenbauer, C.; Stricker, K.; Dhein, J.; Walczak, H.; Debating, K.M.; Krammer, P.H. Sensitization of T Cells to CD95-Mediated Apoptosis by HIV-1 Tat and Gp120. *Nature* **1995**, *375*, 497–500, doi:10.1038/375497a0.
- Xiao, H.; Neuveut, C.; Tiffany, H.L.; Benkirane, M.; Rich, E.A.; Murphy, P.M.; Jeang, K.-T. Selective CXCR4 Antagonism by Tat: Implications for in Vivo Expansion of Coreceptor Use by HIV-1. *Proc. Nat. Acad. Sci. USA* **2000**, *97*, 11466–11471, doi:10.1073/pnas.97.21.11466.
- Croxford, S.; Kitching, A.; Desai, S.; Kall, M.; Edelstein, M.; Skingsley, A.; Burns, F.; Copas, A.; Brown, A.E.; Sullivan, A.K.; et al. Mortality and Causes of Death in People Diagnosed with HIV in the Era of Highly Active Antiretroviral Therapy Compared with the General Population: An Analysis of a National Observational Cohort. *Lancet Public Health* **2017**, *2*, e35–e46, doi:10.1016/S2468-2667(16)30020-2.
- Nishijima, T.; Inaba, Y.; Kawasaki, Y.; Tsukada, K.; Teruya, K.; Kikuchi, Y.; Gatanaga, H.; Oka, S. Mortality and Causes of Death in People Living with HIV in the Era of Combination Antiretroviral Therapy Compared with the General Population in Japan. *AIDS* **2020**, *34*, 913–921, doi:10.1097/QAD.0000000000002498.
- Akbay, B.; Shmakova, A.; Vassetzky, Y.; Dokudovskaya, S. Modulation of MTORC1 Signaling Pathway by HIV-1. *Cells* **2020**, *9*, 1090–1090, doi:10.3390/cells9051090.
- Shiels, M.S.; Engels, E.A. Evolving Epidemiology of HIV-Associated Malignancies. *Curr. Opin. HIV Aids* **2017**, *12*, 6–11, doi:10.1097/COH.0000000000000327.
- Shmakova, A.; Germini, D.; Vassetzky, Y. HIV-1, HAART and Cancer: A Complex Relationship. *Int. J. Cancer* **2020**, *146*, 2666–2679, doi:10.1002/ijc.32730.
- Germini, D.; Tsfasman, T.; Klibi, M.; El-Amine, R.; Pichugin, A.; Iarovaia, O.V.; Bilhou-Nabera, C.; Subra, F.; Bou Saada, Y.; Sukhanova, A.; et al. HIV Tat Induces a Prolonged MYC Relocalization next to IGH in Circulating B-Cells. *Leukemia* **2017**, *31*, 2515–2522, doi:10.1038/leu.2017.106.
- Dolcetti, R.; Giagulli, C.; He, W.; Sella, M.; Caccuri, F.; Eyzaguirre, L.M.; Mazzuca, P.; Corbellini, S.; Campilongo, F.; Marzico, S.; et al. Role of HIV-1 Matrix Protein P17 Variants in Lymphoma Pathogenesis. *Proc. Nat. Acad. Sci. USA* **2015**, *112*, 14331–14336, doi:10.1073/pnas.1514748112.
- Wang, X.; Duan, Z.; Yu, G.; Fan, M.; Scharff, M.D. Human Immunodeficiency Virus Tat Protein Aids V Region Somatic Hypermutation in Human B Cells. *mBio* **2018**, *9*, e02315-17, doi:10.1128/mBio.02315-17.
- El-Amine, R.; Germini, D.; Zakharova, V.V.; Tsfasman, T.; Sheval, E.V.; Louzada, R.A.N.; Dupuy, C.; Bilhou-Nabera, C.; Hamade, A.; Najjar, F.; et al. HIV-1 Tat Protein Induces DNA Damage in Human Peripheral Blood B-Lymphocytes via Mitochondrial ROS Production. *Redox Biol.* **2018**, *15*, 97–108, doi:10.1016/j.redox.2017.11.024.
- Ágnes Márk, M.H.; Zsófia, V.; Sticz, T.B.; Nagy, N.; Csomor, J.; Berczi, L.; Varga, V.; Csóka, M.; Sebestyén, L.K. and A. Characteristic MTOR Activity in Hodgkin-Lymphomas Offers a Potential Therapeutic Target in High Risk Disease – a Combined Tissue Microarray, in Vitro and in Vivo Study. *BMC Cancer* **2013**, *13*, doi:10.1186/1471-2407-13-250.
- Browne, S.H.; Diaz-Perez, J.A.; Preziosi, M.; King, C.C.; Jones, G.A.; Jain, S.; Sun, X.; Reid, E.G.; Vandenberg, S.; Wang, H.Y. MTOR Activity in AIDS-Related Diffuse Large B-Cell Lymphoma. *PLoS ONE* **2017**, *12*, 1–12, doi:10.1371/journal.pone.0170771.

18. Col, J.D.; Zancai, P.; Terrin, L.; Guidoboni, M.; Ponzoni, M.; Pavan, A.; Spina, M.; Bergamin, S.; Rizzo, S.; Tirelli, U.; et al. Distinct Functional Significance of Akt and MTOR Constitutive Activation in Mantle Cell Lymphoma. *Blood* **2008**, *111*, 5142–5151, doi:10.1182/blood-2007-07-103481.
19. El-Salem, M.; Raghunath, P.N.; Marzec, M.; Liu, X.; Kasprzycka, M.; Robertson, E.; Wasik, M.A. Activation of MTORC1 Signaling Pathway in AIDS-Related Lymphomas. *Am. J. Pathol.* **2009**, *175*, 817–824, doi:10.2353/ajpath.2009.080451.
20. Sebestyén, A.; Sticz, T.B.; Márk, Á.; Hajdu, M.; Timár, B.; Nemes, K.; Nagy, N.; Váradi, Z.; Kopper, L. Activity and Complexes of MTOR in Diffuse Large B-Cell Lymphomas—A Tissue Microarray Study. *Mod. Pathol.* **2012**, *25*, 1623–1628, doi:10.1038/modpathol.2012.141.
21. Sekihara, K.; Saitoh, K.; Han, L.; Ciurea, S.; Yamamoto, S.; Kikkawa, M.; Kazuno, S.; Taka, H.; Kaga, N.; Arai, H.; et al. Targeting Mantle Cell Lymphoma Metabolism and Survival through Simultaneous Blockade of MTOR and Nuclear Transporter Exportin-1. *Oncotarget* **2017**, *8*, 34552–34564, doi:10.18632/oncotarget.16602.
22. Le Sage, V.; Cinti, A.; Amorim, R.; Moulund, A. Adapting the Stress Response: Viral Subversion of the MTOR Signaling Pathway. *Viruses* **2016**, *8*, 152–152, doi:10.3390/v8060152.
23. Ma, Y.; Vassetzky, Y.; Dokudovskaya, S. MTORC1 Pathway in DNA Damage Response. *Biochim. Et Biophys. Acta Mol. Cell Res.* **2018**, *1865*, 1293–1311, doi:10.1016/j.bbamcr.2018.06.011.
24. Hoxhaj, G.; Manning, B.D. The PI3K–AKT Network at the Interface of Oncogenic Signalling and Cancer Metabolism. *Nat. Rev. Cancer* **2020**, *20*, 74–88, doi:10.1038/s41568-019-0216-7.
25. Ben-Sahra, I.; Hoxhaj, G.; Ricoult, S.J.H.; Asara, J.M.; Manning, B.D. MTORC1 Induces Purine Synthesis through Control of the Mitochondrial Tetrahydrofolate Cycle. *Science* **2016**, *351*, 728–733, doi:10.1126/science.aad0489.
26. Brunn, G.J.; Hudson, C.C.; Sekulic, A.; Williams, J.M.; Hosoi, H.; Houghton, P.J.; Lawrence, J.C.; Abraham, R.T. Phosphorylation of the Translational Repressor PHAS-I by the Mammalian Target of Rapamycin. *Science* **1997**, *277*, 99–101, doi:10.1126/science.277.5322.99.
27. Dennis, M.D.; Jefferson, L.S.; Kimball, S.R. Role of P70S6K1-Mediated Phosphorylation of EIF4B and PDCD4 Proteins in the Regulation of Protein Synthesis. *J. Biol. Chem.* **2012**, *287*, 42890–42899, doi:10.1074/jbc.M112.404822.
28. Düvel, K.; Yecies, J.L.; Menon, S.; Raman, P.; Lipovsky, A.I.; Souza, A.L.; Triantafellow, E.; Ma, Q.; Gorski, R.; Cleaver, S.; et al. Activation of a Metabolic Gene Regulatory Network Downstream of MTOR Complex 1. *Mol. Cell* **2010**, *39*, 171–183, doi:10.1016/j.molcel.2010.06.022.
29. Haghighat, A.; Sonenberg, N. EIF4G Dramatically Enhances the Binding of EIF4E to the mRNA 5'-Cap Structure. *J. Biol. Chem.* **1997**, *272*, 21677–21680, doi:10.1074/jbc.272.35.21677.
30. Linke, M.; Fritsch, S.D.; Sukhbaatar, N.; Hengstschläger, M.; Weichhart, T. MTORC1 and MTORC2 as Regulators of Cell Metabolism in Immunity. *Febs Lett.* **2017**, *591*, 3089–3103, doi:10.1002/1873-3468.12711.
31. Powell, J.D.; Pollizzi, K.N.; Heikamp, E.B.; Horton, M.R. Regulation of Immune Responses by MTOR. *Annu. Rev. Immunol.* **2012**, *30*, 39–68, doi:10.1146/annurev-immunol-020711-075024.
32. Iwata, T.N.; Ramirez, J.A.; Tsang, M.; Park, H.; Margineantu, D.H.; Hockenbery, D.M.; Iritani, B.M. Conditional Disruption of Raptor Reveals an Essential Role for MTORC1 in B Cell Development, Survival, and Metabolism. *J. Immunol.* **2016**, *197*, 2250–2260, doi:10.4049/jimmunol.1600492.
33. Jones, D.D.; Weiss, B.M.; Allman, D.; Jones, D.D.; Gaudette, B.T.; Wilmore, J.R.; Chernova, I.; Bortnick, A.; Weiss, B.M.; Allman, D. MTOR Has Distinct Functions in Generating versus Sustaining Humoral Immunity. *J. Clin. Invest.* **2016**, *126*, 4250–4261, doi:10.1172/JCI86504.paradoxically.
34. Raybuck, A.L.; Cho, S.H.; Li, J.; Rogers, M.C.; Lee, K.; Williams, C.L.; Shlomchik, M.; Thomas, J.W.; Chen, J.; Williams, J.V.; et al. B Cell-Intrinsic MTORC1 Promotes Germinal Center-Defining Transcription Factor Gene Expression, Somatic Hypermutation, and Memory B Cell Generation in Humoral Immunity. *J. Immunol.* **2018**, *200*, 2627–2639, doi:10.4049/jimmunol.1701321.
35. Zhang, S.; Pruitt, M.; Tran, D.; Du Bois, W.; Zhang, K.; Patel, R.; Hoover, S.; Simpson, R.M.; Simmons, J.; Gary, J.; et al. B Cell-Specific Deficiencies in MTOR Limit Humoral Immune Responses. *J. Immunol.* **2013**, *191*, 1692–1703, doi:10.4049/jimmunol.1201767.
36. Teater, M.; Dominguez, P.M.; Redmond, D.; Chen, Z.; Ennishi, D.; Scott, D.W.; Cimmino, L.; Ghione, P.; Chaudhuri, J.; Gascoyne, R.D.; et al. AICDA Drives Epigenetic Heterogeneity and Accelerates Germinal Center-Derived Lymphomagenesis. *Nat. Commun.* **2018**, *9*, 222–222, doi:10.1038/s41467-017-02595-w.
37. Zhang, J.; Shi, Y.; Zhao, M.; Hu, H.; Huang, H. Activation-Induced Cytidine Deaminase Overexpression in Double-Hit Lymphoma: Potential Target for Novel Anticancer Therapy. *Sci. Rep.* **2020**, *10*, 14164–14164, doi:10.1038/s41598-020-71058-y.
38. Sall, F.B.; El Amine, R.; Markozashvili, D.; Tsfasman, T.; Oksenhendler, E.; Lipinski, M.; Vassetzky, Y.; Germini, D. HIV-1 Tat Protein Induces Aberrant Activation of AICDA in Human B-lymphocytes from Peripheral Blood. *J. Cell. Physiol.* **2019**, *234*, 15678–15685, doi:10.1002/jcp.28219.
39. Gorbacheva, M.A.; Tikhomirova, M.A.; Potashnikova, D.M.; Akbay, B.; Sheval, E.V.; Musinova, Y.R. Production of Stable Cell Lines on the Basis of the Cultured RPMI 8866 B-Cells with Constant and Inducible Expression of the Human Immunodeficiency Virus Tat Protein. *Russ. J. Dev. Biol.* **2019**, *50*, 275–280, doi:10.1134/S1062360419050060.
40. Buccigrossi, V.; Laudiero, G.; Nicastro, E.; Miele, E.; Esposito, F.; Guarino, A. The HIV-1 Transactivator Factor (Tat) Induces Enterocyte Apoptosis through a Redox-Mediated Mechanism. *PLoS ONE* **2011**, *6*, e29436–e29436, doi:10.1371/journal.pone.0029436.

41. Rozzi, S.J.; Borelli, G.; Ryan, K.; Steiner, J.P.; Reglodi, D.; Mocchetti, I.; Avdoshina, V. PACAP27 Is Protective Against Tat-Induced Neurotoxicity. *J. Mol. Neurosci.* **2014**, *54*, 485–493, doi:10.1007/s12031-014-0273-z.
42. Keating, R.; McGargill, M.A. MTOR Regulation of Lymphoid Cells in Immunity to Pathogens. *Front. Immunol.* **2016**, *7*, doi:10.3389/fimmu.2016.00180.
43. Blanchet, F.P.; Moris, A.; Nikolic, D.S.; Lehmann, M.; Cardinaud, S.; Stalder, R.; Garcia, E.; Dinkins, C.; Leuba, F.; Wu, L.; et al. Human Immunodeficiency Virus-1 Inhibition of Immunoamphisomes in Dendritic Cells Impairs Early Innate and Adaptive Immune Responses. *Immunity* **2010**, *32*, 654–669, doi:10.1016/j.immuni.2010.04.011.
44. Cinti, A.; Le Sage, V.; Milev, M.P.; Valiente-Echeverría, F.; Crossie, C.; Miron, M.-J.; Panté, N.; Olivier, M.; Moulard, A.J. HIV-1 Enhances MTORC1 Activity and Repositions Lysosomes to the Periphery by Co-Opting Rag GTPases. *Sci. Rep.* **2017**, *7*, 5515–5515, doi:10.1038/s41598-017-05410-0.
45. Kumar, B.; Arora, S.; Ahmed, S.; Banerjee, A.C. Hyperactivation of Mammalian Target of Rapamycin Complex 1 by HIV-1 Is Necessary for Virion Production and Latent Viral Reactivation. *FASEB J.* **2017**, *31*, 180–191, doi:10.1096/fj.201600813R.
46. Planas, D.; Routy, J.; Ancuta, P.; Planas, D.; Zhang, Y.; Monteiro, P.; Goulet, J.; Gosselin, A. HIV-1 Selectively Targets Gut-Homing Mechanisms Find the Latest Version : HIV-1 Selectively Targets Gut-Homing Mechanisms. **2017**, *2*, 1–21.
47. Rehman, S.; Husain, M.; Yadav, A.; Kasinath, B.S.; Malhotra, A.; Singhal, P.C. HIV-1 Promotes Renal Tubular Epithelial Cell Protein Synthesis: Role of MTOR Pathway. *PLoS ONE* **2012**, *7*, e30071–e30071, doi:10.1371/journal.pone.0030071.
48. Zhang, H.-S.; Zhang, Z.-G.; Zhou, Z.; Du, G.-Y.; Li, H.; Yu, X.-Y.; Huang, Y.-H. PKM2-Mediated Inhibition of Autophagy Facilitates Tat's Inducing HIV-1 Transactivation. *Arch. Biochem. Biophys.* **2017**, 625–626, 17–23, doi:10.1016/j.abb.2017.05.018.
49. Xue, M.; Yao, S.; Hu, M.; Li, W.; Hao, T.; Zhou, F.; Zhu, X.; Lu, H.; Qin, D.; Yan, Q.; et al. HIV-1 Nef and KSHV Oncogene K1 Synergistically Promote Angiogenesis by Inducing Cellular MiR-718 to Regulate the PTEN/AKT/MTOR Signaling Pathway. *Nucleic Acids Res.* **2014**, *42*, 9862–9879, doi:10.1093/nar/gku583.
50. Poggi, A.; Carosio, R.; Fenoglio, D.; Brenci, S.; Murdaca, G.; Setti, M.; Indiveri, F.; Scabini, S.; Ferrero, E.; Zocchi, M.R. Migration of V Delta 1 and V Delta 2 T Cells in Response to CXCR3 and CXCR4 Ligands in Healthy Donors and HIV-1-Infected Patients: Competition by HIV-1 Tat. *Blood* **2004**, *103*, 2205–13, doi:10.1182/blood-2003-08-2928.
51. Kurnaeva, M.A.; Sheval, E.V.; Musinova, Y.R.; Vassetzky, Y.S. Tat Basic Domain: A “Swiss Army Knife” of HIV-1 Tat? *Rev. Med. Virol.* **2019**, *29*, e2031–e2031, doi:10.1002/rmv.2031.
52. Epeldegui, M.; Breen, E.C.; Hung, Y.P.; Boscardin, W.J.; Detels, R.; Martinez-Maza, O. Elevated Expression of Activation Induced Cytidine Deaminase in Peripheral Blood Mononuclear Cells Precedes AIDS-NHL Diagnosis. *AIDS* **2007**, *21*, 2265–2270, doi:10.1097/QAD.0b013e3282ef9f59.
53. Kawamura, K.; Wada, A.; Wang, J.-Y.; Li, Q.; Ishii, A.; Tsujimura, H.; Takagi, T.; Itami, M.; Tada, Y.; Tatsumi, K.; et al. Expression of Activation-Induced Cytidine Deaminase Is Associated with a Poor Prognosis of Diffuse Large B Cell Lymphoma Patients Treated with CHOP-Based Chemotherapy. *J. Cancer Res. Clin. Oncol.* **2016**, *142*, 27–36, doi:10.1007/s00432-015-2001-7.
54. Mdletshe, N.; Nel, A.; Shires, K.; Mowla, S. HIV Nef Enhances the Expression of Oncogenic C-MYC and Activation-Induced Cytidine Deaminase in Burkitt Lymphoma Cells, Promoting Genomic Instability. *Infect Agents Cancer* **2020**, *15*, 54, doi:10.1186/s13027-020-00320-9.
55. Chiu, H.; Jackson, L.V.; Oh, K.L.; Mai, A.; Ronai, Z.A.; Ruggero, D.; Fruman, D.A. The MTORC1/4E-BP/EIF4E Axis Promotes Antibody Class Switching in B Lymphocytes. *J. Immunol.* **2019**, *202*, 579–590, doi:10.4049/jimmunol.1800602.
56. Wu, C.; Fu, Q.; Guo, Q.; Chen, S.; Goswami, S.; Sun, S.; Li, T.; Cao, X.; Chu, F.; Chen, Z.; et al. Lupus-Associated Atypical Memory B Cells Are MTORC1-Hyperactivated and Functionally Dysregulated. *Ann. Rheum. Dis.* **2019**, *78*, 1090–1100, doi:10.1136/annrheumdis-2019-215039.

SUPPLEMENTARY MATERIALS

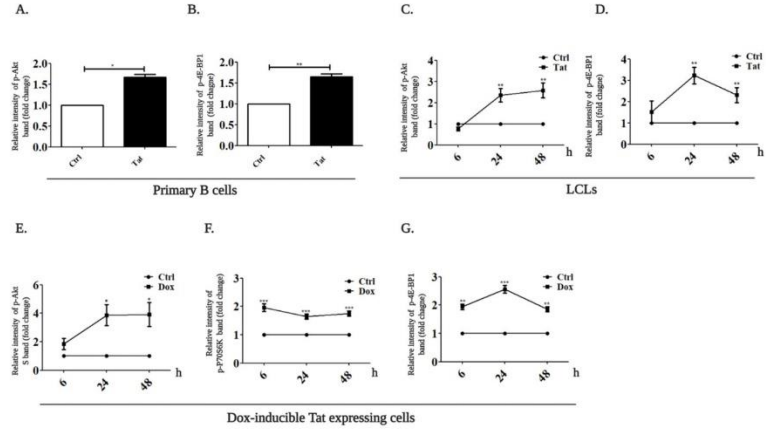


Figure S1. HIV-Tat activates the Akt/mTORC1 pathway in B cells. **A.,B.** Primary B cells purified from the blood of healthy donors were treated (Tat) (or not (Ctrl)) with 250 ng/mL Tat for 48 h. Intensities of p-AKT Ser473 (**A**) and p-4E-BP1 Thr 37/46 (**B**) bands in the Western blots from Figure 2A were quantified with ImageJ software in Tat-treated cells compared to the untreated control (set as 1), after normalization with band intensities of GAPDH (loading control). Relative band intensities on the Y axis are presented as fold change. **C.,D.** Immortalized lymphoblastoid cell lines (LCLs, RPMI-8866) were treated with 250 ng/mL Tat for 6, 24 and 48 h (see Figure 2B). Intensities of p-AKT Ser473 (**C**) and p-4E-BP1 Thr 37/46 (**D**) bands were quantified as described above. **E.,G.** Tat expression in doxycycline-inducible Tat-expressing RPMI-8866 cells was induced (Dox) (or not (Ctrl)) by treating with 1 μ g/mL doxycycline for 6, 24 and 48 h (see Figure 2C). Intensities of p-AKT Ser473 (**E**), p-P70S6K Thr389 (**F**) and p-4E-BP1 Thr 37/46 (**G**) bands were quantified as described above. The statistical analyses were carried out by the one-way ANOVA test. All data are expressed as the mean \pm SEM. The statistical significance was calculated between groups; *** p < 0.001, **0.001 < p < 0.01, *0.01 < p < 0.05.

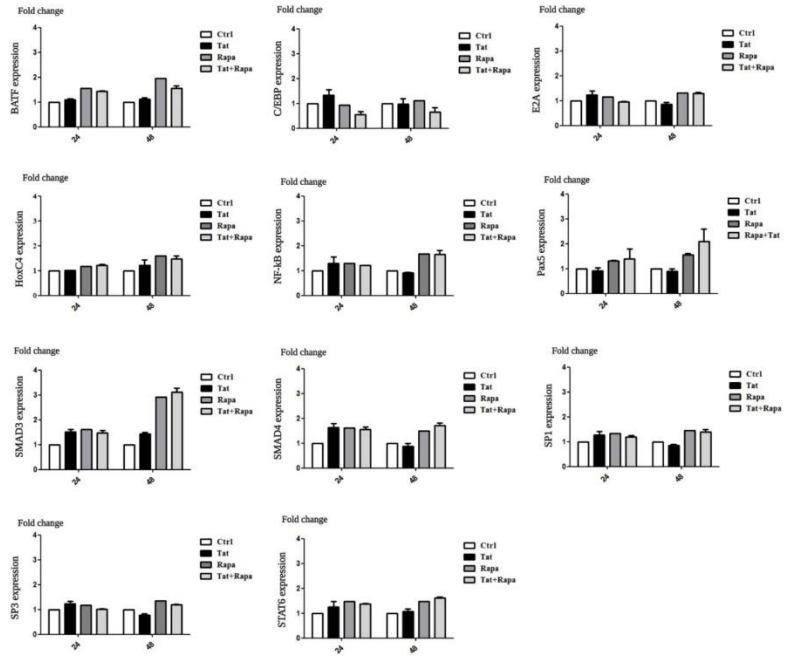


Figure S2. Expression of transcriptional activators of *AICDA* is not affected by Tat induction. mRNA expression levels of *AICDA* activators *BATF*, *C/EBP*, *E2A*, *HoxC4*, *NF-kB*, *Pax5*, *SMAD3*, *SMAD4*, *SP1*, *SP3* and *STAT6* were analyzed by qRT-PCR after 24 and 48 h of Tat expression induction. Expression of target genes in cells treated with 200 nm rapamycin with the untreated control (set as 1) were quantified using the $2^{-\Delta\Delta Ct}$ method after normalization with the *GAPDH* gene expression. The data from three independent experiments are represented as the mean \pm SEM.

Table S1. List of primers used in this study.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
PAX5	ACAGCTCTTCCCTCCCTC	GGGAAGTTGGGCTAGGTCTT
AICDA	TCTTGATGAACCGGAGGAAG	AGCCGTTCCTATTGCGAAGA
BATF	GCAAGGAGATCAAGCAGCTC	GAGCTGACATGAGGTGGTG
C/EBP β	TTTGTCCAAACCAACCGCA	TGCATCAACTTCGAAACCGG
C-Myb	CATTGATCCGCATCCCTG	TCAAAAGTTCAGTGCTGGCC
E2A	ATGGGGCATTTTGTGGGAC	TCCTGTCTACGTCACGATGG
E2F1	GGTCCCTGAGCTGTCTTCT	CCACTCACCTCTCCCATCTC
E2F2	CTCCTGGGTGAGCTGAAGAA	AAGGAGGCTTACATGGTGCT
E2F3	GGTGGGTCAAGACAGATGA	ACCAAGTCCAGTGTGTGTA
E2F4	ACAGTGGTGAGCTCAGTTCA	GAGGTAGAAGGGTTGGGTCC
E2F5	CGGCGTTCTGGATCTCAAAG	TTACAGCCAGCACCTACACC
E2F6	TGTTCAGCTCCCAGAGAAG	TCTTCTTCCTCAGGGCCTTC
E2F7	CGTCTTCAGTGTCCTTGC	TATTGATCCAAGGCCAGGCA
E2F8	GGAGGTGAGACGGTCTTCAA	TGGGAAGGGTGCAGAATTCT
HOXC4	TCCTCTCCCTCCCACTGTA	AAGCCAGACCATCACACCTT
SMAD3	CTCTGGGTGCTTGGGAATA	ATCCAAATGCAGCCAAACGT
SMAD4	ACAAGTCAGCCTGCCAGTAT	GGTGCAGTCTACTTCCAGT
SP1	GAGCAAAACAGCAGACACA	ACTGTTGGTGTCGGATGAT
SP3	TGCCTTGGACGTGGATAGC	GCCCTATCTTGCTGCAGGTA
STAT6	AAGAGCACAGGTTAGGGCAT	TAACCACATGTCCAGACCCC
Tat	CTAGACTAGAGCCCTGGAAGCA	TGAGGAGGTCTTCGTCGCT

2.1. Increased *AICDA* expression by Tat is not prevented by AKT inhibition

We verified if the expression of *AICDA* and its inhibitors, *c-Myb* and *E2F8* could also be dependent on AKT activity. Accordingly, we studied the effects on the expression of these genes after treatment of Doxycycline-inducible Tat-expressing RPMI 8866 cells with specific AKT inhibitor, MK-2206, at various concentrations and different time points. AKT activation was inhibited by MK-2206 starting from 6 hours of treatment (**Figure 24**). *AICDA* expression was increased 24 hours after Tat induction. Interestingly, in contrast to the mTORC1 inhibition, *AICDA* expression was still upregulated after Tat induction even if AKT was inhibited. Yet the *C-Myb* and *E2F8* were behaving the same way during both mTORC1 and AKT inhibition (**Figure 25**). This data indicates that while the whole AKT/mTORC1 axis is inhibited, certain feedback loops might be triggered to maintain the *AICDA* expression or Tat might increase *AICDA* expression by binding to the promoter regions of *AICDA* in response to the AKT/mTORC1 inhibition.

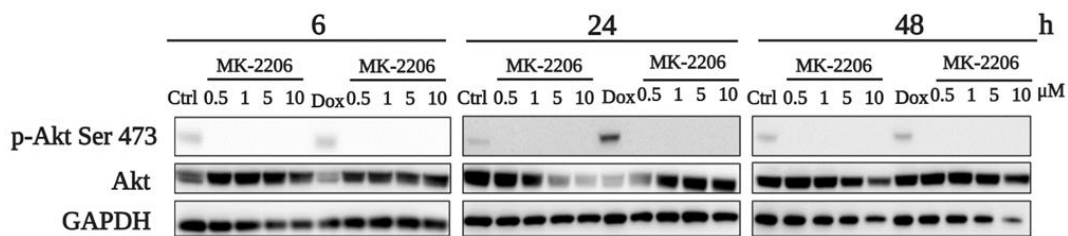


Figure 24. AKT is inhibited by MK-2206. Doxycycline-inducible Tat-expressing RPMI 8866 cells were untreated (Ctrl) or treated (MK-2206) with 0.5, 1, 5, and 10 μ M MK-2206 or treated with both MK2206 and 1 μ g/ml doxycycline (Dox) for 6, 24, and 48 hours. Indicated proteins were detected by Western blot with appropriate specific antibodies. GAPDH was used as the loading control.

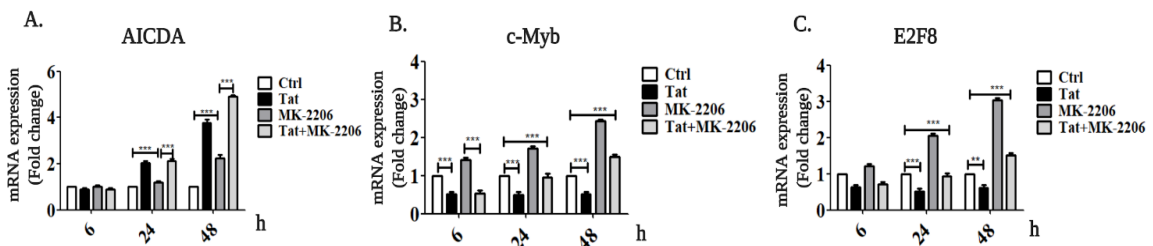


Figure 25. Inhibition of AKT does not prevent *AICDA* overexpression by Tat. Doxycycline-inducible Tat-expressing RPMI 8866 cells were untreated (Ctrl) or treated with 0.5, 1, 5, and 10 μ M MK-2206 (MK-2206), treated with 1 μ g/ml doxycycline (Tat), or with both MK2206 and 1 μ g/ml Doxycycline (Tat+MK-2206) for 6, 24, and 48 hours. *AICDA* (A), *c-Myb* (B) and *E2F8* (C) mRNA expression levels were analyzed by qRT-PCR and quantified by $2^{-\Delta\Delta Ct}$ method comparing the expression of the target gene in Tat-induced, MK-2206-treated, Tat-induced+MK-2206-treated cells with the untreated control (set as 1) after normalization with *GAPDH* gene expression. Results are obtained from three independent experiments. All data are expressed as the mean \pm SEM. The statistical significance was calculated between groups; ***, p value<0.001; **, 0.001 <p< 0.01.

2.2. Exogenous Tat differently regulates downstream targets of mTORC1, p70S6K and 4E-BP1

One interesting observation during my thesis was the differential regulation of mTORC1 targets, P70S6K and 4E-BP1 in RPMI 8866 cells in response to Tat treatment. We noticed that after the treatment the phosphorylation of P70S6K at Thr389 was reduced, while phosphorylation of 4E-BP1 at Thr37/46 was significantly increased (**Figure 26A**). This effect was also observed in primary B cells treated with Tat for 48 hours (**Figure 26B**). P70S6K phosphorylation was irreversibly inhibited by Tat regardless of serum restimulation in response to which 4E-BP1 phosphorylation was strongly stimulated in Tat-treated cells compared to the control (**Figure 26C**). In the majority of cases described so far in the literature both P70S6K and 4E-BP1 phosphorylation is increased in response to mTORC1 activation. One important exception is the case of mTORC1 inhibition by rapamycin, which suppress P70S6K phosphorylation, while 4E-BP1 phosphorylation is largely rapamycin-insensitive. p70S6K1 phosphorylation inhibition by rapamycin leads to initiation of a major feedback loop, which results in PI3K-AKT activation. (147). Indeed, we found increased phosphorylation of AKT S473 in both primary and RPMI 8866 cells treated with Tat (**Figure 26A-B**). These results indicate that upon entry into the B cells Tat selectively suppresses one of the major mTORC1 downstream branches, P70S6K1, to preferentially activate AKT/mTORC1/4E-BP1 branch. Tat might use this signaling axis to exert its oncogenic effects, as AKT/mTORC1/4E-BP1 axis has been described to be highly oncogenic (400–403).

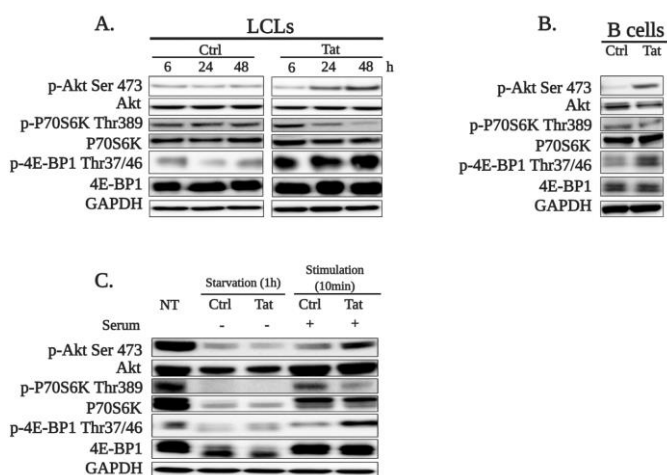


Figure 26. p70S6K and 4E-BP1 are differently regulated by exogenous Tat treatment in B cells. **A.** RPMI 8866 cells were untreated (Ctrl) or treated with 250 ng/ml Tat for 6, 24, and 48 hours (Tat). **B.** Primary B cells were cultured in the absence (Ctrl) and presence of 250 ng/ml Tat for 48 hours (Tat). **C.** RPMI 8866 cells were left untreated (Ctrl) or treated with (Tat) 250 ng/ml Tat for 48 hours. After 48 hours of treatment cells were subjected to serum starvation for 1 hour followed by restimulation with serum for 10 minutes. NT-non treated cells. Indicated proteins were detected by Western blot with appropriate specific antibodies. GAPDH was used as the loading control.

2.3. HIV-Tat inhibits starvation-induced autophagy in B cells

At basal level HIV-Tat does not affect autophagy in B cells. However, when the autophagy is stimulated with amino acid starvation, Tat prevents the induction of autophagy (**Figure 27A**). When Tat expression is induced with doxycycline, it inhibits autophagy as basal level as well as it counteracts starvation-stimulated autophagy induction (**Figure 27B**). Bafilomycin A1 treatment, which blocks autophagosome-lysosome fusion, also resulted in autophagy inhibition both in Tat-treated and Tat-induced cells (**Figure 27**).

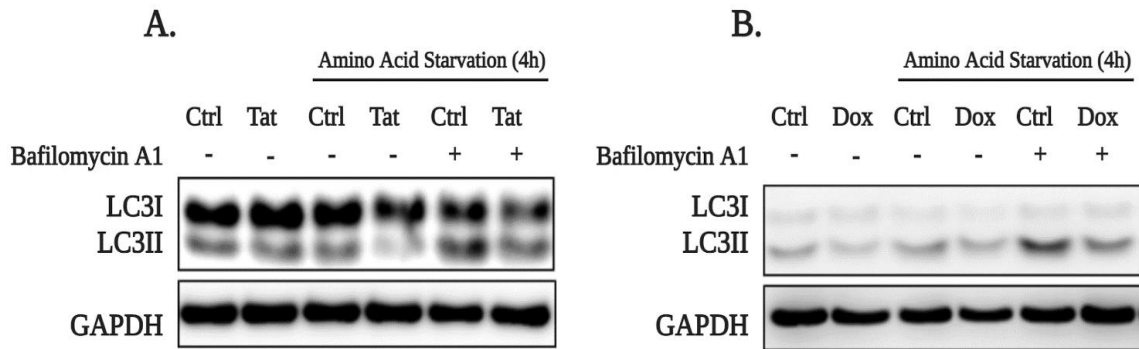


Figure 27. Tat inhibits autophagy induction during amino acid starvation. **A.** RPMI 8866 cells were left untreated (Ctrl) or treated with 250 ng/ml Tat (Tat). **B.** Doxycycline-inducible Tat-expressing RPMI 8866 cells were left untreated (Ctrl) or treated with 1 μ g/ml doxycycline (Dox) for 24 hours. Cells were subjected to amino acid starvation for 4 hours just before the end of the experiment with the absence and presence of Bafilomycin A1 simultaneously added to the culture. Indicated proteins were detected by Western blot with appropriate specific antibodies. GAPDH was used as the loading control.

Discussion

My thesis aimed to understand the mechanisms of B cell lymphomagenesis associated with HIV-1. Patients infected with HIV-1 are at high risk of developing lymphomas of B cell origin, even though the B cells are not infected by HIV-1. In the era of cART the incidence of ARLs is still high in HIV-1 patients and the survival rate of those with lymphomas is about two times lower than in HIV-uninfected patients (100–105).

Several mechanisms have been proposed to explain the mechanisms of lymphomagenesis in HIV-1 patients: 1) infected T lymphocytes escaped from elimination in germinal centers could cause unusual B cell expansion (108); 2) aberrant somatic hyper mutations of immunoglobulin might cause chromosomal translocation leading to malignant transformation (107); 3) chronic inflammation could lead to lymphoproliferation (109). HIV-1 individual proteins which can be secreted and transmitted by the infected cells, could also be involved in the lymphomagenesis (106,112,372). Among them Tat is known to be secreted by infected cells and penetrate other non-infected cells including B cells (80,83–85,404).

Tat can enter the cells either through the passive diffusion due to its small molecular weight or can interact with different ubiquitous cell surface receptors which allow its internalization. Moreover, Tat has a PTD rich in arginine which is characterized by a hydrophobic head that facilitates the penetration of Tat into the cells (60,405). Cell penetrating peptide of Tat fused to other molecules is used in the transfer of proteins, nucleic acids, fluorescent probes and chelators, as well as nanoparticles through the membrane. PTD of Tat is highly efficient in delivery of cargo up to several hundred nanometers into the cells *in vitro* and *in vivo* (86,87,406), and therefore has a potential therapeutic use.

Tat is involved in the oncogenesis in colorectal cancers (407), in the carcinogenesis of cervical cancer (408), AIDS-associated Kaposi's sarcoma (409), and B cell lymphomas (410,411). The exact mechanisms of how Tat induces oncogenesis, especially that of B cell lymphomas is still remaining to be elucidated. Previous studies of our team suggested that Tat induces ROS production, increases DNA damage, promotes AICDA gene overexpression, and brings Myc and IGH loci to a close proximity which presumably leads to their oncogenic translocation (Myc/IGH), a hallmark of Burkitt lymphoma (73,112,399). However, how these events are connected is not well understood.

AKT/mTOR signaling is important during cellular response to various stimuli including viral infection. AKT/mTORC1 pathway has been shown to be essential for successful entry, integration, and replication of HIV-1 in peripheral blood mononuclear cells, memory T cells, dendritic cells (339–344). AKT/mTORC1 activity can be modulated not only by HIV-1 but also by its individual viral proteins. It was shown that HIV-1 Nef and Env proteins can activate mTORC1 pathway in HEK293T, endothelial, and dendritic cells

(343,351). Activation of AKT/mTORC1 by Tat has also been documented in HeLa, Jurkat, and peripheral blood mononuclear cells (341,342). On the other hand, a large number of studies have shown that the AKT/mTORC1 pathway is hyperactive in different subgroups of ARL (106,114,116–118,395,396). Therefore, deregulation of AKT/mTORC1 pathway could be a major factor in Tat oncogenesis and in the ARL development. To the best of our knowledge the regulation of mTORC1 pathway by HIV-Tat in B cells has not been studied so far.

In order to have specific and effective immune responses against pathogens and develop long term immunity, hosts rely on diverse and specific adaptive immunity. To ensure the diversity, B cells exhibit a unique genomic plasticity. During B cell development, rearrangement of germline V/D/J gene segments in the immunoglobulin heavy (H) chain locus and V/J gene segments in the Ig λ or Igk light (L) chain locus generates a diverse B cell receptor (BCR) repertoire through the processes of CSR and SHM (412) which is initiated with the help of *AICDA*. Off-target mutations induced by *AICDA* have been suggested to cause lymphomagenesis. Overexpression of *AICDA* and its involvement in lymphomagenesis have been observed in different subtypes of B cell lymphomas (397,398). Interestingly, mTORC1 inactivation leads to decreased expression of *AICDA* (322,323). In addition, previously our team demonstrated Tat induces *AICDA* overexpression in primary B cells as well as *AICDA* is found to be overexpressed in B cells from HIV-1 infected patients (399).

Based on all these works, we hypothesized that HIV-1 Tat might enter B cells inducing ROS production, leading to DNA damage, further modulating AKT/mTORC1 signaling pathway, and regulating gene expressions, among which *AICDA* gene is of great importance.

Consistent with others and our previous studies (73,413,414), we demonstrated that Tat induces ROS production and DNA damage in B cells. The DNA damage detected at 6 hours of Tat expression, however, was not detectable afterwards, which can be explained that in response to the Tat-induced stresses DNA damage response pathways might be activated, which was evidenced in our study by the increased phosphorylation of Chk2 at 24 hours of Tat expression. Phosphorylation of Chk2 is the downstream signaling event of ATM-Chk2 DNA damage response pathway which can be activated by HIV-1 (415). We also found that there is increased Chk1 phosphorylation (even though not significant) particularly at 24 hours of Tat expression which is the downstream signaling event of ATR-Chk1 DNA damage response pathway. Activation of ATR-Chk1 pathway was recently evidenced by HIV Vpr protein (416,417). Studies on the effects of Tat on the cell response to DNA damage showed that Tat increases Rad51, a protein involved in the DNA repair, thus further increasing the repair of DNA double strand break via homologous recombination (418).

We show for the first time that mTORC1 pathway is activated in B cells in response to Tat expression along with AKT activation which is in line with other studies reporting that AKT/mTORC1 is hyperactivated by Tat (341,342). This observation is also meaningful as AKT/mTORC1 pathway is often upregulated in AIDS-related B cell lymphomas (106,114–118,396). Our results are supported by the other works showing that AKT/mTORC1 pathway is hyperactivated by ROS in order to promote cancer progression (419,420).

During our studies we encountered one controversial but interesting observation. While primary B cells and B cell lines are treated with exogenous Tat, downstream targets of mTORC1, 4E-BP1 and p70S6, were differentially regulated. Tat treatment induced the inhibition of mTORC1/P70S6 branch, while mTORC1/4E-BP1 is stimulated. Both branches (mTORC1/P70S6K, mTORC1/4E-BP1), however, are upregulated in the same manner when Tat is transiently expressed. For this observation we have several explanations: 1) Existence of PI3K/AKT/mTORC1/4E-BP1 activator feedback loop while cells are treated with Tat; 2) Possibility of 4E-BP1 phosphorylation by other kinases like GSK3-beta kinase which was shown to phosphorylate 4E-BP1 at exactly the same residues as mTORC1 (421). Paradoxical activity of mTORC1 is also described in other studies (422), but their observation was the reverse of our case with increased activity of mTORC1/P70S6K and decreased activity of mTORC1/4E-BP1, which was attributed to the downregulation of the AMPK signaling. In this paper it was also shown that phosphorylation of AKT is decreased, which indicates the existence of a positive relation between AKT and mTORC1/4E-BP1 axis. In our case, AKT/mTORC1/4E-BP1 axis is overactivated in both Tat-treated and Tat-induced cells.

Autophagy, one of the downstream readouts of mTORC1 activity, was inhibited in Tat-treated and transiently Tat-expressed cells, which certainly is due to the activation of AKT/mTORC1 pathway, since it is negatively regulated by mTORC1. Inhibition of autophagy by Tat is also described in macrophages through AKT activation (363). It seems that Tat inhibits the autophagy and avoids its degradation after its penetration into the cells, because host cells selectively degrade Tat protein using autophagy (365,366). Regulation of autophagy in B cells by Tat, however, must be explored in detail.

Finally, we looked at the consequences of the activation of AKT/mTORC1 pathway by Tat. In agreement with our previous work (399), we found that the *AICDA* gene is overexpressed in the cells treated with Tat. Other groups had shown that mTORC1 pathway is positively associated with *AICDA* expression (322,323) but without any clear explanation. In my thesis project, we found that Tat-induced *AICDA* expression is mTORC1 dependent. Our results revealed the missing link between mTORC1 and *AICDA* expression. Tat decreases the expression of c-Myb and E2F8, suppressors of *AICDA*, in mTORC1-dependent manner, that might lead to the disruption of the balance between *AICDA* activators and inhibitors (423,424). Interestingly, however, specific inhibition of AKT did not prevent *AICDA* overexpression by Tat while mTORC1 inhibition has drastically reduced its overexpression. It seems that total inhibition of

AKT/mTORC1 axis leads to the activation of some other compensatory mechanisms which might maintain AICDA overexpression level in B cells, such as NF- κ B signaling pathway as it was shown to be involved in the induction of HoxC4, one of the AICDA activators, in mouse B cells (425). In response to AKT/mTORC1 pathway inhibition Tat might bind directly to the promoter regions of AICDA to maintain high level of AICDA (397,398).

Conclusions and perspectives

In this work we showed that in B cells HIV-1 Tat activates the AKT/mTORC1 pathway and increases 4E-BP phosphorylation, while suppresses P70S6K phosphorylation. It would be interesting to explore this phenomenon 1) by verifying the PI3K/AKT activator feedback loop using new generation mTORC1 inhibitor RapaLink-1 which specifically inhibits phosphorylation of both 4E-BP1 and p70S6K. If RapaLink-1 downregulates 4E-BP1 phosphorylation, that would prove the existence of the feedback loop; 2) by studying the other upstream regulators of PI3K/AKT (e.g. PTEN), functioning of TSC, and other independent signaling pathways like AMPK and p53 signaling. AKT/mTORC1/4E-BP1 signaling axis might be oncogenic.

We demonstrated that AKT/mTORC1 activation is associated with DNA damage induced by Tat-generated ROS. DNA damage is recovered rapidly through the activation of DNA damage repair pathway. It would be interesting to explore DDR by studying p53 signaling pathway and the key DDR proteins like Ku proteins, DNA-PKs, and Artemis.

We also demonstrated that Tat treatment and transient Tat expression inhibited autophagy, in order to avoid its degradation by this process. However, we need to further explore the regulation of B cell autophagy by Tat in more detail by studying the behavior of other key proteins like ULK and p62, by analyzing autophagic structures using electron microscopy and monitoring GFP-LC3 degradation with flow cytometer and mRFP-GFP-LC3 Tandem Fluorescent Protein Quenching Assay.

Another interesting finding is that AKT/mTORC1 activation by Tat leads to the overexpression of *AICDA*, which was shown to be oncogenic, through the downregulation of its inhibitors. The disruption of the balance of between *AICDA* activators and inhibitors due to their suppression might relieve the competition for the binding sites preferentially allowing the activators to bind to the *AICDA* sequence with its final overexpression. So, it should be confirmed using techniques like DNA immunoprecipitation (DIP-chip) by treating the cells with Tat or by inducing its expression to see if *AICDA* activators binding capacity would increase. We observe that *AICDA* overexpression is completely blocked by mTORC1 inhibition confirming the dependency of *AICDA* expression on mTORC1, while AKT inhibition did not prevent the Tat-induced *AICDA* overexpression, which also implies that while the whole AKT/mTORC1 pathway is blocked, some other mechanisms like NF- κ B signaling pathway might be activated as a compensation. Therefore, it would be interesting to study the other mechanisms of *AICDA* overexpression by Tat.

By summarizing all the results, we can draw a conclusion that HIV-Tat integrates three “oncogenics” - the activation of AKT/mTORC1, the overexpression of *AICDA*, and the translocation of MYC/IG - to exert its oncogenic effects on B cells, leading the development of B cell lymphomas.

Conferences and Publications

1. Conferences

Akbay Burkitkan, Yegor Vassetzky, Svetlana Dokudovskaya. “Regulation of mTORC1 pathway and autophagy by HIV transcriptional activator Tat in B cells: a novel mechanism of AIDS-related oncogenesis?”. 9-th Scientific days of CFATG in collaboration with the German and Swiss networks for Autophagy Research. November 20-22th ,2019 (**Oral presentation**).

Akbay Burkitkan, Yegor Vassetzky, Svetlana Dokudovskaya. “HIV-Tat induced mTORC1 pathway deregulation in B cells: A novel mechanism of AIDS-related oncogenesis”. October 27-30th 2020. International Conference on Innovative Solutions: Cancer, Aging and Genetic diseases. World Wide Live Remote Conference (**Oral presentation**). **CIPREVO-sponsored ICIS 2020 Prize winner**.

2. Publications

1- MA Gorbacheva, MA Tikhomirova, DM Potashnikova, **B Akbay**, EV Sheval, YR Musinova. “Production of Stable Cell Lines on the Basis of the Cultured RPMI 8866 B-Cells with Constant and Inducible Expression of the Human Immunodeficiency Virus Tat Protein” (**Article** published in Russian Journal of Developmental Biology 2019).

2- **Burkitkan Akbay**, Anna Shmakova, Yegor Vassetzky, Svetlana Dokudovskaya. “Modulation of mTORC1 Signaling Pathway by HIV-1” (**Review** published in Cells 2020).

3- **Burkitkan Akbay**, Diego Germini, Amangeldy K. Bissenbaev, Yana R. Musinova, Evgeny V. Sheval, Yegor Vassetzky and Svetlana Dokudovskaya. “HIV-1 Tat activates AKT/mTORC1 pathway and AICDA expression by downregulating its transcriptional inhibitors in B cells” (**Invited article** accepted to the Special Issue “mTOR Signaling network in Cell Biology and Disease” International Journal of Molecular Sciences)

References

1. Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M. Human viruses: Discovery and emergence. *Philos Trans R Soc B Biol Sci* (2012) **367**:2864–2871. doi:10.1098/rstb.2011.0354
2. Mettenleiter TC. *The First “Virus Hunters.”* 1st ed. Elsevier Inc. (2017). doi:10.1016/bs.aivir.2017.07.005
3. Ryu W-S. “Virus Structure,” in *Molecular Virology of Human Pathogenic Viruses* (Elsevier), 21–29. doi:10.1016/B978-0-12-800838-6.00002-3
4. Lucas W. “Viral Capsids and Envelopes: Structure and Function,” in *Encyclopedia of Life Sciences* (Chichester, UK: John Wiley & Sons, Ltd), 1–7. doi:10.1002/9780470015902.a0001091.pub2
5. López-García P, Moreira D. Viruses in Biology. *Evol Educ Outreach* (2012) **5**:389–398. doi:10.1007/s12052-012-0441-y
6. HR G. *Structure and Classification of Viruses*. 4th ed. , ed. S Baron Galveston (TX): University of Texas Medical Branch at Galveston (1996). Available at: <https://www.ncbi.nlm.nih.gov/books/NBK8174/>
7. Simmonds P, Aiewsakun P. Virus classification – where do you draw the line? *Arch Virol* (2018) **163**:2037–2046. doi:10.1007/s00705-018-3938-z
8. Guttman BS. “Virus,” in *Encyclopedia of Genetics* (Elsevier), 2108–2114. doi:10.1006/rwgn.2001.1371
9. Maginnis MS. Virus–Receptor Interactions: The Key to Cellular Invasion. *J Mol Biol* (2018) **430**:2590–2611. doi:10.1016/j.jmb.2018.06.024
10. Marsh M, Helenius A. Virus entry: Open sesame. *Cell* (2006) **124**:729–740. doi:10.1016/j.cell.2006.02.007
11. Grove J, Marsh M. The cell biology of receptor-mediated virus entry. *J Cell Biol* (2011) **195**:1071–1082. doi:10.1083/jcb.201108131
12. Dimitrov DS. Virus entry: molecular mechanisms and biomedical applications. *Nat Rev Microbiol* (2004) **2**:109–122. doi:10.1038/nrmicro817
13. Thorley JA, McKeating JA, Rappoport JZ. Mechanisms of viral entry: sneaking in the front door. *Protoplasma* (2010) **244**:15–24. doi:10.1007/s00709-010-0152-6
14. Yamauchi Y, Helenius A. Virus entry at a glance. *J Cell Sci* (2013) **126**:1289–1295. doi:10.1242/jcs.119685
15. Doms RW. “Basic Concepts,” in *Viral Pathogenesis* (Elsevier), 29–40. doi:10.1016/B978-0-12-800964-2.00003-3
16. Cosset F-L, Lavillette D. “Cell Entry of Enveloped Viruses,” in *Advances in Genetics*, 121–183. doi:10.1016/B978-0-12-380860-8.00004-5
17. den Boon JA, Diaz A, Ahlquist P. Cytoplasmic Viral Replication Complexes. *Cell Host Microbe* (2010) **8**:77–85. doi:10.1016/j.chom.2010.06.010

18. Dou D, Revol R, Östbye H, Wang H, Daniels R. Influenza A Virus Cell Entry, Replication, Virion Assembly and Movement. *Front Immunol* (2018) **9**:1–17. doi:10.3389/fimmu.2018.01581
19. Rampersad S, Tennant P. “Replication and Expression Strategies of Viruses,” in *Viruses* (Elsevier), 55–82. doi:10.1016/B978-0-12-811257-1.00003-6
20. Soderstrom K. “Viral Replication,” in *xPharm: The Comprehensive Pharmacology Reference* (Elsevier), 1–5. doi:10.1016/B978-008055232-3.60230-2
21. Perlmutter JD, Hagan MF. Mechanisms of Virus Assembly. *Annu Rev Phys Chem* (2015) **66**:217–239. doi:10.1146/annurev-physchem-040214-121637
22. Rixon FJ, Chiu W. “Studying Large Viruses,” in, 379–408. doi:10.1016/S0065-3233(03)01011-8
23. Payne S. “Virus Interactions With the Cell,” in *Viruses* (Elsevier), 23–35. doi:10.1016/B978-0-12-803109-4.00003-9
24. Gallo R, Sarin P, Gelmann E, Robert-Guroff M, Richardson E, Kalyanaraman V, Mann D, Sidhu G, Stahl R, Zolla-Pazner S, et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science* (80-) (1983) **220**:865–867. doi:10.1126/science.6601823
25. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. *Nature* (1999) **397**:436–441. doi:10.1038/17130
26. Faria NR, Rambaut A, Suchard MA, Baele G, Bedford T, Ward MJ, Tatem AJ, Sousa JD, Arinaminpathy N, Pépin J, et al. The early spread and epidemic ignition of HIV-1 in human populations. *Science* (80-) (2014) **346**:56–61. doi:10.1126/science.1256739
27. Turner BG, Summers MF. Structural biology of HIV 1. *J Mol Biol* (1999) **285**:1–32. doi:10.1006/jmbi.1998.2354
28. Votteler J, Schubert U. “Human Immunodeficiency Viruses: Molecular Biology,” in *Encyclopedia of Virology* (Elsevier), 517–525. doi:10.1016/B978-012374410-4.00428-3
29. Broder CC, Collman RG. Chemokine receptors and HIV. *J Leukoc Biol* (1997) **62**:20–29. doi:10.1002/jlb.62.1.20
30. Knight SC, Macatonia SE, Patterson S. HIV I Infection of Dendritic Cells. *Int Rev Immunol* (1990) **6**:163–175. doi:10.3109/08830189009056627
31. Berger EA, Murphy PM, Farber JM. CHEMOKINE RECEPTORS AS HIV-1 CORECEPTORS: Roles in Viral Entry, Tropism, and Disease. *Annu Rev Immunol* (1999) **17**:657–700. doi:10.1146/annurev.immunol.17.1.657
32. Weiss RA. HIV Receptors and Cellular Tropism. *IUBMB Life* (2002) **53**:201–205. doi:10.1080/15216540290098927
33. Lobritz MA, Ratcliff AN, Arts EJ. HIV-1 Entry, Inhibitors, and Resistance. *Viruses* (2010) **2**:1069–1105. doi:10.3390/v2051069
34. Chen B. Molecular Mechanism of HIV-1 Entry. *Trends Microbiol* (2019) **27**:878–891. doi:10.1016/j.tim.2019.06.002

35. Chen P, Chen BK, Mosoian A, Hays T, Ross MJ, Klotman PE, Klotman ME. Virological synapses allow HIV-1 uptake and gene expression in renal tubular epithelial cells. *J Am Soc Nephrol* (2011) **22**:496–507. doi:10.1681/ASN.2010040379
36. Liu Y, Liu H, Kim BO, Gattone VH, Li J, Nath A, Blum J, He JJ. CD4-Independent Infection of Astrocytes by Human Immunodeficiency Virus Type 1: Requirement for the Human Mannose Receptor. *J Virol* (2004) **78**:4120–4133. doi:10.1128/JVI.78.8.4120-4133.2004
37. Russell RA, Chojnacki J, Jones DM, Johnson E, Do T, Eggeling C, Padilla-Parra S, Sattentau QJ. Astrocytes Resist HIV-1 Fusion but Engulf Infected Macrophage Material. *Cell Rep* (2017) **18**:1473–1483. doi:10.1016/j.celrep.2017.01.027
38. Hatsukari I, Singh P, Hitosugi N, Messmer D, Valderrama E, Teichberg S, Chaung W, Gross E, Schmidtmayerova H, Singhal PC. DEC-205–Mediated Internalization of HIV-1 Results in the Establishment of Silent Infection in Renal Tubular Cells. *J Am Soc Nephrol* (2007) **18**:780–787. doi:10.1681/ASN.2006121307
39. Burdick RC, Li C, Munshi MH, Rawson JMO, Nagashima K, Hu WS, Pathak VK. HIV-1 uncoats in the nucleus near sites of integration. *Proc Natl Acad Sci U S A* (2020) **117**:5486–5493. doi:10.1073/pnas.1920631117
40. Hu W-S, Hughes SH. HIV-1 Reverse Transcription. *Cold Spring Harb Perspect Med* (2012) **2**:a006882–a006882. doi:10.1101/cshperspect.a006882
41. Craigie R, Bushman FD. HIV DNA Integration. *Cold Spring Harb Perspect Med* (2012) **2**:a006890–a006890. doi:10.1101/cshperspect.a006890
42. Lucic B, Lusic M. Connecting HIV-1 integration and transcription: a step toward new treatments. *FEBS Lett* (2016) **590**:1927–1939. doi:10.1002/1873-3468.12226
43. Coiras M, López-Huertas MR, Pérez-Olmeda M, Alcamí J. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nat Rev Microbiol* (2009) **7**:798–812. doi:10.1038/nrmicro2223
44. Lusic M. Coloring hidden viruses. *Elife* (2018) **7**:3–5. doi:10.7554/eLife.37732
45. Peng K, Muranyi W, Glass B, Laketa V, Yant SR, Tsai L, Cihlar T, Müller B, Kräusslich H-G. Quantitative microscopy of functional HIV post-entry complexes reveals association of replication with the viral capsid. *Elife* (2014) **3**:e04114. doi:10.7554/eLife.04114
46. Kao S-Y, Calman AF, Luciw PA, Peterlin BM. Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product. *Nature* (1987) **330**:489–493. doi:10.1038/330489a0
47. Kirchhoff F. “HIV Life Cycle: Overview,” in *Encyclopedia of AIDS*, eds. T. J. Hope, M. Stevenson, D. Richman (New York, NY: Springer New York), 1–9. doi:10.1007/978-1-4614-9610-6_60-1
48. Liu R, Wu J, Shao R, Xue Y. Mechanism and factors that control HIV-1 transcription and latency activation. *J Zhejiang Univ Sci B* (2014) **15**:455–465. doi:10.1631/jzus.B1400059
49. Seitz R. Human Immunodeficiency Virus (HIV). *Transfus Med Hemotherapy* (2016) **43**:203–222. doi:10.1159/000445852
50. Ganser-Pornillos BK, Yeager M, Sundquist WI. The structural biology of HIV assembly. *Curr Opin Struct Biol* (2008) **18**:203–217. doi:10.1016/j.sbi.2008.02.001

51. Sundquist WI, Krausslich H-G. HIV-1 Assembly, Budding, and Maturation. *Cold Spring Harb Perspect Med* (2012) **2**:a006924–a006924. doi:10.1101/cshperspect.a006924
52. Freed EO. HIV-1 assembly, release and maturation. *Nat Rev Microbiol* (2015) **13**:484–496. doi:10.1038/nrmicro3490
53. Dayton AGS, Craig A. Rosen, Wei Chun Goh A, Haseltine WA. The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. *Cell* (1986) **44**:941–947. doi:10.1016/0092-8674(86)90017-6
54. Hauber J, Perkins A, Heimer EP, Cullen BR. Trans-activation of human immunodeficiency virus gene expression is mediated by nuclear events. *Proc Natl Acad Sci U S A* (1987) **84**:6364–6368. doi:10.1073/pnas.84.18.6364
55. Das AT, Harwig A, Berkhout B. The HIV-1 Tat Protein Has a Versatile Role in Activating Viral Transcription. *J Virol* (2011) **85**:9506–9516. doi:10.1128/JVI.00650-11
56. Clark E, Nava B, Caputi M. Tat is a multifunctional viral protein that modulates cellular gene expression and functions. *Oncotarget* (2017) **8**:27569–27581. doi:10.18632/oncotarget.15174
57. Ne E, Palstra RJ, Mahmoudi T. *Transcription: Insights From the HIV-1 Promoter*. 1st ed. Elsevier Inc. (2017). doi:10.1016/bs.ircmb.2017.07.011
58. Chiozzini C, Toschi E. HIV-1 TAT and IMMUNE DYSREGULATION in AIDS PATHOGENESIS: a THERAPEUTIC TARGET. *Curr Drug Targets* (2015) **17**:33–45. doi:10.2174/1389450116666150825110658
59. Kurnaeva MA, Sheval E V., Musinova YR, Vassetzky YS. Tat basic domain: A “Swiss army knife” of HIV-1 Tat? *Rev Med Virol* (2019) **29**:e2031. doi:10.1002/rmv.2031
60. Musinova YR, Sheval E V., Dib C, Germini D, Vassetzky YS. Functional roles of HIV-1 Tat protein in the nucleus. *Cell Mol Life Sci* (2016) **73**:589–601. doi:10.1007/s00018-015-2077-x
61. Stauber RH, Pavlakis GN. Intracellular Trafficking and Interactions of the HIV-1 Tat Protein. *Virology* (1998) **252**:126–136. doi:10.1006/viro.1998.9400
62. Fischer WH, Fang S-M, Jones KA, Wei P, Garber ME. A Novel CDK9-Associated C-Type Cyclin Interacts Directly with HIV-1 Tat and Mediates Its High-Affinity, Loop-Specific Binding to TAR RNA. *Cell* (1998) **92**:451–462. Available at: http://ac.els-cdn.com/S0092867400809393/1-s2.0-S0092867400809393-main.pdf?_tid=932d212a-dc81-11e4-8f42-00000aacb361&acdnat=1428341160_5fc92f25862e477fa4652bf3eb7c881f
63. Parada CA, Roeder RG. Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain. *Nature* (1996) **384**:375–378. doi:10.1038/384375a0
64. Vardabasso C, Manganaro L, Lusic M, Marcello A, Giacca M. The histone chaperone protein Nucleosome Assembly Protein-1 (hNAP-1) binds HIV-1 Tat and promotes viral transcription. *Retrovirology* (2008) **5**:8. doi:10.1186/1742-4690-5-8
65. Raha T, Cheng SWG, Green MR. HIV-1 Tat stimulates transcription complex assembly through recruitment of TBP in the absence of TAFs. *PLoS Biol* (2005) **3**:0221–0230. doi:10.1371/journal.pbio.0030044
66. Carvallo L, Lopez L, Fajardo JE, Jaureguiberry-Bravo M, Fiser A, Berman JW. HIV-Tat regulates

macrophage gene expression in the context of neuroAIDS. *PLoS One* (2017) **12**:e0179882. doi:10.1371/journal.pone.0179882

67. Dhamija N, Choudhary D, Ladha JS, Pillai B, Mitra D. Tat predominantly associates with host promoter elements in HIV-1-infected T-cells - regulatory basis of transcriptional repression of c-Rel. *FEBS J* (2015) **282**:595–610. doi:10.1111/febs.13168
68. Kim N, Kukkonen S, Gupta S, Aldovini A. Association of Tat with Promoters of PTEN and PP2A Subunits Is Key to Transcriptional Activation of Apoptotic Pathways in HIV-Infected CD4+ T Cells. *PLoS Pathog* (2010) **6**:e1001103. doi:10.1371/journal.ppat.1001103
69. Reeder JE, Kwak Y-T, McNamara RP, Forst C V., D’Orso I. HIV Tat controls RNA Polymerase II and the epigenetic landscape to transcriptionally reprogram target immune cells. *Elife* (2015) **4**:1–44. doi:10.7554/eLife.08955
70. Buonaguro L, Buonaguro FM, Giraldo G, Ensoli B. The human immunodeficiency virus type 1 Tat protein transactivates tumor necrosis factor beta gene expression through a TAR-like structure. *J Virol* (1994) **68**:2677–2682. doi:10.1128/JVI.68.4.2677-2682.1994
71. Thangaraj A, Periyasamy P, Liao K, Bendi VS, Callen S, Pendyala G, Buch S. HIV-1 TAT-mediated microglial activation: role of mitochondrial dysfunction and defective mitophagy. *Autophagy* (2018) **14**:1596–1619. doi:10.1080/15548627.2018.1476810
72. Wu X, Dong H, Ye X, Zhong L, Cao T, Xu Q, Wang J, Zhang Y, Xu J, Wang W, et al. HIV-1 Tat increases BAG3 via NF- κ B signaling to induce autophagy during HIV-associated neurocognitive disorder. *Cell Cycle* (2018) **17**:1614–1623. doi:10.1080/15384101.2018.1480219
73. El-Amine R, Germini D, Zakharova V V., Tsfasman T, Sheval E V., Louzada RAN, Dupuy C, Bilhou-Nabera C, Hamade A, Najjar F, et al. HIV-1 Tat protein induces DNA damage in human peripheral blood B-lymphocytes via mitochondrial ROS production. *Redox Biol* (2018) **15**:97–108. doi:10.1016/j.redox.2017.11.024
74. Lien K, Mayer W, Herrera R, Rosbe K, Tugizov SM. HIV-1 proteins gp120 and tat induce the epithelial–mesenchymal transition in oral and genital mucosal epithelial cells. *PLoS One* (2019) **14**:1–36. doi:10.1371/journal.pone.0226343
75. Sil S, Hu G, Liao K, Niu F, Callen S, Periyasamy P, Fox HS, Buch S. HIV-1 Tat-mediated astrocytic amyloidosis involves the HIF-1 α /lncRNA BACE1-AS axis. *PLOS Biol* (2020) **18**:e3000660. doi:10.1371/journal.pbio.3000660
76. Manes TL, Simenauer A, Geohring JL, Flemming J, Brehm M, Cota-Gomez A. The HIV-Tat protein interacts with Sp3 transcription factor and inhibits its binding to a distal site of the sod2 promoter in human pulmonary artery endothelial cells. *Free Radic Biol Med* (2020) **147**:102–113. doi:10.1016/j.freeradbiomed.2019.12.015
77. Simenauer A, Assefa B, Rios-Ochoa J, Geraci K, Hybertson B, Gao B, McCord J, Elajaili H, Nozik-Grayck E, Cota-Gomez A. Repression of Nrf2/ARE regulated antioxidant genes and dysregulation of the cellular redox environment by the HIV Transactivator of Transcription. *Free Radic Biol Med* (2019) **141**:244–252. doi:10.1016/j.freeradbiomed.2019.06.015
78. Santerre M, Bagashev A, Gorecki L, Lysek KZ, Wang Y, Shrestha J, Carpio-Cano F Del, Mukerjee R, Sawaya BE. HIV-1 Tat protein promotes neuronal dysregulation by inhibiting E2F transcription factor 3 (E2F3). *J Biol Chem* (2019) **294**:3618–3633. doi:10.1074/jbc.RA118.003744

79. ZAULI G, MILANI D, MIRANDOLA P, MAZZONI M, SECCHIERO P, MISCIA S, CAPITANI S. HIV-1 Tat protein down-regulates CREB transcription factor expression in PC12 neuronal cells through a phosphatidylinositol 3-kinase/AKT/cyclic nucleoside phosphodiesterase pathway. *FASEB J* (2001) **15**:483–491. doi:10.1096/fj.00-0354com
80. Chang HC, Samaniego F, Nair BC, Buonaguro L, Ensoli B. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. *Aids* (1997) **11**:1421–1431. doi:10.1097/00002030-199712000-00006
81. Rabouille C. Pathways of Unconventional Protein Secretion. *Trends Cell Biol* (2017) **27**:230–240. doi:10.1016/j.tcb.2016.11.007
82. Mele AR, Marino J, Chen K, Pirrone V, Janetopoulos C, Wigdahl B, Klase Z, Nonnemacher MR. Defining the molecular mechanisms of HIV-1 Tat secretion: PtdIns(4,5)P₂ at the epicenter. *Traffic* (2018) **19**:655–665. doi:10.1111/tra.12578
83. Xiao H, Neuveut C, Tiffany HL, Benkirane M, Rich EA, Murphy PM, Jeang K-T. Selective CXCR4 antagonism by Tat: Implications for in vivo expansion of coreceptor use by HIV-1. *Proc Natl Acad Sci* (2000) **97**:11466–11471. doi:10.1073/pnas.97.21.11466
84. Choi JY, Hightower GK, Wong JK, Heaton R, Woods S, Grant I, Marcotte TD, Ellis RJ, Letendre SL, Collier AC, et al. Genetic features of cerebrospinal fluid-derived subtype B HIV-1 tat. *J Neurovirol* (2012) **18**:81–90. doi:10.1007/s13365-011-0059-9
85. Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, Debating KM, Krammer PH. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* (1995) **375**:497–500. doi:10.1038/375497a0
86. Koizumi K, Nakamura H, Iijima M, Matsuzaki T, Somiya M, Kumasawa K, Kimura T, Kuroda S. In vivo uterine local gene delivery system using TAT-displaying bionanocapsules. *J Gene Med* (2019) **21**: doi:10.1002/jgm.3140
87. Shan S, Jia S, Lawson T, Yan L, Lin M, Liu Y. The use of TAT peptide-functionalized graphene as a highly nuclear-targeting carrier system for suppression of choroidal melanoma. *Int J Mol Sci* (2019) **20**: doi:10.3390/ijms20184454
88. Chauhan A, Tikoo A, Kapur AK, Singh M. The taming of the cell penetrating domain of the HIV Tat: Myths and realities. *J Control Release* (2007) **117**:148–162. doi:10.1016/j.jconrel.2006.10.031
89. Urbinati C, Nicoli S, Giacca M, David G, Fiorentini S, Caruso A, Alfano M, Cassetta L, Presta M, Rusnati M. HIV-1 Tat and heparan sulfate proteoglycan interaction: A novel mechanism of lymphocyte adhesion and migration across the endothelium. *Blood* (2009) **114**:3335–3342. doi:10.1182/blood-2009-01-198945
90. Liu Y, Jones M, Hingtgen CM, Bu G, Laribee N, Tanzi RE, Moir RD, Nath A, He JJ. Uptake of HIV-1 Tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat Med* (2000) **6**:1380–1387. doi:10.1038/82199
91. Gutheil WG, Subramanyam M, Flentke GR, Sanford DG, Munoz E, Huber BT, Bachovchin WW. Human immunodeficiency virus 1 Tat binds to dipeptidyl aminopeptidase IV (CD26): A possible mechanism for Tat's immunosuppressive activity. *Proc Natl Acad Sci U S A* (1994) **91**:6594–6598. doi:10.1073/pnas.91.14.6594

92. Hussein HAM, Walker LR, Abdel-Raouf UM, Desouky SA, Montasser AKM, Akula SM. Beyond RGD: virus interactions with integrins. *Arch Virol* (2015) **160**:2669–2681. doi:10.1007/s00705-015-2579-8
93. Vendeville A, Rayne F, Bonhoure A, Bettache N, Montcourrier P, Beaumelle B. HIV-1 Tat Enters T Cells Using Coated Pits before Translocating from Acidified Endosomes and Eliciting Biological Responses. *Mol Biol Cell* (2004) **15**:2347–2360. doi:10.1091/mbc.e03-12-0921
94. Debaisieux S, Rayne F, Yezid H, Beaumelle B. The Ins and Outs of HIV-1 Tat. *Traffic* (2012) **13**:355–363. doi:10.1111/j.1600-0854.2011.01286.x
95. Shiels MS, Engels EA. Evolving epidemiology of HIV-associated malignancies. *Curr Opin HIV AIDS* (2017) **12**:6–11. doi:10.1097/COH.0000000000000327
96. Vangipuram R, Tying SK. “AIDS-Associated Malignancies,” in *Cancer Treatment and Research*, ed. C. Meyers (Cham: Springer International Publishing), 1–21. doi:10.1007/978-3-030-03502-0_1
97. Palau L, Menez S, Rodriguez-Sanchez J, Novick T, Delsante M, McMahon BA, Atta MG. HIV-associated nephropathy: links, risks and management. *HIV/AIDS - Res Palliat Care* (2018) **Volume 10**:73–81. doi:10.2147/HIV.S141978
98. Eggers C, Arendt G, Hahn K, Husstedt IW, Maschke M, Neuen-Jacob E, Obermann M, Rosenkranz T, Schielke E, Straube E. HIV-1-associated neurocognitive disorder: epidemiology, pathogenesis, diagnosis, and treatment. *J Neurol* (2017) **264**:1715–1727. doi:10.1007/s00415-017-8503-2
99. Shmakova A, Germini D, Vassetzky Y. HIV-1, HAART and cancer: A complex relationship. *Int J Cancer* (2020) **146**:2666–2679. doi:10.1002/ijc.32730
100. Engels EA, Pfeiffer RM, Goedert JJ, Virgo P, McNeel TS, Scoppa SM, Biggar RJ. Trends in cancer risk among people with AIDS in the United States 1980–2002. *AIDS* (2006) **20**:1645–1654. doi:10.1097/01.aids.0000238411.75324.59
101. Dal Maso L, Polesel J, Serraino D, Lise M, Piselli P, Falcini F, Russo A, Intrieri T, Vercelli M, Zambon P, et al. Pattern of cancer risk in persons with AIDS in Italy in the HAART era. *Br J Cancer* (2009) **100**:840–847. doi:10.1038/sj.bjc.6604923
102. Franceschi S, Lise M, Clifford GM, Rickenbach M, Levi F, Maspoli M, Bouchardy C, Dehler S, Jundt G, Ess S, et al. Changing patterns of cancer incidence in the early- and late-HAART periods: the Swiss HIV Cohort Study. *Br J Cancer* (2010) **103**:416–422. doi:10.1038/sj.bjc.6605756
103. Gibson TM, Morton LM, Shiels MS, Clarke CA, Engels EA. Risk of non-Hodgkin lymphoma subtypes in HIV-infected people during the HAART era. *AIDS* (2014) **28**:2313–2318. doi:10.1097/QAD.0000000000000428
104. Shiels MS, Koritzinsky EH, Clarke CA, Suneja G, Morton LM, Engels EA. Prevalence of HIV Infection among U.S. Hodgkin Lymphoma Cases. *Cancer Epidemiol Biomarkers Prev* (2014) **23**:274–281. doi:10.1158/1055-9965.EPI-13-0865
105. Maso LD, Suligoi B, Franceschi S, Braga C, Buzzoni C, Polesel J, Zucchetto A, Piselli P, Falcini F, Caldarella A, et al. Survival After Cancer in Italian Persons With AIDS, 1986–2005. *JAIDS J Acquir Immune Defic Syndr* (2014) **66**:428–435. doi:10.1097/QAI.0000000000000184
106. Dolcetti R, Giagulli C, He W, Selleri M, Caccuri F, Eyzaguirre LM, Mazzuca P, Corbellini S,

- Campilongo F, Marsico S, et al. Role of HIV-1 matrix protein p17 variants in lymphoma pathogenesis. *Proc Natl Acad Sci* (2015) **112**:14331–14336. doi:10.1073/pnas.1514748112
107. Wang X, Duan Z, Yu G, Fan M, Scharff MD. Human Immunodeficiency Virus Tat Protein Aids V Region Somatic Hypermutation in Human B Cells. *MBio* (2018) **9**:e02315-17. doi:10.1128/mBio.02315-17
 108. Totonchy J, Cesarman E. Does persistent HIV replication explain continued lymphoma incidence in the era of effective antiretroviral therapy? *Curr Opin Virol* (2016) **20**:71–77. doi:10.1016/j.coviro.2016.09.001
 109. Hunt PW. HIV and Inflammation: Mechanisms and Consequences. *Curr HIV/AIDS Rep* (2012) **9**:139–147. doi:10.1007/s11904-012-0118-8
 110. Carbone A. Emerging pathways in the development of AIDS-related lymphomas. *Lancet Oncol* (2003) **4**:22–29. doi:10.1016/S1470-2045(03)00957-4
 111. Gaidano G, Carbone A, Dalla-Favera R. Genetic Basis of Acquired Immunodeficiency Syndrome-Related Lymphomagenesis. *JNCI Monogr* (1998) **1998**:95–100. doi:10.1093/oxfordjournals.jncimonographs.a024181
 112. Germini D, Tsfasman T, Klibi M, El-Amine R, Pichugin A, Iarovaia O V., Bilhou-Nabera C, Subra F, Bou Saada Y, Sukhanova A, et al. HIV Tat induces a prolonged MYC relocalization next to IGH in circulating B-cells. *Leukemia* (2017) **31**:2515–2522. doi:10.1038/leu.2017.106
 113. El-Salem M, Raghunath PN, Marzec M, Liu X, Kasprzycka M, Robertson E, Wasik MA. Activation of mTORC1 signaling pathway in AIDS-related lymphomas. *Am J Pathol* (2009) **175**:817–824. doi:10.2353/ajpath.2009.080451
 114. Sebestyén A, Sticz TB, Márk Á, Hajdu M, Timár B, Nemes K, Nagy N, Váradi Z, Kopper L. Activity and complexes of mTOR in diffuse large B-cell lymphomas - A tissue microarray study. *Mod Pathol* (2012) **25**:1623–1628. doi:10.1038/modpathol.2012.141
 115. Ágnes Márk MH, Zsófia V, Sticz TB, Nagy N, Csomor J, Berczi L, Varga V, Csóka M, Sebestyén LK and A. Characteristic mTOR activity in Hodgkin-lymphomas offers a potential therapeutic target in high risk disease – a combined tissue microarray, in vitro and in vivo study. *BMC Cancer* (2013) **13**: doi:10.1186/1471-2407-13-250
 116. Browne SH, Diaz-Perez JA, Preziosi M, King CC, Jones GA, Jain S, Sun X, Reid EG, Vandenberg S, Wang HY. mTOR activity in AIDS-related diffuse large B-cell lymphoma. *PLoS One* (2017) **12**:1–12. doi:10.1371/journal.pone.0170771
 117. Col JD, Zancai P, Terrin L, Guidoboni M, Ponzoni M, Pavan A, Spina M, Bergamin S, Rizzo S, Tirelli U, et al. Distinct functional significance of Akt and mTOR constitutive activation in mantle cell lymphoma. *Blood* (2008) **111**:5142–5151. doi:10.1182/blood-2007-07-103481
 118. Sekihara K, Saitoh K, Han L, Ciurea S, Yamamoto S, Kikkawa M, Kazuno S, Taka H, Kaga N, Arai H, et al. Targeting mantle cell lymphoma metabolism and survival through simultaneous blockade of mTOR and nuclear transporter exportin-1. *Oncotarget* (2017) **8**:34552–34564. doi:10.18632/oncotarget.16602
 119. Grogg KL, Miller RF, Dogan A. HIV infection and lymphoma. *J Clin Pathol* (2006) **60**:1365–1372. doi:10.1136/jcp.2007.051953

120. Clayton A, Mughal T. The changing face of HIV-associated lymphoma: what can we learn about optimal therapy in the post highly active antiretroviral therapy era? *Hematol Oncol* (2004) **22**:111–120. doi:10.1002/hon.735
121. MacMahon E, Charache P, Glass D, Mann RB, Becker PS, Hayward D, McArthur J, Ambinder RF. Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet* (1991) **338**:969–973. doi:10.1016/0140-6736(91)91837-K
122. Narkhede M, Arora S, Ujjani C. Primary effusion lymphoma: current perspectives. *Onco Targets Ther* (2018) **Volume 11**:3747–3754. doi:10.2147/OTT.S167392
123. Castillo JJ, Bibas M, Miranda RN. The biology and treatment of plasmablastic lymphoma. *Blood* (2015) **125**:2323–2330. doi:10.1182/blood-2014-10-567479
124. Oksenhendler E, Boutboul D, Fajgenbaum D, Mirouse A, Fieschi C, Malphettes M, Vercellino L, Meignin V, Gérard L, Galicier L. The full spectrum of Castleman disease: 273 patients studied over 20 years. *Br J Haematol* (2018) **180**:206–216. doi:10.1111/bjh.15019
125. Spina M, Carbone A, Gloghini A, Serraino D, Berretta M, Tirelli U. Hodgkin's Disease in Patients with HIV Infection. *Adv Hematol* (2011) **2011**:1–7. doi:10.1155/2011/402682
126. VÉZINA C, KUDELSKI A, SEHGAL SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* (1975) **28**:721–726. doi:10.7164/antibiotics.28.721
127. Martel RR, Klicius J, Galet S. Inhibition of the immune response by rapamycin, a new antifungal antibiotic. *Can J Physiol Pharmacol* (1977) **55**:48–51. doi:10.1139/y77-007
128. Eng CP, Sehgal SN, Vézina C. Activity of rapamycin (ay-22,989) against transplanted tumors. *J Antibiot (Tokyo)* (1984) **37**:1231–1237. doi:10.7164/antibiotics.37.1231
129. Heitman J, Movva N, Hall M. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* (80-) (1991) **253**:905–909. doi:10.1126/science.1715094
130. Brown EJ, Albers MW, Bum Shin T, Ichikawa K, Keith CT, Lane WS, Schreiber SL. A mammalian protein targeted by G1-arresting rapamycin–receptor complex. *Nature* (1994) **369**:756–758. doi:10.1038/369756a0
131. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* (1994) **78**:35–43. doi:10.1016/0092-8674(94)90570-3
132. Chiu MI, Katz H, Berlin V. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc Natl Acad Sci* (1994) **91**:12574–12578. doi:10.1073/pnas.91.26.12574
133. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, Abraham RT. Isolation of a Protein Target of the FKBP12-Rapamycin Complex in Mammalian Cells. *J Biol Chem* (1995) **270**:815–822. doi:10.1074/jbc.270.2.815
134. Bierer BE, Mattila PS, Standaert RF, Herzenberg LA, Burakoff SJ, Crabtree G, Schreiber SL. Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. *Proc Natl Acad Sci* (1990) **87**:9231–9235.

doi:10.1073/pnas.87.23.9231

135. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science* (80-) (1996) **273**:239–242. doi:10.1126/science.273.5272.239
136. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* (2006) **124**:471–484. doi:10.1016/j.cell.2006.01.016
137. Hall MN. On mTOR nomenclature. *Biochem Soc Trans* (2013) **41**:887–888. doi:10.1042/BST20130092
138. Kim D-H, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR Interacts with Raptor to Form a Nutrient-Sensitive Complex that Signals to the Cell Growth Machinery. *Cell* (2002) **110**:163–175. doi:10.1016/S0092-8674(02)00808-5
139. Nojima H, Tokunaga C, Eguchi S, Oshiro N, Hidayat S, Yoshino K, Hara K, Tanaka N, Avruch J, Yonezawa K. The Mammalian Target of Rapamycin (mTOR) Partner, Raptor, Binds the mTOR Substrates p70 S6 Kinase and 4E-BP1 through Their TOR Signaling (TOS) Motif. *J Biol Chem* (2003) **278**:15461–15464. doi:10.1074/jbc.C200665200
140. Kim D-H, Sarbassov DD, Ali SM, Latek RR, Guntur KVP, Erdjument-Bromage H, Tempst P, Sabatini DM. GβL, a Positive Regulator of the Rapamycin-Sensitive Pathway Required for the Nutrient-Sensitive Interaction between Raptor and mTOR. *Mol Cell* (2003) **11**:895–904. doi:10.1016/S1097-2765(03)00114-X
141. Thedieck K, Polak P, Kim ML, Molle KD, Cohen A, Jenö P, Arrieumerlou C, Hall MN. PRAS40 and PRR5-Like Protein Are New mTOR Interactors that Regulate Apoptosis. *PLoS One* (2007) **2**:e1217. doi:10.1371/journal.pone.0001217
142. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM. DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells and Required for Their Survival. *Cell* (2009) **137**:873–886. doi:10.1016/j.cell.2009.03.046
143. Varusai TM, Nguyen LK. Dynamic modelling of the mTOR signalling network reveals complex emergent behaviours conferred by DEPTOR. *Sci Rep* (2018) **8**:643. doi:10.1038/s41598-017-18400-z
144. Takahara T, Amemiya Y, Sugiyama R, Maki M, Shibata H. Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *J Biomed Sci* (2020) **27**:87. doi:10.1186/s12929-020-00679-2
145. Qian J, Su S, Liu P. Experimental Approaches in Delineating mTOR Signaling. *Genes (Basel)* (2020) **11**:738. doi:10.3390/genes11070738
146. Fu W, Hall MN. Regulation of mTORC2 Signaling. *Genes (Basel)* (2020) **11**:1045. doi:10.3390/genes11091045
147. Ma Y, Vassetzky Y, Dokudovskaya S. mTORC1 pathway in DNA damage response. *Biochim Biophys Acta - Mol Cell Res* (2018) **1865**:1293–1311. doi:10.1016/j.bbamcr.2018.06.011
148. Laplante M, Sabatini DM. mTOR Signaling in Growth Control and Disease. *Cell* (2012) **149**:274–293. doi:10.1016/j.cell.2012.03.017
149. Oshiro N, Yoshino K, Hidayat S, Tokunaga C, Hara K, Eguchi S, Avruch J, Yonezawa K.

Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes to Cells* (2004) **9**:359–366. doi:10.1111/j.1356-9597.2004.00727.x

150. Wang L, Lawrence JC, Sturgill TW, Harris TE. Mammalian Target of Rapamycin Complex 1 (mTORC1) Activity Is Associated with Phosphorylation of Raptor by mTOR. *J Biol Chem* (2009) **284**:14693–14697. doi:10.1074/jbc.C109.002907
151. Scaiola A, Mangia F, Imseng S, Boehringer D, Berneiser K, Shimobayashi M, Stüttfeld E, Hall MN, Ban N, Maier T. The 3.2-Å resolution structure of human mTORC2. *Sci Adv* (2020) **6**:eabc1251. doi:10.1126/sciadv.abc1251
152. García-Martínez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J* (2008) **416**:375–385. doi:10.1042/BJ20081668
153. Lu M, Wang J, Jones KT, Ives HE, Feldman ME, Yao L, Shokat KM, Ashrafi K, Pearce D. mTOR Complex-2 Activates ENaC by Phosphorylating SGK1. *J Am Soc Nephrol* (2010) **21**:811–818. doi:10.1681/ASN.2009111168
154. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B. SIN1/MIP1 Maintains rictor-mTOR Complex Integrity and Regulates Akt Phosphorylation and Substrate Specificity. *Cell* (2006) **127**:125–137. doi:10.1016/j.cell.2006.08.033
155. Gleason CE, Osés-Prieto JA, Li KH, Saha B, Situ G, Burlingame AL, Pearce D. Phosphorylation at distinct subcellular locations underlies specificity in mTORC2-mediated activation of SGK1 and Akt. *J Cell Sci* (2019) **132**:jcs224931. doi:10.1242/jcs.224931
156. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM. Ablation in Mice of the mTORC Components raptor, rictor, or mLST8 Reveals that mTORC2 Is Required for Signaling to Akt-FOXO and PKC α , but Not S6K1. *Dev Cell* (2006) **11**:859–871. doi:10.1016/j.devcel.2006.10.007
157. Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME. Protein Kinase SGK Mediates Survival Signals by Phosphorylating the Forkhead Transcription Factor FKHRL1 (FOXO3a). *Mol Cell Biol* (2001) **21**:952–965. doi:10.1128/MCB.21.3.952-965.2001
158. Dos D, Sarbassov, Ali SM, Kim D-H, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a Novel Binding Partner of mTOR, Defines a Rapamycin-Insensitive and Raptor-Independent Pathway that Regulates the Cytoskeleton. *Curr Biol* (2004) **14**:1296–1302. doi:10.1016/j.cub.2004.06.054
159. Sen B, Xie Z, Case N, Thompson WR, Uzer G, Styner M, Rubin J. mTORC2 Regulates Mechanically Induced Cytoskeletal Reorganization and Lineage Selection in Marrow-Derived Mesenchymal Stem Cells. *J Bone Miner Res* (2014) **29**:78–89. doi:10.1002/jbmr.2031
160. Huang H, Long L, Zhou P, Chapman NM, Chi H. mTOR signaling at the crossroads of environmental signals and T-cell fate decisions. *Immunol Rev* (2020) **295**:15–38. doi:10.1111/imr.12845
161. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. *Cell* (2017) **169**:381–405. doi:10.1016/j.cell.2017.04.001
162. Cantley LC. The Phosphoinositide 3-Kinase Pathway. *Science* (80-) (2002) **296**:1655–1657. doi:10.1126/science.296.5573.1655

163. Papa, Pandolfi. The PTEN–PI3K Axis in Cancer. *Biomolecules* (2019) **9**:153. doi:10.3390/biom9040153
164. Nitulescu G, Van De Venter M, Nitulescu G, Ungurianu A, Juzenas P, Peng Q, Olaru O, Grădinaru D, Tsatsakis A, Tsoukalas D, et al. The Akt pathway in oncology therapy and beyond (Review). *Int J Oncol* (2018) **53**:2319–2331. doi:10.3892/ijo.2018.4597
165. Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, Finan PM, Kwiatkowski DJ, Murphy LO, Manning BD. TBC1D7 Is a Third Subunit of the TSC1-TSC2 Complex Upstream of mTORC1. *Mol Cell* (2012) **47**:535–546. doi:10.1016/j.molcel.2012.06.009
166. Cai S-L, Tee AR, Short JD, Bergeron JM, Kim J, Shen J, Guo R, Johnson CL, Kiguchi K, Walker CL. Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *J Cell Biol* (2006) **173**:279–289. doi:10.1083/jcb.200507119
167. Haar E Vander, Lee S, Bandhakavi S, Griffin TJ, Kim D-H. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* (2007) **9**:316–323. doi:10.1038/ncb1547
168. Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, Yang H, Hild M, Kung C, Wilson C, et al. Bidirectional Transport of Amino Acids Regulates mTOR and Autophagy. *Cell* (2009) **136**:521–534. doi:10.1016/j.cell.2008.11.044
169. Watanabe-Asano T, Kuma A, Mizushima N. Cycloheximide inhibits starvation-induced autophagy through mTORC1 activation. *Biochem Biophys Res Commun* (2014) **445**:334–339. doi:10.1016/j.bbrc.2014.01.180
170. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 Senses Lysosomal Amino Acids Through an Inside-Out Mechanism That Requires the Vacuolar H⁺-ATPase. *Science* (80-) (2011) **334**:678–683. doi:10.1126/science.1207056
171. Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, Kim HK, Ha SH, Ryu SH, Kim S. Leucyl-tRNA Synthetase Is an Intracellular Leucine Sensor for the mTORC1-Signaling Pathway. *Cell* (2012) **149**:410–424. doi:10.1016/j.cell.2012.02.044
172. Chantranupong L, Wolfson RL, Orozco JM, Saxton RA, Scaria SM, Bar-Peled L, Spooner E, Isasa M, Gygi SP, Sabatini DM. The Sestrins Interact with GATOR2 to Negatively Regulate the Amino-Acid-Sensing Pathway Upstream of mTORC1. *Cell Rep* (2014) **9**:1–8. doi:10.1016/j.celrep.2014.09.014
173. Parmigiani A, Nourbakhsh A, Ding B, Wang W, Kim YC, Akopiants K, Guan K-L, Karin M, Budanov A V. Sestrins Inhibit mTORC1 Kinase Activation through the GATOR Complex. *Cell Rep* (2014) **9**:1281–1291. doi:10.1016/j.celrep.2014.10.019
174. Kim JS, Ro S-H, Kim M, Park H-W, Semple IA, Park H, Cho U-S, Wang W, Guan K-L, Karin M, et al. Sestrin2 inhibits mTORC1 through modulation of GATOR complexes. *Sci Rep* (2015) **5**:9502. doi:10.1038/srep09502
175. Shen K, Sabatini DM. Regulator and SLC38A9 activate the Rag GTPases through noncanonical GEF mechanisms. *Proc Natl Acad Sci* (2018) **115**:9545–9550. doi:10.1073/pnas.1811727115
176. Chantranupong L, Scaria SM, Saxton RA, Gygi MP, Shen K, Wyant GA, Wang T, Harper JW, Gygi SP, Sabatini DM. The CASTOR Proteins Are Arginine Sensors for the mTORC1 Pathway. *Cell* (2016) **165**:153–164. doi:10.1016/j.cell.2016.02.035

177. Baba M, Hong S-B, Sharma N, Warren MB, Nickerson ML, Iwamatsu A, Esposito D, Gillette WK, Hopkins RF, Hartley JL, et al. Folliculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. *Proc Natl Acad Sci* (2006) **103**:15552–15557. doi:10.1073/pnas.0603781103
178. Hasumi H, Baba M, Hong S-B, Hasumi Y, Huang Y, Yao M, Valera VA, Linehan WM, Schmidt LS. Identification and characterization of a novel folliculin-interacting protein FNIP2. *Gene* (2008) **415**:60–67. doi:10.1016/j.gene.2008.02.022
179. Tsun Z-Y, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, Spooner E, Sabatini DM. The Folliculin Tumor Suppressor Is a GAP for the RagC/D GTPases That Signal Amino Acid Levels to mTORC1. *Mol Cell* (2013) **52**:495–505. doi:10.1016/j.molcel.2013.09.016
180. Dokudovskaya S, Waharte F, Schlessinger A, Pieper U, Devos DP, Cristea IM, Williams R, Salamero J, Chait BT, Sali A, et al. A Conserved Coatomer-related Complex Containing Sec13 and Seh1 Dynamically Associates With the Vacuole in *Saccharomyces cerevisiae*. *Mol Cell Proteomics* (2011) **10**:M110.006478. doi:10.1074/mcp.M110.006478
181. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM. A Tumor Suppressor Complex with GAP Activity for the Rag GTPases That Signal Amino Acid Sufficiency to mTORC1. *Science* (80-) (2013) **340**:1100–1106. doi:10.1126/science.1232044
182. Saxton RA, Knockenhauer KE, Wolfson RL, Chantranupong L, Pacold ME, Wang T, Schwartz TU, Sabatini DM. Structural basis for leucine sensing by the Sestrin2-mTORC1 pathway. *Science* (80-) (2016) **351**:53–58. doi:10.1126/science.aad2087
183. Wolfson RL, Chantranupong L, Saxton RA, Shen K, Scaria SM, Cantor JR, Sabatini DM. Sestrin2 is a leucine sensor for the mTORC1 pathway. Available at: <http://science.sciencemag.org/content/sci/351/6268/43.full.pdf> [Accessed March 5, 2018]
184. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag Complex Targets mTORC1 to the Lysosomal Surface and Is Necessary for Its Activation by Amino Acids. *Cell* (2010) **141**:290–303. doi:10.1016/j.cell.2010.02.024
185. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator Is a GEF for the Rag GTPases that Signal Amino Acid Levels to mTORC1. *Cell* (2012) **150**:1196–1208. doi:10.1016/j.cell.2012.07.032
186. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases Bind Raptor and Mediate Amino Acid Signaling to mTORC1. *Science* (80-) (2008) **320**:1496–1501. doi:10.1126/science.1157535
187. Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nat Rev Mol Cell Biol* (2007) **8**:917–929. doi:10.1038/nrm2272
188. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 Senses Lysosomal Amino Acids Through an Inside-Out Mechanism That Requires the Vacuolar H⁺-ATPase. *Science* (80-) (2011) **334**:678–683. doi:10.1126/science.1207056
189. Marechal A, Zou L. DNA Damage Sensing by the ATM and ATR Kinases. *Cold Spring Harb Perspect Biol* (2013) **5**:a012716–a012716. doi:10.1101/cshperspect.a012716
190. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage

Response. *Mol Cell* (2017) **66**:801–817. doi:10.1016/j.molcel.2017.05.015

191. Andrs M, Korabecny J, Jun D, Hodny Z, Bartek J, Kuca K. Phosphatidylinositol 3-Kinase (PI3K) and Phosphatidylinositol 3-Kinase-Related Kinase (PIKK) Inhibitors: Importance of the Morpholine Ring. *J Med Chem* (2015) **58**:41–71. doi:10.1021/jm501026z
192. Mavragani I V., Nikitaki Z, Kalospyros SA, Georgakilas AG. Ionizing Radiation and Complex DNA Damage: From Prediction to Detection Challenges and Biological Significance. *Cancers (Basel)* (2019) **11**:1789. doi:10.3390/cancers11111789
193. Cheung-Ong K, Giaever G, Nislow C. DNA-Damaging Agents in Cancer Chemotherapy: Serendipity and Chemical Biology. *Chem Biol* (2013) **20**:648–659. doi:10.1016/j.chembiol.2013.04.007
194. Cadet J, Wagner JR. DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation. *Cold Spring Harb Perspect Biol* (2013) **5**:a012559–a012559. doi:10.1101/cshperspect.a012559
195. Mayr M, Hu Y, Hainaut P, Xu Q. Mechanical Stress-induced DNA damage and rac-p38MAPK Signal Pathways Mediate p53-dependent Apoptosis in Vascular Smooth Muscle Cells. *FASEB J* (2002) **16**:1423–1425. doi:10.1096/fj.02-0042fje
196. Arya R, Bassing CH. V(D)J Recombination Exploits DNA Damage Responses to Promote Immunity. *Trends Genet* (2017) **33**:479–489. doi:10.1016/j.tig.2017.04.006
197. Papavasiliou FN, Schatz DG. Cell-cycle-regulated DNA double-strand breaks in somatic hypermutation of immunoglobulin genes. *Nature* (2000) **408**:216–221. doi:10.1038/35041599
198. Roth DB, Nakajima PB, Menetski JP, Bosma MJ, Gellert M. V(D)J recombination in mouse thymocytes: Double-strand breaks near T cell receptor δ rearrangement signals. *Cell* (1992) **69**:41–53. doi:10.1016/0092-8674(92)90117-U
199. Baretić D, Williams RL. PIKKs — the solenoid nest where partners and kinases meet. *Curr Opin Struct Biol* (2014) **29**:134–142. doi:10.1016/j.sbi.2014.11.003
200. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. *Environ Mol Mutagen* (2017) **58**:235–263. doi:10.1002/em.22087
201. Lanz MC, Dibitetto D, Smolka MB. <scp>DNA</scp> damage kinase signaling: checkpoint and repair at 30 years. *EMBO J* (2019) **38**: doi:10.15252/embj.2019101801
202. Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM Phosphorylates Histone H2AX in Response to DNA Double-strand Breaks. *J Biol Chem* (2001) **276**:42462–42467. doi:10.1074/jbc.C100466200
203. Tanaka T, Kurose A, Huang X, Dai W, Darzynkiewicz Z. ATM activation and histone H2AX phosphorylation as indicators of DNA damage by DNA topoisomerase I inhibitor topotecan and during apoptosis. *Cell Prolif* (2006) **39**:49–60. doi:10.1111/j.1365-2184.2006.00364.x
204. Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ. Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. *Proc Natl Acad Sci* (2000) **97**:10389–10394. doi:10.1073/pnas.190030497
205. Chehab NH, Malikzay A, Appel M, Halazonetis TD. Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53. *Genes Dev* (2000) **14**:278–288. doi:10.1101/gad.14.3.278

206. Hirao A. DNA Damage-Induced Activation of p53 by the Checkpoint Kinase Chk2. *Science* (80-) (2000) **287**:1824–1827. doi:10.1126/science.287.5459.1824
207. Lindsay HD, Griffiths DJF, Edwards RJ, Christensen PU, Murray JM, Osman F, Walworth N, Carr AM. S-phase-specific activation of Cds1 kinase defines a subpathway of the checkpoint response in *Schizosaccharomyces pombe*. *Genes Dev* (1998) **12**:382–395. doi:10.1101/gad.12.3.382
208. Mordes DA, Glick GG, Zhao R, Cortez D. TopBP1 activates ATR through ATRIP and a PIKK regulatory domain. *Genes Dev* (2008) **22**:1478–1489. doi:10.1101/gad.1666208
209. Zhao H, Piwnicka-Worms H. ATR-Mediated Checkpoint Pathways Regulate Phosphorylation and Activation of Human Chk1. *Mol Cell Biol* (2001) **21**:4129–4139. doi:10.1128/MCB.21.13.4129-4139.2001
210. Okita N, Minato S, Ohmi E, Tanuma S, Higami Y. DNA damage-induced CHK1 autophosphorylation at Ser296 is regulated by an intramolecular mechanism. *FEBS Lett* (2012) **586**:3974–3979. doi:10.1016/j.febslet.2012.09.048
211. Bennett LN, Larkin C, Gillespie DA, Clarke PR. Claspin is phosphorylated in the Chk1-binding domain by a kinase distinct from Chk1. *Biochem Biophys Res Commun* (2008) **369**:973–976. doi:10.1016/j.bbrc.2008.02.154
212. Uto K, Inoue D, Shimuta K, Nakajo N, Sagata N. Chk1, but not Chk2, inhibits Cdc25 phosphatases by a novel common mechanism. *EMBO J* (2004) **23**:3386–3396. doi:10.1038/sj.emboj.7600328
213. Lee J, Kumagai A, Dunphy WG. Positive Regulation of Wee1 by Chk1 and 14-3-3 Proteins. *Mol Biol Cell* (2001) **12**:551–563. doi:10.1091/mbc.12.3.551
214. Patil M, Pabla N, Dong Z. Checkpoint kinase 1 in DNA damage response and cell cycle regulation. *Cell Mol Life Sci* (2013) **70**:4009–4021. doi:10.1007/s00018-013-1307-3
215. Jette N, Lees-Miller SP. The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. *Prog Biophys Mol Biol* (2015) **117**:194–205. doi:10.1016/j.pbiomolbio.2014.12.003
216. Graham TGW, Walter JC, Loparo JJ. Two-Stage Synapsis of DNA Ends during Non-homologous End Joining. *Mol Cell* (2016) **61**:850–858. doi:10.1016/j.molcel.2016.02.010
217. Li Z, Otevrel T, Gao Y, Cheng H-L, Seed B, Stamato TD, Taccioli GE, Alt FW. The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. *Cell* (1995) **83**:1079–1089. doi:10.1016/0092-8674(95)90135-3
218. Ahnesorg P, Smith P, Jackson SP. XLF Interacts with the XRCC4-DNA Ligase IV Complex to Promote DNA Nonhomologous End-Joining. *Cell* (2006) **124**:301–313. doi:10.1016/j.cell.2005.12.031
219. Critchlow SE, Bowater RP, Jackson SP. Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. *Curr Biol* (1997) **7**:588–598. doi:10.1016/S0960-9822(06)00258-2
220. Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin Opening and Overhang Processing by an Artemis/DNA-Dependent Protein Kinase Complex in Nonhomologous End Joining and V(D)J Recombination. *Cell* (2002) **108**:781–794. doi:10.1016/S0092-8674(02)00671-2
221. Li J, Stern DF. Regulation of CHK2 by DNA-dependent Protein Kinase. *J Biol Chem* (2005)

222. Lin Y-F, Shih H-Y, Shang Z, Matsunaga S, Chen BP. DNA-PKcs is required to maintain stability of Chk1 and Claspin for optimal replication stress response. *Nucleic Acids Res* (2014) **42**:4463–4473. doi:10.1093/nar/gku116
223. Gatei M, Sloper K, Sörensen C, Syljuäsen R, Falck J, Hobson K, Savage K, Lukas J, Zhou B-B, Bartek J, et al. Ataxia-telangiectasia-mutated (ATM) and NBS1-dependent Phosphorylation of Chk1 on Ser-317 in Response to Ionizing Radiation. *J Biol Chem* (2003) **278**:14806–14811. doi:10.1074/jbc.M210862200
224. Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ. Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. *Proc Natl Acad Sci* (2000) **97**:10389–10394. doi:10.1073/pnas.190030497
225. Shiotani B, Zou L. Single-Stranded DNA Orchestrates an ATM-to-ATR Switch at DNA Breaks. *Mol Cell* (2009) **33**:547–558. doi:10.1016/j.molcel.2009.01.024
226. Stiff T, Walker SA, Cersaletti K, Goodarzi AA, Petermann E, Concannon P, O’Driscoll M, Jeggo PA. ATR-dependent phosphorylation and activation of ATM in response to UV treatment or replication fork stalling. *EMBO J* (2006) **25**:5775–5782. doi:10.1038/sj.emboj.7601446
227. Zhou Y, Lee J-H, Jiang W, Crowe JL, Zha S, Paull TT. Regulation of the DNA Damage Response by DNA-PKcs Inhibitory Phosphorylation of ATM. *Mol Cell* (2017) **65**:91–104. doi:10.1016/j.molcel.2016.11.004
228. Chen BPC, Uematsu N, Kobayashi J, Lerenthal Y, Krempler A, Yajima H, Löbrich M, Shiloh Y, Chen DJ. Ataxia Telangiectasia Mutated (ATM) Is Essential for DNA-PKcs Phosphorylations at the Thr-2609 Cluster upon DNA Double Strand Break. *J Biol Chem* (2007) **282**:6582–6587. doi:10.1074/jbc.M611605200
229. Yajima H, Lee K-J, Chen BPC. ATR-Dependent Phosphorylation of DNA-Dependent Protein Kinase Catalytic Subunit in Response to UV-Induced Replication Stress. *Mol Cell Biol* (2006) **26**:7520–7528. doi:10.1128/MCB.00048-06
230. Alexander A, Cai SL, Kim J, Nanez A, Sahin M, MacLean KH, Inoki K, Guan KL, Shen J, Person MD, et al. Correction for Alexander et al., ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. *Proc Natl Acad Sci* (2012) **109**:8352–8352. doi:10.1073/pnas.1206201109
231. Zhang J, Kim J, Alexander A, Cai S, Tripathi DN, Dere R, Tee AR, Tait-Mulder J, Di Nardo A, Han JM, et al. A tuberous sclerosis complex signalling node at the peroxisome regulates mTORC1 and autophagy in response to ROS. *Nat Cell Biol* (2013) **15**:1186–1196. doi:10.1038/ncb2822
232. Cam H, Easton JB, High A, Houghton PJ. mTORC1 Signaling under Hypoxic Conditions Is Controlled by ATM-Dependent Phosphorylation of HIF-1 α . *Mol Cell* (2010) **40**:509–520. doi:10.1016/j.molcel.2010.10.030
233. Kim S-T. Involvement of the cohesin protein, Smc1, in Atm-dependent and independent responses to DNA damage. *Genes Dev* (2002) **16**:560–570. doi:10.1101/gad.970602
234. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J, et al. FoxO3 Controls Autophagy in Skeletal Muscle In Vivo. *Cell Metab* (2007)

6:458–471. doi:10.1016/j.cmet.2007.11.001

235. Tsai W-B, Chung YM, Takahashi Y, Xu Z, Hu MCT. Functional interaction between FOXO3a and ATM regulates DNA damage response. *Nat Cell Biol* (2008) **10**:460–467. doi:10.1038/ncb1709
236. Zhao M, Klionsky DJ. AMPK-Dependent Phosphorylation of ULK1 Induces Autophagy. *Cell Metab* (2011) **13**:119–120. doi:10.1016/j.cmet.2011.01.009
237. Alexander A, Cai SL, Kim J, Nanez A, Sahin M, MacLean KH, Inoki K, Guan KL, Shen J, Person MD, et al. Correction for Alexander et al., ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. *Proc Natl Acad Sci* (2012) **109**:8352–8352. doi:10.1073/pnas.1206201109
238. Selvarajah J, Elia A, Carroll VA, Moumen A. DNA damage-induced S and G2/M cell cycle arrest requires mTORC2-dependent regulation of Chk1. *Oncotarget* (2015) **6**:427–440. doi:10.18632/oncotarget.2813
239. Selvarajah J, Elia A, Carroll VA, Moumen A. DNA damage-induced S and G2/M cell cycle arrest requires mTORC2-dependent regulation of Chk1. *Oncotarget* (2015) **6**:427–440. doi:10.18632/oncotarget.2813
240. Ma Y, Silveri L, LaCava J, Dokudovskaya S. Tumor suppressor NPRL2 induces ROS production and DNA damage response. *Sci Rep* (2017) **7**:15311. doi:10.1038/s41598-017-15497-0
241. Shen C, Houghton PJ. The mTOR pathway negatively controls ATM by up-regulating miRNAs. *Proc Natl Acad Sci* (2013) **110**:11869–11874. doi:10.1073/pnas.1220898110
242. Zhou X, Liu W, Hu X, Dorrance A, Garzon R, Houghton PJ, Shen C. Regulation of CHK1 by mTOR contributes to the evasion of DNA damage barrier of cancer cells. *Sci Rep* (2017) **7**:1535. doi:10.1038/s41598-017-01729-w
243. Feng Z, Hu W, de Stanchina E, Teresky AK, Jin S, Lowe S, Levine AJ. The Regulation of AMPK β 1, TSC2, and PTEN Expression by p53: Stress, Cell and Tissue Specificity, and the Role of These Gene Products in Modulating the IGF-1-AKT-mTOR Pathways. *Cancer Res* (2007) **67**:3043–3053. doi:10.1158/0008-5472.CAN-06-4149
244. Sakai Y, Kassai H, Nakayama H, Fukaya M, Maeda T, Nakao K, Hashimoto K, Sakagami H, Kano M, Aiba A. Hyperactivation of mTORC1 disrupts cellular homeostasis in cerebellar Purkinje cells. *Sci Rep* (2019) **9**:2799. doi:10.1038/s41598-019-38730-4
245. Zhang S, Readinger JA, DuBois W, Janka-Junttila M, Robinson R, Pruitt M, Bliskovsky V, Wu JZ, Sakakibara K, Patel J, et al. Constitutive reductions in mTOR alter cell size, immune cell development, and antibody production. *Blood* (2011) **117**:1228–1238. doi:10.1182/blood-2010-05-287821
246. Fingar DC. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev* (2002) **16**:1472–1487. doi:10.1101/gad.995802
247. Blommaert EFC, Luiken JJFP, Blommaert PJE, van Woerkom GM, Meijer AJ. Phosphorylation of Ribosomal Protein S6 Is Inhibitory for Autophagy in Isolated Rat Hepatocytes. *J Biol Chem* (1995) **270**:2320–2326. doi:10.1074/jbc.270.5.2320
248. Hara K, Yonezawa K, Weng Q-P, Kozlowski MT, Belham C, Avruch J. Amino Acid Sufficiency and mTOR Regulate p70 S6 Kinase and eIF-4E BP1 through a Common Effector Mechanism. *J Biol*

Chem (1998) **273**:14484–14494. doi:10.1074/jbc.273.23.14484

249. Schalm SS, Blenis J. Identification of a Conserved Motif Required for mTOR Signaling. *Curr Biol* (2002) **12**:632–639. doi:10.1016/S0960-9822(02)00762-5
250. Schalm SS, Fingar DC, Sabatini DM, Blenis J. TOS Motif-Mediated Raptor Binding Regulates 4E-BP1 Multisite Phosphorylation and Function. *Curr Biol* (2003) **13**:797–806. doi:10.1016/S0960-9822(03)00329-4
251. Gingras A-C. Regulation of translation initiation by FRAP/mTOR. *Genes Dev* (2001) **15**:807–826. doi:10.1101/gad.887201
252. Sailaxmi G, Lalitha K. Impact of a stress management program on stress perception of nurses working with psychiatric patients. *Asian J Psychiatr* (2015) **14**:42–45. doi:10.1016/j.ajp.2015.01.002
253. Brunn GJ, Hudson CC, Sekulić A, Williams JM, Hosoi H, Houghton PJ, Lawrence JC, Abraham RT. Phosphorylation of the Translational Repressor PHAS-I by the Mammalian Target of Rapamycin. *Science* (80-) (1997) **277**:99–101. doi:10.1126/science.277.5322.99
254. Dennis MD, Jefferson LS, Kimball SR. Role of p70S6K1-mediated Phosphorylation of eIF4B and PDCD4 Proteins in the Regulation of Protein Synthesis. *J Biol Chem* (2012) **287**:42890–42899. doi:10.1074/jbc.M112.404822
255. Ma XM, Yoon S-O, Richardson CJ, Jülich K, Blenis J. SKAR Links Pre-mRNA Splicing to mTOR/S6K1-Mediated Enhanced Translation Efficiency of Spliced mRNAs. *Cell* (2008) **133**:303–313. doi:10.1016/j.cell.2008.02.031
256. Ben-Sahra I, Hoxhaj G, Ricoult SJH, Asara JM, Manning BD. mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science* (80-) (2016) **351**:728–733. doi:10.1126/science.aad0489
257. Ben-Sahra I, Howell JJ, Asara JM, Manning BD. Stimulation of de Novo Pyrimidine Synthesis by Growth Signaling Through mTOR and S6K1. *Science* (80-) (2013) **339**:1323–1328. doi:10.1126/science.1228792
258. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, et al. Activation of a Metabolic Gene Regulatory Network Downstream of mTOR Complex 1. *Mol Cell* (2010) **39**:171–183. doi:10.1016/j.molcel.2010.06.022
259. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden KL, Carpenter AE, Finck BN, et al. mTOR Complex 1 Regulates Lipin 1 Localization to Control the SREBP Pathway. *Cell* (2011) **146**:408–420. doi:10.1016/j.cell.2011.06.034
260. Rousseau A, Bertolotti A. An evolutionarily conserved pathway controls proteasome homeostasis. *Nature* (2016) **536**:184–189. doi:10.1038/nature18943
261. Zhao J, Zhai B, Gygi SP, Goldberg AL. mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. *Proc Natl Acad Sci* (2015) **112**:15790–15797. doi:10.1073/pnas.1521919112
262. Xie Y, Li J, Kang R, Tang D. Interplay Between Lipid Metabolism and Autophagy. *Front Cell Dev Biol* (2020) **8**: doi:10.3389/fcell.2020.00431
263. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, Iemura S, Natsume T, Takehana K,

- Yamada N, et al. Nutrient-dependent mTORC1 Association with the ULK1–Atg13–FIP200 Complex Required for Autophagy. *Mol Biol Cell* (2009) **20**:1981–1991. doi:10.1091/mbc.e08-12-1248
264. Martina J a., Chen Y, Gucek M, Puertollano R. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* (2012) **8**:903–914. doi:10.4161/auto.19653
 265. Roczniak-Ferguson A, Petit CS, Froehlich F, Qian S, Ky J, Angarola B, Walther TC, Ferguson SM. The Transcription Factor TFEB Links mTORC1 Signaling to Transcriptional Control of Lysosome Homeostasis. *Sci Signal* (2012) **5**:ra42–ra42. doi:10.1126/scisignal.2002790
 266. Nicotra G, Mercalli F, Peracchio C, Castino R, Follo C, Valente G, Isidoro C. Autophagy-active beclin-1 correlates with favourable clinical outcome in non-Hodgkin lymphomas. *Mod Pathol* (2010) **23**:937–950. doi:10.1038/modpathol.2010.80
 267. Huang J-J, Zhu Y-J, Lin T-Y, Jiang W-Q, Huang H-Q, Li Z-M. Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Hum Pathol* (2011) **42**:1459–1466. doi:10.1016/j.humpath.2010.12.014
 268. Bertolo C, Roa S, Sagardoy A, Mena-Varas M, Robles EF, Martinez-Ferrandis JI, Sagaert X, Tousseyn T, Orta A, Lossos IS, et al. LITAF , a BCL6 target gene, regulates autophagy in mature B-cell lymphomas. *Br J Haematol* (2013) **162**:621–630. doi:10.1111/bjh.12440
 269. Cheng C, Wang T, Song Z, Peng L, Gao M, Hermine O, Rousseaux S, Khochbin S, Mi J-Q, Wang J. Induction of autophagy and autophagy-dependent apoptosis in diffuse large B-cell lymphoma by a new antimalarial artemisinin derivative, SM1044. *Cancer Med* (2018) **7**:380–396. doi:10.1002/cam4.1276
 270. Chen Z, Cheng L qiu. Eupafolin induces autophagy and apoptosis in B-cell non-Hodgkin lymphomas. *J Pharm Pharmacol* (2021) **73**:241–246. doi:10.1093/jpp/rgaa011
 271. Xu L, Gao X, Yang P, Sang W, Jiao J, Niu M, Liu M, Qin Y, Yan D, Song X, et al. EHMT2 inhibitor BIX-01294 induces endoplasmic reticulum stress mediated apoptosis and autophagy in diffuse large B-cell lymphoma cells. *J Cancer* (2021) **12**:1011–1022. doi:10.7150/jca.48310
 272. Zhang MY, Calin GA, Yuen KS, Jin DY, Chim CS. Epigenetic silencing of miR-342-3p in B cell lymphoma and its impact on autophagy. *Clin Epigenetics* (2020) **12**:150. doi:10.1186/s13148-020-00926-1
 273. Yuan H, He M, Cheng F, Bai R, da Silva SR, Aguiar RCT, Gao S-J. Tenovin-6 inhibits proliferation and survival of diffuse large B-cell lymphoma cells by blocking autophagy. *Oncotarget* (2017) **8**:14912–14924. doi:10.18632/oncotarget.14741
 274. Zhang L, Zhou S, Zhou T, Yuan K, Li X, Tang J. S100A8 promotes chemoresistance via augmenting autophagy in B-cell lymphoma cells. *Oncol Rep* (2020) **45**:151–158. doi:10.3892/or.2020.7841
 275. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of Immune Responses by mTOR. *Annu Rev Immunol* (2012) **30**:39–68. doi:10.1146/annurev-immunol-020711-075024
 276. Linke M, Fritsch SD, Sukhbaatar N, Hengstschläger M, Weichhart T. mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett* (2017) **591**:3089–3103. doi:10.1002/1873-3468.12711

277. Turvey SE, Broide DH. Innate immunity. *J Allergy Clin Immunol* (2010) **125**:S24–S32. doi:10.1016/j.jaci.2009.07.016
278. Bonilla FA, Oettgen HC. Adaptive immunity. *J Allergy Clin Immunol* (2010) **125**:S33–S40. doi:10.1016/j.jaci.2009.09.017
279. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* (2010) **125**:S73–S80. doi:10.1016/j.jaci.2009.11.017
280. Turvey SE, Broide DH. Innate immunity. *J Allergy Clin Immunol* (2010) **125**:S24–S32. doi:10.1016/j.jaci.2009.07.016
281. Murray PJ. Macrophage Polarization. *Annu Rev Physiol* (2017) **79**:541–566. doi:10.1146/annurev-physiol-022516-034339
282. Ruytinx P, Proost P, Van Damme J, Struyf S. Chemokine-Induced Macrophage Polarization in Inflammatory Conditions. *Front Immunol* (2018) **9**:1–12. doi:10.3389/fimmu.2018.01930
283. Haloul M, Oliveira ERA, Kader M, Wells JZ, Tominello TR, El Andaloussi A, Yates CC, Ismail N. mTORC1-mediated polarization of M1 macrophages and their accumulation in the liver correlate with immunopathology in fatal ehrlichiosis. *Sci Rep* (2019) **9**:14050. doi:10.1038/s41598-019-50320-y
284. Byles V, Covarrubias AJ, Ben-Sahra I, Lamming DW, Sabatini DM, Manning BD, Horng T. The TSC-mTOR pathway regulates macrophage polarization. *Nat Commun* (2013) **4**:2834. doi:10.1038/ncomms3834
285. Gomez-Cambronero J. Rapamycin inhibits GM-CSF-induced neutrophil migration. *FEBS Lett* (2003) **550**:94–100. doi:10.1016/S0014-5793(03)00828-7
286. Fernández N, González A, Valera I, Alonso S, Crespo MS. Mannan and peptidoglycan induce COX-2 protein in human PMN via the mammalian target of rapamycin. *Eur J Immunol* (2007) **37**:2572–2582. doi:10.1002/eji.200737262
287. Chen F, Cao A, Yao S, Evans-Marin HL, Liu H, Wu W, Carlsen ED, Dann SM, Soong L, Sun J, et al. mTOR Mediates IL-23 Induction of Neutrophil IL-17 and IL-22 Production. *J Immunol* (2016) **196**:4390–4399. doi:10.4049/jimmunol.1501541
288. Kim M-S, Kuehn HS, Metcalfe DD, Gilfillan AM. Activation and Function of the mTORC1 Pathway in Mast Cells. *J Immunol* (2008) **180**:4586–4595. doi:10.4049/jimmunol.180.7.4586
289. Rakhmanova V, Jin M, Shin J. Inhibition of Mast Cell Function and Proliferation by mTOR Activator MHY1485. *Immune Netw* (2018) **18**:1–8. doi:10.4110/in.2018.18.e18
290. Sathaliyawala T, O’Gorman WE, Greter M, Bogunovic M, Konjufca V, Hou ZE, Nolan GP, Miller MJ, Merad M, Reizis B. Mammalian Target of Rapamycin Controls Dendritic Cell Development Downstream of Flt3 Ligand Signaling. *Immunity* (2010) **33**:597–606. doi:10.1016/j.immuni.2010.09.012
291. Wang Y, Huang G, Zeng H, Yang K, Lamb RF, Chi H. Tuberous sclerosis 1 (Tsc1)-dependent metabolic checkpoint controls development of dendritic cells. *Proc Natl Acad Sci* (2013) **110**:E4894–E4903. doi:10.1073/pnas.1308905110
292. Weichhart T, Costantino G, Poglitsch M, Rosner M, Zeyda M, Stuhlmeier KM, Kolbe T, Stulnig TM, Hörl WH, Hengstschläger M, et al. The TSC-mTOR Signaling Pathway Regulates the Innate

Inflammatory Response. *Immunity* (2008) **29**:565–577. doi:10.1016/j.immuni.2008.08.012

293. Ohtani M, Hoshii T, Fujii H, Koyasu S, Hirao A, Matsuda S. Cutting Edge: mTORC1 in Intestinal CD11c + CD11b + Dendritic Cells Regulates Intestinal Homeostasis by Promoting IL-10 Production. *J Immunol* (2012) **188**:4736–4740. doi:10.4049/jimmunol.1200069
294. Haidinger M, Poglitsch M, Geyeregger R, Kasturi S, Zeyda M, Zlabinger GJ, Pulendran B, Hörl WH, Säemann MD, Weichhart T. A Versatile Role of Mammalian Target of Rapamycin in Human Dendritic Cell Function and Differentiation. *J Immunol* (2010) **185**:3919–3931. doi:10.4049/jimmunol.1000296
295. Hua W, Liu H, Xia L-X, Tian B-P, Huang H-Q, Chen Z-Y, Ju Z-Y, Li W, Chen Z-H, Shen H-H. Rapamycin inhibition of eosinophil differentiation attenuates allergic airway inflammation in mice. *Respirology* (2015) **20**:1055–1065. doi:10.1111/resp.12554
296. Zhu C, Xia L, Li F, Zhou L, Weng Q, Li Z, Wu Y, Mao Y, Zhang C, Wu Y, et al. mTOR complexes differentially orchestrates eosinophil development in allergy. *Sci Rep* (2018) **8**:6883. doi:10.1038/s41598-018-25358-z
297. Yang C, Tsaih S-W, Lemke A, Flister MJ, Thakar MS, Malarkannan S. mTORC1 and mTORC2 differentially promote natural killer cell development. *Elife* (2018) **7**:1–25. doi:10.7554/eLife.35619
298. Marçais A, Marotel M, Degouve S, Koenig A, Fauteux-Daniel S, Drouillard A, Schlums H, Viel S, Besson L, Allatif O, et al. High mTOR activity is a hallmark of reactive natural killer cells and amplifies early signaling through activating receptors. *Elife* (2017) **6**:1–21. doi:10.7554/eLife.26423
299. Marçais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J, et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. *Nat Immunol* (2014) **15**:749–757. doi:10.1038/ni.2936
300. Nandagopal N, Ali AK, Komal AK, Lee S-H. The Critical Role of IL-15–PI3K–mTOR Pathway in Natural Killer Cell Effector Functions. *Front Immunol* (2014) **5**:1–12. doi:10.3389/fimmu.2014.00187
301. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. *Allergy, Asthma Clin Immunol* (2018) **14**:49. doi:10.1186/s13223-018-0278-1
302. Bonilla FA, Oettgen HC. Adaptive immunity. *J Allergy Clin Immunol* (2010) **125**:S33–S40. doi:10.1016/j.jaci.2009.09.017
303. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. *Allergy, Asthma Clin Immunol* (2018) **14**:49. doi:10.1186/s13223-018-0278-1
304. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC, Powell JD. The mTOR Kinase Differentially Regulates Effector and Regulatory T Cell Lineage Commitment. *Immunity* (2009) **30**:832–844. doi:10.1016/j.immuni.2009.04.014
305. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF, Powell JD. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* (2011) **12**:295–303. doi:10.1038/ni.2005
306. Kurebayashi Y, Nagai S, Ikejiri A, Ohtani M, Ichiyama K, Baba Y, Yamada T, Egami S, Hoshii T,

Hirao A, et al. PI3K-Akt-mTORC1-S6K1/2 Axis Controls Th17 Differentiation by Regulating Gfi1 Expression and Nuclear Translocation of ROR γ . *Cell Rep* (2012) **1**:360–373. doi:10.1016/j.celrep.2012.02.007

307. Yang K, Shrestha S, Zeng H, Karmaus PWF, Neale G, Vogel P, Guertin DA, Lamb RF, Chi H. T Cell Exit from Quiescence and Differentiation into Th2 Cells Depend on Raptor-mTORC1-Mediated Metabolic Reprogramming. *Immunity* (2013) **39**:1043–1056. doi:10.1016/j.immuni.2013.09.015
308. Park Y, Jin H-S, Lopez J, Elly C, Kim G, Murai M, Kronenberg M, Liu Y-C. TSC1 regulates the balance between effector and regulatory T cells. *J Clin Invest* (2013) **123**:5165–5178. doi:10.1172/JCI69751
309. Sinclair L V., Rolf J, Emslie E, Shi Y-B, Taylor PM, Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat Immunol* (2013) **14**:500–508. doi:10.1038/ni.2556
310. Nakaya M, Xiao Y, Zhou X, Chang J-H, Chang M, Cheng X, Blonska M, Lin X, Sun S-C. Inflammatory T Cell Responses Rely on Amino Acid Transporter ASCT2 Facilitation of Glutamine Uptake and mTORC1 Kinase Activation. *Immunity* (2014) **40**:692–705. doi:10.1016/j.immuni.2014.04.007
311. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish Treg-cell function. *Nature* (2013) **499**:485–490. doi:10.1038/nature12297
312. De Rosa V, Galgani M, Porcellini A, Colamatteo A, Santopaolo M, Zuchegna C, Romano A, De Simone S, Procaccini C, La Rocca C, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. *Nat Immunol* (2015) **16**:1174–1184. doi:10.1038/ni.3269
313. Gerriets VA, Kishton RJ, Johnson MO, Cohen S, Siska PJ, Nichols AG, Warmoes MO, de Cubas AA, MacIver NJ, Locasale JW, et al. Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat Immunol* (2016) **17**:1459–1466. doi:10.1038/ni.3577
314. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, Wu C, Vogel P, Neale G, Green DR, et al. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. *Nat Immunol* (2016) **17**:277–285. doi:10.1038/ni.3365
315. Zeng H, Cohen S, Guy C, Shrestha S, Neale G, Brown SA, Cloer C, Kishton RJ, Gao X, Youngblood B, et al. mTORC1 and mTORC2 Kinase Signaling and Glucose Metabolism Drive Follicular Helper T Cell Differentiation. *Immunity* (2016) **45**:540–554. doi:10.1016/j.immuni.2016.08.017
316. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed R. mTOR regulates memory CD8 T-cell differentiation. *Nature* (2009) **460**:108–112. doi:10.1038/nature08155
317. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang L-S, Jones RG, Choi Y. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* (2009) **460**:103–107. doi:10.1038/nature08097
318. Rao RR, Li Q, Odunsi K, Shrikant PA. The mTOR Kinase Determines Effector versus Memory CD8 + T Cell Fate by Regulating the Expression of Transcription Factors T-bet and Eomesodermin. *Immunity* (2010) **32**:67–78. doi:10.1016/j.immuni.2009.10.010

319. Allen CDC, Okada T, Cyster JG. Germinal-Center Organization and Cellular Dynamics. *Immunity* (2007) **27**:190–202. doi:10.1016/j.immuni.2007.07.009
320. Steele EJ. Mechanism of somatic hypermutation: Critical analysis of strand biased mutation signatures at A:T and G:C base pairs. *Mol Immunol* (2009) **46**:305–320. doi:10.1016/j.molimm.2008.10.021
321. Iwata TN, Ramírez JA, Tsang M, Park H, Margineantu DH, Hockenbery DM, Iritani BM. Conditional Disruption of Raptor Reveals an Essential Role for mTORC1 in B Cell Development, Survival, and Metabolism. *J Immunol* (2016) **197**:2250–2260. doi:10.4049/jimmunol.1600492
322. Raybuck AL, Cho SH, Li J, Rogers MC, Lee K, Williams CL, Shlomchik M, Thomas JW, Chen J, Williams J V., et al. B Cell–Intrinsic mTORC1 Promotes Germinal Center–Defining Transcription Factor Gene Expression, Somatic Hypermutation, and Memory B Cell Generation in Humoral Immunity. *J Immunol* (2018) **200**:2627–2639. doi:10.4049/jimmunol.1701321
323. Zhang S, Pruitt M, Tran D, Du Bois W, Zhang K, Patel R, Hoover S, Simpson RM, Simmons J, Gary J, et al. B Cell–Specific Deficiencies in mTOR Limit Humoral Immune Responses. *J Immunol* (2013) **191**:1692–1703. doi:10.4049/jimmunol.1201767
324. Jones DD, Weiss BM, Allman D, Jones DD, Gaudette BT, Wilmore JR, Chernova I, Bortnick A, Weiss BM, Allman D. mTOR has distinct functions in generating versus sustaining humoral immunity. *J Clin Invest* (2016) **126**:4250–4261. doi:10.1172/JCI86504.paradoxically
325. Gaudette BT, Jones DD, Bortnick A, Argon Y, Allman D. mTORC1 coordinates an immediate unfolded protein response-related transcriptome in activated B cells preceding antibody secretion. *Nat Commun* (2020) **11**:723. doi:10.1038/s41467-019-14032-1
326. Zheng Y, Li R, Liu S. Immunoregulation with mTOR inhibitors to prevent COVID-19 severity: A novel intervention strategy beyond vaccines and specific antiviral medicines. *J Med Virol* (2020) **92**:1495–1500. doi:10.1002/jmv.26009
327. Le Sage V, Cinti A, Amorim R, Mouland A. Adapting the Stress Response: Viral Subversion of the mTOR Signaling Pathway. *Viruses* (2016) **8**:152. doi:10.3390/v8060152
328. O’Shea C, Klupsch K, Choi S, Bagus B, Soria C, Shen J, McCormick F, Stokoe D. Adenoviral proteins mimic nutrient/growth signals to activate the mTOR pathway for viral replication. *EMBO J* (2005) **24**:1211–1221. doi:10.1038/sj.emboj.7600597
329. Moody CA, Scott RS, Amirghahari N, Nathan C-A, Young LS, Dawson CW, Sixbey JW. Modulation of the Cell Growth Regulator mTOR by Epstein-Barr Virus-Encoded LMP2A. *J Virol* (2005) **79**:5499–5506. doi:10.1128/JVI.79.9.5499-5506.2005
330. Shives KD, Beatman EL, Chamanian M, O’Brien C, Hobson-Peters J, Beckham JD. West Nile Virus-Induced Activation of Mammalian Target of Rapamycin Complex 1 Supports Viral Growth and Viral Protein Expression. *J Virol* (2014) **88**:9458–9471. doi:10.1128/JVI.01323-14
331. Aoyagi M, Gaspar M, Shenk TE. Human cytomegalovirus UL69 protein facilitates translation by associating with the mRNA cap-binding complex and excluding 4EBP1. *Proc Natl Acad Sci* (2010) **107**:2640–2645. doi:10.1073/pnas.0914856107
332. Abdallah C, Lejamtel C, Benzoubir N, Battaglia S, Sidahmed-Adrar N, Desterke C, Lemasson M, Rosenberg AR, Samuel D, Bréchet C, et al. Hepatitis C virus core protein targets 4E-BP1 expression and phosphorylation and potentiates Myc-induced liver carcinogenesis in transgenic mice. *Oncotarget*

(2017) **8**:56228–56242. doi:10.18632/oncotarget.17280

333. Ranadheera C, Coombs KM, Kobasa D. Comprehending a Killer: The Akt/mTOR Signaling Pathways Are Temporally High-Jacked by the Highly Pathogenic 1918 Influenza Virus. *EBioMedicine* (2018) **32**:142–163. doi:10.1016/j.ebiom.2018.05.027
334. Luo Q, Zhang L, Wei F, Fang Q, Bao F, Mi S, Li N, Wang C, Liu Y, Tu C. mTORC1 Negatively Regulates the Replication of Classical Swine Fever Virus Through Autophagy and IRES-Dependent Translation. *iScience* (2018) **3**:87–101. doi:10.1016/j.isci.2018.04.010
335. Heredia A, Amoroso A, Davis C, Le N, Reardon E, Dominique JK, Klingebiel E, Gallo RC, Redfield RR. Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV β -chemokines: An approach to suppress R5 strains of HIV-1. *Proc Natl Acad Sci* (2003) **100**:10411–10416. doi:10.1073/PNAS.1834278100
336. Donia M, McCubrey JA, Bendtzen K, Nicoletti F. Potential use of rapamycin in HIV infection. *Br J Clin Pharmacol* (2010) **70**:784–793. doi:10.1111/j.1365-2125.2010.03735.x
337. Nicoletti F, Fagone P, Meroni P, Mccubrey J, Bendtzen K. mTOR as a multifunctional therapeutic target in HIV infection. *Drug Discov Today* (2011) **16**:715–721. doi:10.1016/j.drudis.2011.05.008
338. Heredia A, Le N, Gartenhaus RB, Sausville E, Medina-Moreno S, Zapata JC, Davis C, Gallo RC, Redfield RR. Targeting of mTOR catalytic site inhibits multiple steps of the HIV-1 lifecycle and suppresses HIV-1 viremia in humanized mice. *Proc Natl Acad Sci* (2015) **112**:9412–9417. doi:10.1073/pnas.1511144112
339. Planas D, Routy J, Ancuta P, Planas D, Zhang Y, Monteiro P, Goulet J, Gosselin A. HIV-1 selectively targets gut-homing mechanisms Find the latest version : HIV-1 selectively targets gut-homing mechanisms. (2017) **2**:1–21.
340. Cinti A, Le Sage V, Milev MP, Valiente-Echeverría F, Crossie C, Miron M-J, Panté N, Olivier M, Mouland AJ. HIV-1 enhances mTORC1 activity and repositions lysosomes to the periphery by co-opting Rag GTPases. *Sci Rep* (2017) **7**:5515. doi:10.1038/s41598-017-05410-0
341. Zhang H-S, Zhang Z-G, Zhou Z, Du G-Y, Li H, Yu X-Y, Huang Y-H. PKM2-mediated inhibition of autophagy facilitates Tat's inducing HIV-1 transactivation. *Arch Biochem Biophys* (2017) **625–626**:17–23. doi:10.1016/j.abb.2017.05.018
342. Kumar B, Arora S, Ahmed S, Banerjee AC. Hyperactivation of mammalian target of rapamycin complex 1 by HIV-1 is necessary for virion production and latent viral reactivation. *FASEB J* (2017) **31**:180–191. doi:10.1096/fj.201600813R
343. Blanchet FP, Moris A, Nikolic DS, Lehmann M, Cardinaud S, Stalder R, Garcia E, Dinkins C, Leuba F, Wu L, et al. Human immunodeficiency virus-1 inhibition of immunoamphisomes in dendritic cells impairs early innate and adaptive immune responses. *Immunity* (2010) **32**:654–669. doi:10.1016/j.immuni.2010.04.011
344. Rehman S, Husain M, Yadav A, Kasinath BS, Malhotra A, Singhal PC. HIV-1 Promotes Renal Tubular Epithelial Cell Protein Synthesis: Role of mTOR Pathway. *PLoS One* (2012) **7**:e30071. doi:10.1371/journal.pone.0030071
345. Heredia A, Amoroso A, Davis C, Le N, Reardon E, Dominique JK, Klingebiel E, Gallo RC, Redfield RR. Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV β -chemokines : An

approach to suppress R5 strains of HIV-1. (2003)2–7. Available at:
<https://doi.org/10.1073/pnas.1834278100>

346. Heredia A, Latinovic O, Gallo RC, Melikyan G, Reitz M, Le N, Redfield RR. Reduction of CCR5 with low-dose rapamycin enhances the antiviral activity of vicriviroc against both sensitive and drug-resistant HIV-1. (2008) **105**:1–6.
347. Taylor HE, Calantone N, Lichon D, Hudson H, Clerc I, Campbell EM, D'Aquila RT. mTOR Overcomes Multiple Metabolic Restrictions to Enable HIV-1 Reverse Transcription and Intracellular Transport. *Cell Rep* (2020) **31**:107810. doi:10.1016/j.celrep.2020.107810
348. Campbell GR, Bruckman RS, Herns SD, Joshi S, Durden DL, Spector SA. Induction of autophagy by PI3K/MTOR and PI3K/MTOR/BRD4 inhibitors suppresses HIV-1 replication. *J Biol Chem* (2018) **293**:5808–5820. doi:10.1074/jbc.RA118.002353
349. Saxton RA, Sabatini DM. Erratum: mTOR Signaling in Growth, Metabolism, and Disease (*Cell* (2017) 168(6) (960–976) (S0092867417301824) (10.1016/j.cell.2017.02.004)). *Cell* (2017) **169**:361–371. doi:10.1016/j.cell.2017.03.035
350. Molle D, Segura-Morales C, Camus G, Berlioz-Torrent C, Kjems J, Basyuk E, Bertrand E. Endosomal trafficking of HIV-1 gag and genomic RNAs regulates viral egress. *J Biol Chem* (2009) **284**:19727–43. doi:10.1074/jbc.M109.019844
351. Xue M, Yao S, Hu M, Li W, Hao T, Zhou F, Zhu X, Lu H, Qin D, Yan Q, et al. HIV-1 Nef and KSHV oncogene K1 synergistically promote angiogenesis by inducing cellular miR-718 to regulate the PTEN/AKT/mTOR signaling pathway. *Nucleic Acids Res* (2014) **42**:9862–9879. doi:10.1093/nar/gku583
352. Li J, Wang W, Tong P, Leung C-K, Yang G, Li Z, Li N, Sun X, Han Y, Lu C, et al. Autophagy Induction by HIV-Tat and Methamphetamine in Primary Midbrain Neuronal Cells of Tree Shrews via the mTOR Signaling and ATG5/ATG7 Pathway. *Front Neurosci* (2018) **12**:1–15. doi:10.3389/fnins.2018.00921
353. Poggi A, Carosio R, Fenoglio D, Brenci S, Murdaca G, Setti M, Indiveri F, Scabini S, Ferrero E, Zocchi MR. Migration of V delta 1 and V delta 2 T cells in response to CXCR3 and CXCR4 ligands in healthy donors and HIV-1-infected patients: competition by HIV-1 Tat. *Blood* (2004) **103**:2205–13. doi:10.1182/blood-2003-08-2928
354. Lahiri V, Hawkins WD, Klionsky DJ. Watch What You (Self-) Eat: Autophagic Mechanisms that Modulate Metabolism. *Cell Metab* (2019) **29**:803–826. doi:10.1016/j.cmet.2019.03.003
355. Dossou AS, Basu A. The Emerging Roles of mTORC1 in Macromanaging Autophagy. *Cancers (Basel)* (2019) **11**:1422. doi:10.3390/cancers11101422
356. van Beek N, Klionsky DJ, Reggiori F. Genetic aberrations in macroautophagy genes leading to diseases. *Biochim Biophys Acta - Mol Cell Res* (2018) **1865**:803–816. doi:10.1016/j.bbamcr.2018.03.002
357. Nardacci R, Ciccocanti F, Marsella C, Ippolito G, Piacentini M, Fimia GM. Role of autophagy in HIV infection and pathogenesis. *J Intern Med* (2017) **281**:422–432. doi:10.1111/joim.12596
358. Liu Z, Xiao Y, Torresilla C, Rassart É, Barbeau B. Implication of Different HIV-1 Genes in the Modulation of Autophagy. *Viruses* (2017) **9**:389. doi:10.3390/v9120389

359. Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, Wu L, Kominami E, Ueno T, Yamamoto A, et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol* (2009) **186**:255–268. doi:10.1083/jcb.200903070
360. Campbell GR, Rawat P, Bruckman RS, Spector SA. Human Immunodeficiency Virus Type 1 Nef Inhibits Autophagy through Transcription Factor EB Sequestration. *PLOS Pathog* (2015) **11**:e1005018. doi:10.1371/journal.ppat.1005018
361. Castro-Gonzalez S, Shi Y, Colomer-Lluch M, Song Y, Mowery K, Almodovar S, Bansal A, Kirchhoff F, Sparrer K, Liang C, et al. HIV-1 Nef counteracts autophagy restriction by enhancing the association between BECN1 and its inhibitor BCL2 in a PRKN-dependent manner. *Autophagy* (2020) **8627**:1–25. doi:10.1080/15548627.2020.1725401
362. Chang C, Young LN, Morris KL, von Bülow S, Schöneberg J, Yamamoto-Imoto H, Oe Y, Yamamoto K, Nakamura S, Stjepanovic G, et al. Bidirectional Control of Autophagy by BECN1 BARA Domain Dynamics. *Mol Cell* (2019) **73**:339–353.e6. doi:10.1016/j.molcel.2018.10.035
363. van Grol J, Subauste C, Andrade RM, Fujinaga K, Nelson J, Subauste CS. HIV-1 inhibits autophagy in bystander macrophage/ monocytic cells through Src-Akt and STAT3. *PLoS One* (2010) **5**: doi:10.1371/journal.pone.0011733
364. Espert L, Denizot M, Grimaldi M, Robert-Hebmann V, Gay B, Varbanov M, Codogno P, Biard-Piechaczyk M. Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *J Clin Invest* (2006) **116**:2161–2172. doi:10.1172/JCI26185
365. Espert L, Varbanov M, Robert-Hebmann V, Sagnier S, Robbins I, Sanchez F, Lafont V, Biard-Piechaczyk M. Differential role of autophagy in CD4 T cells and macrophages during X4 and R5 HIV-1 infection. *PLoS One* (2009) **4**: doi:10.1371/journal.pone.0005787
366. Sagnier S, Daussy CF, Borel S, Robert-Hebmann V, Faure M, Blanchet FP, Beaumelle B, Biard-Piechaczyk M, Espert L. Autophagy Restricts HIV-1 Infection by Selectively Degrading Tat in CD4 + T Lymphocytes . *J Virol* (2015) **89**:615–625. doi:10.1128/jvi.02174-14
367. Alfaisal J, Machado A, Galais M, Robert-Hebmann V, Arnauné-Pelloquin L, Espert L, Biard-Piechaczyk M. HIV-1 Vpr inhibits autophagy during the early steps of infection of CD4 T cells. *Biol Cell* (2019) **111**:308–318. doi:10.1111/boc.201900071
368. Borel S, Robert-Hebmann V, Alfaisal J, Jain A, Faure M, Espert L, Chaloin L, Paillart J-C, Johansen T, Biard-Piechaczyk M. HIV-1 viral infectivity factor interacts with microtubule-associated protein light chain 3 and inhibits autophagy. *AIDS* (2015) **29**:275–286. doi:10.1097/QAD.0000000000000554
369. Yakasai AM. Impact of Symptomatic HIV- Related Neurocognitive Disorders in Survival of HIV- Infected Individuals: A Systematic Review and Meta-Analyses. *J Neuroinfectious Dis* (2015) **06**:1–7. doi:10.4172/2314-7326.1000166
370. Fields J, Dumaop W, Elueteri S, Campos S, Serger E, Trejo M, Kosberg K, Adame A, Spencer B, Rockenstein E, et al. HIV-1 Tat Alters Neuronal Autophagy by Modulating Autophagosome Fusion to the Lysosome: Implications for HIV-Associated Neurocognitive Disorders. *J Neurosci* (2015) **35**:1921–1938. doi:10.1523/jneurosci.3207-14.2015
371. Bruno AP, De Simone FI, Iorio V, De Marco M, Khalili K, Sariyer IK, Capunzo M, Nori SL, Rosati A. HIV-1 Tat protein induces glial cell autophagy through enhancement of BAG3 protein levels. *Cell Cycle* (2014) **13**:3640–3644. doi:10.4161/15384101.2014.952959

372. Wu X, Dong H, Ye X, Zhong L, Cao T, Xu Q, Wang J, Zhang Y, Xu J, Wang W, et al. HIV-1 Tat increases BAG3 via NF- κ B signaling to induce autophagy during HIV-associated neurocognitive disorder. *Cell Cycle* (2018) **17**:1614–1623. doi:10.1080/15384101.2018.1480219
373. Zeng XF, Li Q, Li J, Wong N, Li Z, Huang J, Yang G, Sham PC, Li S Bin, Lu G. HIV-1 Tat and methamphetamine co-induced oxidative cellular injury is mitigated by N-acetylcysteine amide (NACA) through rectifying mTOR signaling. *Toxicol Lett* (2018) **299**:159–171. doi:10.1016/j.toxlet.2018.09.009
374. Qi L, Gang L, Hang KW, Ling CH, Xiaofeng Z, Zhen L, David Wai Y, Sang PW. Programmed neuronal cell death induced by HIV-1 tat and methamphetamine. *Microsc Res Tech* (2011) **74**:1139–1144. doi:10.1002/jemt.21006
375. Thangaraj A, Periyasamy P, Liao K, Bendi VS, Callen S, Pendyala G, Buch S. HIV-1 TAT-mediated microglial activation: role of mitochondrial dysfunction and defective mitophagy. *Autophagy* (2018) **14**:1596–1619. doi:10.1080/15548627.2018.1476810
376. Cary DC, Fujinaga K, Peterlin BM. Molecular mechanisms of HIV latency. *J Clin Invest* (2016) **126**:448–454. doi:10.1172/JCI80565
377. Donahue DA, Wainberg MA. Cellular and molecular mechanisms involved in the establishment of HIV-1 latency. *Retrovirology* (2013) **10**:11. doi:10.1186/1742-4690-10-11
378. Karn J. The molecular biology of HIV latency: Breaking and restoring the Tat-dependent transcriptional circuit. *Curr Opin HIV AIDS* (2011) **6**:4–11. doi:10.1097/COH.0b013e328340ffbb
379. Donahue DA, Bastarache SM, Sloan RD, Wainberg MA. Latent HIV-1 Can Be Reactivated by Cellular Superinfection in a Tat-Dependent Manner, Which Can Lead to the Emergence of Multidrug-Resistant Recombinant Viruses. *J Virol* (2013) **87**:9620–9632. doi:10.1128/jvi.01165-13
380. Forsythe SS, McGreevey W, Whiteside A, Shah M, Cohen J, Hecht R, Bollinger LA, Kinghorn A. Twenty Years Of Antiretroviral Therapy For People Living With HIV: Global Costs, Health Achievements, Economic Benefits. *Health Aff* (2019) **38**:1163–1172. doi:10.1377/hlthaff.2018.05391
381. Eisele E, Siliciano RF. Redefining the Viral Reservoirs that Prevent HIV-1 Eradication. *Immunity* (2012) **37**:377–388. doi:10.1016/j.immuni.2012.08.010
382. Blankson JN, Persaud D, Siliciano RF. The Challenge of Viral Reservoirs in HIV-1 Infection. *Annu Rev Med* (2002) **53**:557–593. doi:10.1146/annurev.med.53.082901.104024
383. Eisele E, Siliciano RF. Redefining the Viral Reservoirs that Prevent HIV-1 Eradication. *Immunity* (2012) **37**:377–388. doi:10.1016/j.immuni.2012.08.010
384. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4⁺ T cells during primary HIV-1 infection. *Proc Natl Acad Sci U S A* (1998) **95**:8869–8873. doi:10.1073/pnas.95.15.8869
385. Vanhamel J, Bruggemans A, Debyser Z. Establishment of latent HIV-1 reservoirs: what do we really know? *J virus Erad* (2019) **5**:3–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/30800420> <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6362902>
386. Abner E, Jordan A. HIV “shock and kill” therapy: In need of revision. *Antiviral Res* (2019) **166**:19–34.

doi:10.1016/j.antiviral.2019.03.008

387. Elsheikh MM, Tang Y, Li D, Jiang G. Deep latency: A new insight into a functional HIV cure. *EBioMedicine* (2019) **45**:624–629. doi:10.1016/j.ebiom.2019.06.020
388. Spivak AM, Planelles V. Novel Latency Reversal Agents for HIV-1 Cure. *Annu Rev Med* (2018) **69**:421–436. doi:10.1146/annurev-med-052716-031710
389. Besnard E, Hakre S, Kampmann M, Lim HW, Hosmane NN, Martin A, Bassik MC, Verschueren E, Battivelli E, Chan J, et al. The mTOR Complex Controls HIV Latency. *Cell Host Microbe* (2016) **20**:785–797. doi:10.1016/j.chom.2016.11.001
390. Martin AR, Pollack RA, Capoferri A, Ambinder RF, Durand CM, Siliciano RF. Rapamycin-mediated mTOR inhibition uncouples HIV-1 latency reversal from cytokine-associated toxicity. *J Clin Invest* (2017) **127**:651–656. doi:10.1172/JCI89552
391. Chowdhury FZ, Ouyang Z, Buzon M, Walker BD, Lichterfeld M, Yu XG. Metabolic pathway activation distinguishes transcriptional signatures of CD8+ T cells from HIV-1 elite controllers. *AIDS* (2018) **32**:2669–2677. doi:10.1097/QAD.0000000000002007
392. Jin S, Liao Q, Chen J, Zhang L, He Q, Zhu H, Zhang X, Xu J. TSC1 and DEPDC5 regulate HIV-1 latency through the mTOR signaling pathway. *Emerg Microbes Infect* (2018) **7**: doi:10.1038/s41426-018-0139-5
393. van Montfort T, van der Sluis R, Darcis G, Beaty D, Groen K, Pasternak AO, Pollakis G, Vink M, Westerhout EM, Hamdi M, et al. Dendritic cells potently purge latent HIV-1 beyond TCR-stimulation, activating the PI3K-Akt-mTOR pathway. *EBioMedicine* (2019) **42**:97–108. doi:10.1016/j.ebiom.2019.02.014
394. Reeves DB, Duke ER, Wagner TA, Palmer SE, Spivak AM, Schiffer JT. A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation. *Nat Commun* (2018) **9**:4811. doi:10.1038/s41467-018-06843-5
395. Márk Á, Hajdu M, Váradi Z, Sticz TB, Nagy N, Csomor J, Berczi L, Varga V, Csóka M, Kopper L, et al. Characteristic mTOR activity in Hodgkin-lymphomas offers a potential therapeutic target in high risk disease - a combined tissue microarray, in vitro and in vivo study. *BMC Cancer* (2013) **13**: doi:10.1186/1471-2407-13-250
396. El-Salem M, Raghunath PN, Marzec M, Liu X, Kasprzycka M, Robertson E, Wasik MA. Activation of mTORC1 signaling pathway in AIDS-related lymphomas. *Am J Pathol* (2009) **175**:817–824. doi:10.2353/ajpath.2009.080451
397. Teater M, Dominguez PM, Redmond D, Chen Z, Ennishi D, Scott DW, Cimmino L, Ghione P, Chaudhuri J, Gascoyne RD, et al. AICDA drives epigenetic heterogeneity and accelerates germinal center-derived lymphomagenesis. *Nat Commun* (2018) **9**:222. doi:10.1038/s41467-017-02595-w
398. Zhang J, Shi Y, Zhao M, Hu H, Huang H. Activation-induced cytidine deaminase overexpression in double-hit lymphoma: potential target for novel anticancer therapy. *Sci Rep* (2020) **10**:14164. doi:10.1038/s41598-020-71058-y
399. Sall FB, El Amine R, Markozashvili D, Tsfasman T, Oksenhendler E, Lipinski M, Vassetzky Y, Germini D. HIV-1 Tat protein induces aberrant activation of AICDA in human B-lymphocytes from peripheral blood. *J Cell Physiol* (2019) **234**:15678–15685. doi:10.1002/jcp.28219

400. Rutkovsky AC, Yeh ES, Guest ST, Findlay VJ, Muise-Helmericks RC, Armeson K, Ethier SP. Eukaryotic initiation factor 4E-binding protein as an oncogene in breast cancer. *BMC Cancer* (2019) **19**:491. doi:10.1186/s12885-019-5667-4
401. She Q-B, Halilovic E, Ye Q, Zhen W, Shirasawa S, Sasazuki T, Solit DB, Rosen N. 4E-BP1 Is a Key Effector of the Oncogenic Activation of the AKT and ERK Signaling Pathways that Integrates Their Function in Tumors. *Cancer Cell* (2010) **18**:39–51. doi:10.1016/j.ccr.2010.05.023
402. Hsieh AC, Costa M, Zollo O, Davis C, Feldman ME, Testa JR, Meyuhas O, Shokat KM, Ruggero D. Genetic Dissection of the Oncogenic mTOR Pathway Reveals Druggable Addiction to Translational Control via 4EBP-eIF4E. *Cancer Cell* (2010) **17**:249–261. doi:10.1016/j.ccr.2010.01.021
403. Asimomytis A, Karanikou M, Rodolakis A, Vaiopoulou A, Tsetsa P, Creatsas G, Stefos T, Antsaklis A, Patsouris E, Rassidakis GZ. mTOR downstream effectors, 4EBP1 and eIF4E, are overexpressed and associated with HPV status in precancerous lesions and carcinomas of the uterine cervix. *Oncol Lett* (2016) **12**:3234–3240. doi:10.3892/ol.2016.5056
404. Romani B, Engelbrecht S, Glashoff RH. Functions of Tat: the versatile protein of human immunodeficiency virus type 1. *J Gen Virol* (2010) **91**:1–12. doi:10.1099/vir.0.016303-0
405. Zhao M, Weissleder R. Intracellular cargo delivery using tat peptide and derivatives. *Med Res Rev* (2004) **24**:1–12. doi:10.1002/med.10056
406. Chauhan A, Tikoo A, Kapur AK, Singh M. The taming of the cell penetrating domain of the HIV Tat: Myths and realities. *J Control Release* (2007) **117**:148–162. doi:10.1016/j.jconrel.2006.10.031
407. Duy Huynh, Elizabeth Vincan, Theo Mantamadiotis, Damian Purcell, Chee-Kai Chan, Robert Ramsay. Oncogenic Properties of HIV-Tat in Colorectal Cancer Cells. *Curr HIV Res* (2007) **5**:403–409. doi:10.2174/157016207781023974
408. Nyagol J, Leucci E, Omnis A, De Falco G, Tigli C, Sanseverino F, Torricelli M, Palummo N, Pacenti L, Santopietro R, et al. The effects of HIV-1 Tat protein on cell cycle during cervical carcinogenesis. *Cancer Biol Ther* (2006) **5**:684–690. doi:10.4161/cbt.5.6.2907
409. Chen X, Cheng L, Jia X, Zeng Y, Yao S, Lv Z, Qin D, Fang X, Lei Y, Lu C. Human Immunodeficiency Virus Type 1 Tat Accelerates Kaposi Sarcoma-Associated Herpesvirus Kaposin A-Mediated Tumorigenesis of Transformed Fibroblasts In Vitro as well as in Nude and Immunocompetent Mice. *Neoplasia* (2009) **11**:1272–1284. doi:10.1593/neo.09494
410. Curreli S, Krishnan S, Reitz M, Lunardi-Iskandar Y, Lafferty MK, Garzino-Demo A, Zella D, Gallo RC, Bryant J. B cell lymphoma in hiv transgenic mice. *Retrovirology* (2013) **10**:92. doi:10.1186/1742-4690-10-92
411. Kundu RK, Sangiorgi F, Wu L-Y, Pattengale PK, Hinton DR, Gill PS, Maxson R. Expression of the Human Immunodeficiency Virus-Tat Gene in Lymphoid Tissues of Transgenic Mice Is Associated With B-Cell Lymphoma. *Blood* (1999) **94**:275–282. doi:10.1182/blood.V94.1.275.413a30_275_282
412. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class Switch Recombination and Hypermutation Require Activation-Induced Cytidine Deaminase (AID), a Potential RNA Editing Enzyme. *Cell* (2000) **102**:553–563. doi:10.1016/S0092-8674(00)00078-7
413. Buccigrossi V, Laudiero G, Nicastro E, Miele E, Esposito F, Guarino A. The HIV-1 Transactivator Factor (Tat) Induces Enterocyte Apoptosis through a Redox-Mediated Mechanism. *PLoS One* (2011)

414. Rozzi SJ, Borelli G, Ryan K, Steiner JP, Reglodi D, Mocchetti I, Avdoshina V. PACAP27 is Protective Against Tat-Induced Neurotoxicity. *J Mol Neurosci* (2014) **54**:485–493. doi:10.1007/s12031-014-0273-z
415. Lau A, Swinbank KM, Ahmed PS, Taylor DL, Jackson SP, Smith GCM, O'Connor MJ. Suppression of HIV-1 infection by a small molecule inhibitor of the ATM kinase. *Nat Cell Biol* (2005) **7**:493–500. doi:10.1038/ncb1250
416. Li D, Lopez A, Sandoval C, Nichols Doyle R, Fregoso OI. HIV Vpr Modulates the Host DNA Damage Response at Two Independent Steps to Damage DNA and Repress Double-Strand DNA Break Repair. *MBio* (2020) **11**:1–18. doi:10.1128/mBio.00940-20
417. Fregoso OI, Emerman M. Activation of the DNA Damage Response Is a Conserved Function of HIV-1 and HIV-2 Vpr That Is Independent of SLX4 Recruitment. *MBio* (2016) **7**:1–10. doi:10.1128/mBio.01433-16
418. Chipitsyna G, Slonina D, Siddiqui K, Peruzzi F, Skorski T, Reiss K, Sawaya BE, Khalili K, Amini S. HIV-1 Tat increases cell survival in response to cisplatin by stimulating Rad51 gene expression. *Oncogene* (2004) **23**:2664–2671. doi:10.1038/sj.onc.1207417
419. Silva A, Gírio A, Cebola I, Santos CI, Antunes F, Barata JT. Intracellular reactive oxygen species are essential for PI3K/Akt/mTOR-dependent IL-7-mediated viability of T-cell acute lymphoblastic leukemia cells. *Leukemia* (2011) **25**:960–967. doi:10.1038/leu.2011.56
420. Yalcin S, Marinkovic D, Mungamuri SK, Zhang X, Tong W, Sellers R, Ghaffari S. ROS-mediated amplification of AKT/mTOR signalling pathway leads to myeloproliferative syndrome in Foxo3^{-/-} mice. *EMBO J* (2010) **29**:4118–4131. doi:10.1038/emboj.2010.292
421. Batool A, Aashaq S, Andrabi KI. Reappraisal to the study of 4E-BP1 as an mTOR substrate – A normative critique. *Eur J Cell Biol* (2017) **96**:325–336. doi:10.1016/j.ejcb.2017.03.013
422. Mirzoev T, Tyganov S, Vilchinskaya N, Lomonosova Y, Shenkman B. Key Markers of mTORC1-Dependent and mTORC1-Independent Signaling Pathways Regulating Protein Synthesis in Rat Soleus Muscle During Early Stages of Hindlimb Unloading. *Cell Physiol Biochem* (2016) **39**:1011–1020. doi:10.1159/000447808
423. Tran TH, Nakata M, Suzuki K, Begum NA, Shinkura R, Fagarasan S, Honjo T, Nagaoka H. B cell-specific and stimulation-responsive enhancers derepress Aicda by overcoming the effects of silencers. *Nat Immunol* (2010) **11**:148–154. doi:10.1038/ni.1829
424. Huong LT, Kobayashi M, Nakata M, Shioi G, Miyachi H, Honjo T, Nagaoka H. In Vivo Analysis of Aicda Gene Regulation: A Critical Balance between Upstream Enhancers and Intronic Silencers Governs Appropriate Expression. *PLoS One* (2013) **8**:e61433. doi:10.1371/journal.pone.0061433
425. Park S-R, Kim P-H, Lee K-S, Lee S-H, Seo G-Y, Yoo Y-C, Lee J, Casali P. APRIL stimulates NF- κ B-mediated HoxC4 induction for AID expression in mouse B cells. *Cytokine* (2013) **61**:608–613. doi:10.1016/j.cyto.2012.10.018

Titre : Régulation de la voie Akt / mTORC1 par l'activateur transcriptionnel du VIH Tat dans les cellules B

Mots clés : VIH, Akt/ mTOR, Lymphome de cellules B, Tat

Résumé : Les lymphomes agressifs à cellules B sont la principale cause de décès chez les personnes infectées par le VIH-1, bien que les cellules B ne soient pas ciblées par le virus. Les mécanismes exacts de la lymphomagenèse ne sont pas connus. Des études antérieures de notre équipe ont révélé que le HIV-1 Tat peut pénétrer les cellules B, où il peut induire la production de ROS, endommager l'ADN et augmenter les chances de translocations oncogènes spécifiques au lymphome de Burkitt. En outre, dans de nombreuses cellules immunitaires, le VIH-1 et ses protéines (e.g. Tat) peuvent réguler la voie AKT/mTORC1, un intégrateur central de signaux intra et extracellulaires, y compris l'infection virale et les dommages à l'ADN. Cependant, aucune étude n'a examiné la régulation de la voie AKT/mTORC1 par Tat dans les cellules B.

J'ai testé dans cette thèse l'hypothèse selon laquelle Tat pourrait produire des effets oncogènes dans les cellules B en modulant la voie de signalisation AKT/mTORC1 et en régulant l'expression des gènes impliqués dans la lymphomagenèse. J'ai découvert que HIV-1 Tat activait la voie de signalisation AKT/mTORC1, ce qui entraîne une activation aberrante de l'AICDA (cytidine désaminase induite par l'activation) en raison de l'inhibition des répresseurs transcriptionnels c-Myb et E2F8 de l'AICDA. Ces perturbations peuvent finalement conduire à une instabilité génomique accrue et à une prolifération qui pourrait provoquer des malignités des cellules B.

Title : Regulation of the Akt/mTORC1 pathway by HIV transcriptional activator Tat in B cells

Keywords : HIV, Akt/mTOR, B cell lymphoma, Tat

Abstract : Aggressive B cell lymphomas are the main cause of death in HIV-1 infected individuals, although B cells are not targeted by virus. The exact mechanisms of lymphomagenesis are not known. Previous studies of our team revealed that HIV-1 Tat can penetrate B cells, where it can induce ROS production, DNA damage and increase the chances of the oncogenic translocations specific for Burkitt lymphoma. In addition, in many immune cells HIV-1 and its proteins (e.g. Tat) can regulate AKT/mTORC1 pathway, a central integrator of many intra and extracellular signals including viral infection and DNA damage. However, no studies have examined the regulation of AKT/mTORC1 pathway by Tat in B cells.

In this thesis I have tested the hypothesis that HIV-1 Tat might produce oncogenic effects in B cells by modulating AKT/mTORC1 signaling pathway and regulating expression of genes involved in lymphomagenesis. I found that HIV-1 Tat activated AKT/mTORC1 signaling pathway, which leads to aberrant activation of AICDA (activation induced cytidine deaminase) due to inhibition of AICDA transcriptional repressors c-Myb and E2F8. These perturbations may ultimately lead to an increased genomic instability and proliferation that might cause B cell malignancies.