INTRODUCTION

HEPATITIS C VIRUS INFECTION

Since its discovery in 1989, hepatitis C virus (HCV) has been recognized as a major cause of chronic liver disease worldwide.[1] The most recent World Health Organization estimate of the prevalence of HCV infection is 3%, representing approximately 170-200 million people. Although HCV is endemic worldwide, there is geographic variability in its distribution.[2] Countries with the highest prevalence rates are located in Africa and Asia; areas with lower prevalence include the industrialized nations in North America, northern and western Europe, and Australia.[3] In Egypt, the prevalence of HCV is the highest worldwide ranging from 6% to more than 40% with an average of 14.7% among regions and demographic groups (according to the recently published Egyptian Demographic Health Survey in 2009).[4] In populations of blood transfusion recipients over the age of 30, this proportion has been reported to be as high as 73%, and in the general population aged 40–60 years, it can be as high as 55%.[5,6]

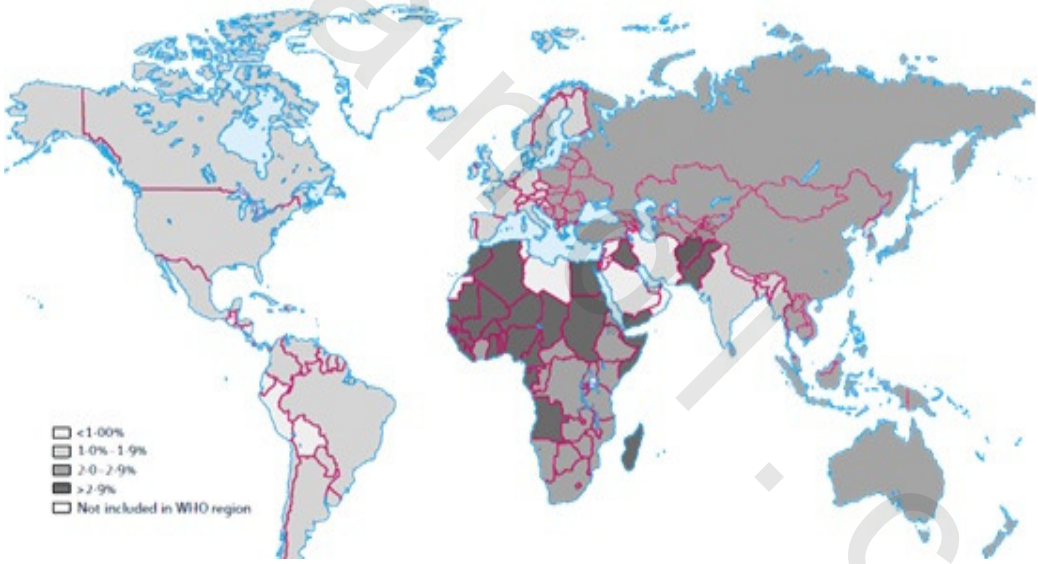

Figure 1 : Estimated prevalence of HCV infection by World Health Organization region.[3]

Molecular biology of HCV:

Hepatitis C virus, a member of the *Flaviviridae* family of the genus *Hepacivirus*, is a hepatotropic, positive-sense, single stranded, ribonucleic acid (RNA) virus. The HCV genome contains a single open reading frame with the potential to encode a protein of 3000 amino acids in length. This open reading frame is flanked by 5' and 3' untranslated regions (URT), each of which contains conserved RNA structures essential for the translation of virus protein and genome replication.[7] The HCV polyprotein is co- and post translationally processed by cellular and virally encoded proteases to produce at least 10 polypeptides

including the mature structural (Core, E1, E2, and p7) and non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Figure 2).[8] In addition to their role in regulation of viral life cycle and replication, HCV proteins interact with many of host cell factors and affect a wide range of cellular activities including cell signaling, transcriptional modulation, transformation, apoptosis, membrane rearrangements, vesicular trafficking, and translational regulation.[9]

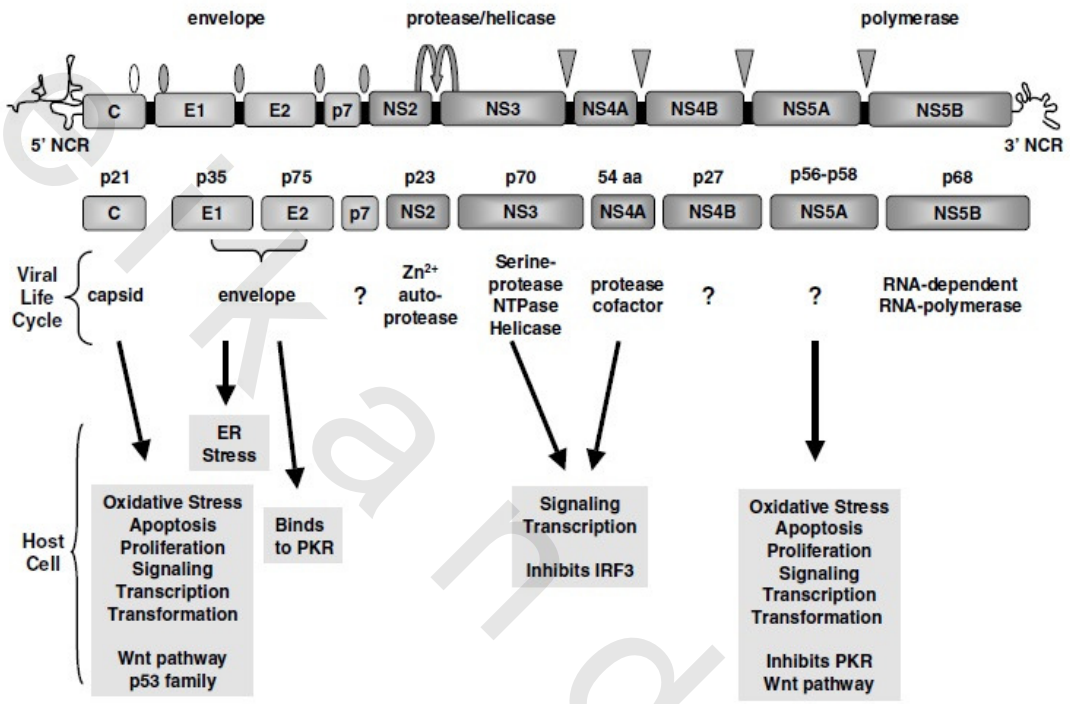

Figure 2: Hepatitis C virus (HCV) genome and proteins coded by its RNA.[15]

HCV proteins:

The first structural protein from the N-terminus of the polyprotein, is ***the core protein***. It constitutes the virion nucleocapsid and most likely interacts with the viral RNA. The full-length core protein has been shown to localize on the cytoplasmic side of the endoplasmic reticulum (ER), but some of its truncated forms have been found in the nucleus.[10] Core protein has been extensively studied and appears to play multiple roles in various cellular signaling pathways, and potentially in viral oncongenesis.[11] The next two proteins are ***the envelope glycoproteins E1 and E2***. They are essential for virus entry and in the assembly of infectious particles. The E2 protein mediates viral binding to the cells, as shown by a decrease of infectivity by incubation of the virus with anti-E2 antibodies. The E1 and E2 contain both an ER retention signal, which limits their intracellular localization to the ER.[12,13] The E2 contains two hypervariable regions (HVR), HVR1 and HVR2, which are under constant selection for mutation probably because they are targets for neutralizing antibodies. The genetic heterogeneity of the HVR1 may enable virus to evade

the immune system and facilitate establishment of chronic infection.[14] The ***p7*** is located between HCV E2 and NS2 genes in the ER. It belongs to a family of viral proteins called viroporins that form ion channels required for the production of infectious virus particles.[15]

The ***NS proteins*** have various functions involved in viral RNA replication or proteolytic processing of the polyprotein.[16] The ***NS2*** and ***NS3*** are the two viral proteases responsible for the cleavage of all the NS proteins. Furthermore, NS3 has a helicase and an NTPase activity, suggesting that it plays a role in RNA replication as well.[17,18] Also, NS3 serine protease influences the innate cellular host defense.[19] The ***NS4A*** is a cofactor for NS3, with which it forms a heterodimer.[20] The ***NS4B*** is an integral ER membrane protein. Its function is not yet known, but it may play a role in the anchorage of the replication complex to membrane as observed for the replication of other RNA viruses.[21] The ***NS5A*** is a hydrophilic phosphoprotein, which has RNA-binding activity and plays an important role in viral replication. This protein contains a region called the interferon (IFN)-α sensitivity-determining region, which confers resistance of the virus to IFN treatment.[22,23] Finally, ***NS5B*** is the viral RNA-dependent RNA polymerase (RdRp). It can copy a full-length HCV genomic RNA. Since NS5B lacks a "proofreading" function, complex mutant swarms are generated due to a high rate of error-prone replication.[24]

HCV genotypes and quasispecies:

Comparisons of HCV nucleotide sequences derived from individuals from different geographical regions revealed the presence of six major ***HCV genotypes*** with a large number of subtypes within each genotype. Sequence divergence of genotypes and subtypes is 20% and 30% respectively. HCV strains belonging to the major genotypes 1, 2, 4, and 5 are found in sub-Saharan Africa whereas genotypes 3 and 6 are detected with extremely high diversity in South East Asia.[25] In Egypt genotype 4 represent 90% of cases.[26] Besides epidemiological aspects, determination of the HCV genotype plays an important role for the initiation of anti-HCV treatment since the response of different genotypes varies significantly with regard to specific antiviral drug regimens, e.g., genotype 1 is most resistant to the current therapy of the combination of pegylated IFN-α and ribavirin.[27]

One of the important characteristics of HCV is that its genome exhibits significant genetic heterogeneity as a result of the accumulation of mutations during viral replication.[28] This high mutation rate can be attributed to an error-prone RdRp that lacks proofreading activity.[24] It has been shown that, like many other RNA viruses, HCV circulates in an infected individual as a population of closely related, yet heterogeneous, sequences: referred to as ***quasispecies***.[29] The high mutation rate of RNA viruses contributes to their rapid evolution, changes in virulence and development of resistance to antiviral agents.[30]

HCV life cycle:

Adsorption of HCV to its target cell is the first step of viral entry.[31] Binding is possibly initiated by the interaction of the HCV E2 envelope glycoprotein and the host cell

receptors including the tetraspanin cluster of differentiation (CD)81, low-density lipoprotein receptor, scavenger receptor class B type I, glycosaminglycan heparan sulfate, members of the claudin family and mannose-binding lectins.[31-33] After binding different host cell factors, HCV enters the cell in a pH-dependent manner indicating that the virus is internalized via clathrin-mediated endocytosis.[34] Several cellular as well as viral factors have been reported to be part of the HCV RNA replication complex. One important viral factor for the formation of the replication complex appears to be NS4B, which is able to induce an ER-derived membranous web containing most of the nonstructural HCV proteins including NS5B.[35] The NS5B RdRp uses the previously released genomic positive-stranded HCV RNA as a template for the synthesis of an intermediate minus-stranded RNA. After the viral polymerase has bound to its template, the NS3 helicase is assumed to unwind putative secondary structures of the template RNA in order to facilitate the synthesis of minus-strand RNA.[24] In turn, again with the assistance of the NS3 helicase, the newly synthesized antisense RNA molecule serves as the template for the synthesis of numerous plus-stranded RNA.[18] The resulting positive sense RNA may be used subsequently as genomic RNA for HCV progeny as well as for polyprotein translation. After the viral proteins and the genomic HCV RNA have been synthesized, viral assembly takes place within the ER and lipid droplets are involved in particle formation through its association with core protein and NS5A.[36] Nucleocapsids are enveloped and matured into the Golgi apparatus before newly produced virions are released in the pericellular space by exocytosis (Figure 3).[37]

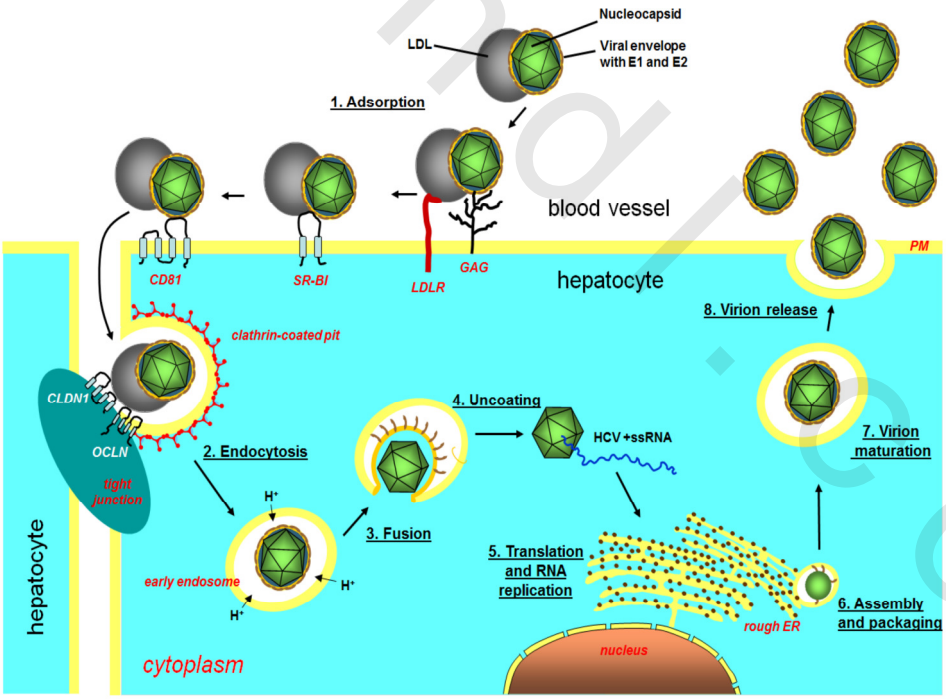

Figure 3: Current model of the HCV life cycle.[37]

HEPATITIS C VIRUS-RELATED CHRONIC LIVER DISEASE

Because HCV infection is rarely cleared in the acute phase of infection, most patients develop chronic hepatitis which eventually progress to liver cirrhosis and hepatocellular carcinoma (HCC).[38] Acute HCV infection is often asymptomatic in 70-80% of cases, making it a very difficult to detect at an early stage.[39] About 60–80% of HCV-infected patients develop disease chronicity. HCV-related chronic liver disease often progresses and becomes more severe with the time, although most patients remain asymptomatic for 10-30 years, even though they have persistently elevated alanine aminotransferase (ALT) levels and moderate or severe liver disease. This is why chronic HCV is named the "*silent epidemic*".[1] Approximately 10–20% of patients with chronic hepatitis C (CHC) develop cirrhosis over a time period of 20–30 years; of these, 6% will decompensate to end-stage liver disease and an additional 1-4% will develop HCC per year (Figure 4).[40]

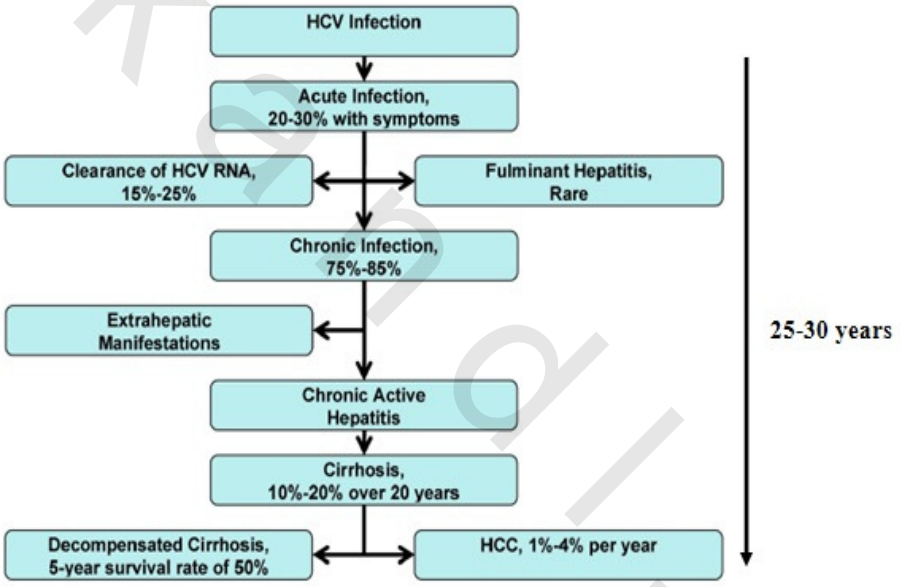

Figure 4: Natural history of hepatitis C virus.[1]

Chronic hepatitis C:

Chronic hepatitis C develops in the majority of persons with acute HCV infection. Patients who develop chronicity are less likely to have symptoms and jaundice during acute infection than those with resolving acute hepatitis C.[41] Probably the evolution of quasispecies during the acute phase has a relationship with chronicity.[42] The diagnosis of CHC is marked by the persistence of HCV RNA in the blood for at least 6 months after onset of acute infection.[43] Serum ALT levels are usually continuously or intermittently elevated, but the height of elevations correlates poorly with disease activity. However, up to one third of infected persons have persistently normal ALT levels when tested serially

over a 6-month observation period and may show histologic evidence of minimal or mild chronic inflammation in the majority of cases.[44] The apparent discordance between ALT levels and liver injury may be related to the proportion of liver cell damage and death due to apoptosis (which limits the inflammatory response) and necrosis (which promotes the inflammatory response).[45]

Most patients with CHC are asymptomatic or have only mild non-specific symptoms. The most frequent complaint is chronic fatigue. Less common manifestations are nausea, weakness, myalgia, arthralgia, right upper quadrant pain, poor appetite and weight loss.[40] Around 30 to 40% of patients with CHC have an extra-hepatic manifestations of HCV including hematologic manifestations (essential mixed cryoglobulinemia, lymphoma), autoimmune disorders (thyroiditis, presence of various autoantibodies), renal disease (membranoproliferative glomerulonephritis), dermatologic disease (porphyria cutaneatarda, lichen planus), diabetes mellitus and sicca syndrome.[46]

Almost all patients with chronic HCV infection have some degree of necroinflammatory disease on liver biopsy, but the severity of disease (activity or "grade") and the amount of structural damage (fibrosis or "stage") varies considerably.[47] One of the few validated scoring systems is called the METAVIR scoring system.[118] This system assesses histological lesions in CHC using two separate scores, one for necroinflammatory grade (A for activity from A0 to A3) and another for the stage of fibrosis (F from F0 to F4 cirrhosis).[48] HCV-associated chronic inflammation seems to result from the induction of the pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 [49] and chemokines including CXCL10, CXCL9, CXCL11, and CCL5 produced by hepatocytes, sinusoidal endothelial cells and biliary epithelium.[50] Another common histological feature of liver biopsies from HCV-infected individuals is hepatic steatosis, which is independently associated with fibrosis severity.[51] In genotype 3 infection, the presence of steatosis correlates with intrahepatic viral replication, is commonly present in non-obese patients and resolves with successful viral clearance, implying it is a direct consequence of the virus itself.[52] In contrast, in non-genotype 3 infections, steatosis is highly correlated with obesity and insulin resistance and is unresponsive to antiviral therapy.[53]

HCV-related liver fibrosis and cirrhosis:

Chronic HCV infection is a major risk factor for development of liver fibrosis culminating in cirrhosis.[54] Chronic infection with HCV typically induces injury and inflammation of the liver, which appear to be responsible for the associated fibrogenesis.[55] During the course of HCV infection, many consequences that evolve secondary to liver injury, inflammation, oxidative stress, steatosis and necrosis, appear to be responsible for the associated fibrogenesis and the progressive replacement of the hepatocytes by abundant extracellular matrix (ECM).[56] Although fibrosis stage and inflammatory grade are correlated, there is discordance in approximately one-third of patients. In fact, fibrosis alone is the best marker of ongoing fibrogenesis.[57] Fibrosis progression in patients with CHC is a dynamic process, occurring throughout a period of one or more decades, subject to changes emerging from the interplay of cell matrix interface, which in essence results in

the deposition of fibrotic tissue.[58] The fibrosis development rate observed (direct) is defined as the ratio of the fibrosis stages difference between two biopsies expressed as METAVIR units and the interval between these two biopsies in years. The estimated fibrosis development rate (indirect) is defined as the ratio between fibrosis stage in METAVIR units and the estimated infection duration in years.[54] The distribution of fibrosis progression rates suggests the presence of at least three populations: a population of '*rapid* fibrosers', a population of '*intermediate* fibrosers' and a population of '*slow* fibrosers'. These discrepancies reflect the great heterogenecity of HCV infection as to its severity and outcome and the many factors that can influence its course and progression.[57,59] Time to fibrosis progression may be shorter in the older patients and in the presence of co-factors like alcohol or metabolic abnormalities leading to accumulation of steatosis in the liver.[60]

Approximately one fifth (20–30%) of patients with CHC develop cirrhosis over a time period of 20-30 years.[61] The annual rate of developing cirrhosis is very variable, ranging from 0 to 8%.[62] Patterns of progression to cirrhosis are very different: *fast* if it occurs in less than 20 years, *intermediate* in 20-50 years, *slow* in more than 50 years; moreover, in some patients there is no progression at all.[60] The progression to cirrhosis is often clinically silent, and some patients are not known to have CHC until they present with the complications of end-stage liver disease or HCC. The annual rate of decompensation among patients with cirrhosis is estimated to be about 4%.[63] While in most patients the first decompensation consists of a single complication (i.e. ascites), other present more than one complication at their initial decompensation including upper gastrointestinal bleeding secondary to varices or portal hypertensive gastropathy, hepatorenal syndrome and hepatic encephalopathy.[64] The prognosis of decompensated HCV-related cirrhosis is poor, with a 5-year survival rate of only 50%.[65]

HCV-related hepatocellular carcinoma:

Chronic HCV infection is a major risk factor for development of HCC.[66] The oncogenic process of HCV infection is slow and insidious and probably requires multiple steps of genetic and epigenetic alterations, the activation of cellular oncogenes, the inactivation of tumor suppressor genes, and dysregulation of multiple signal transduction pathways.[67] The annual HCC development rates range between 1-4% in cirrhotic patients with HCV infection and almost a quarter of chronically-infected patients develop HCC within 30 years.[66] In a meta-analysis of 21 case-control studies, the risk for HCC was increased 17-fold in HCV-infected patients compared to HCV-negative controls.[68] Development of HCC is clearly the main cause of death in patients with HCV-related cirrhosis.[69] Virtually most HCV-related HCCs occur among patients with cirrhosis.[70] Chronic inflammation, necrosis, regeneration and cirrhosis enhance mutagenesis in regenerating hepatocytes, the accumulation of which culminates in HCC. However, cases of HCC in patients with chronic hepatitis without cirrhosis have been reported but remains very scarce.[71] The rare occurrence of HCC in non-cirrhotic liver has directed the attention towards the possibility of a direct role of the virus in the pathogenesis of HCC. Although HCV is unable to reverse transcribe its genome and to integrate it into the host genome, viral proteins and their evoked host responses contribute mostly to the viral oncogenic

processes where they are involved in a wide range of activities including cell signaling, transcription, cell proliferation, apoptosis, oxidative stress, membrane rearrangements, vesicular trafficking and translational regulation.[72]

Factors influencing the evolution of chronic HCV infection:

The rate and speed of progression of HCV-related liver disease varies markedly from individual to individual and are strongly influenced by a number of co-factors.[57,60,68] Factors related to host and environment/lifestyle appears to be more important than viral factors in determining progression to cirrhosis.[73] These factors include older age at the time of acquisition of infection, male gender,[74] heavy alcohol intake (>50g/day),[75] non-alcoholic steatohepatitis (NASH), hyper-insulinemia/insulin resistance,[76] obesity,[77] and coinfection with human immunodeficiency virus (HIV) [78] or hepatitis B virus (HBV).[79] There is no strong evidence that HCV viral factors like genotype, viral load, or quasispecies are important in determining the risk of progression to cirrhosis or HCC.[80,81] Recently, polymorphism within the IL-28B region is more prevalent in patients with viral cirrhosis due to HCV in comparison to other etiologies.[82]

Immune evasion in chronic HCV infection:

The mechanisms that determine the outcome of HCV infection are not well understood, although it is widely assumed that immune responses play an important role.[83] Failure to generate sufficiently effective immune responses during the acute phase of infection is considered a key factor in developing chronic HCV infection. In a majority of HCV-infected patients, virus infection does not resolve naturally and the virus mutates to escape immune surveillance.[84] HCV appears to use several escape mechanisms to subvert the host immune response against the virus and it is likely that several different mechanisms are operational.[85] Several HCV structural and nonstructural proteins have been shown to interfere with the innate immune response at different levels. HCV-derived proteins are involved in the suppression of the IFN signaling pathways and IFN stimulated genes expression by blocking toll-like receptor (TLR) 3 and retinoic acid–inducible gene I signaling.[86] Also, HCV proteins inhibit dendritic cell (DC) maturation and functions.[87] In chronic HCV infection, the frequency of plasmacytoid DCs in the blood, and their ability to produce IFN-α are reduced while the ability of myeloid DC to stimulate allogenic T-lymphocytes is reduced despite no reduction in overall frequency of these cells.[88] Alternatively, HCV may directly suppress the function of natural killer (NK) cells and contribute to virus persistence.[89] HCV envelope protein E2 exerts an inhibitory effect on NK cells through engagement of CD81, thereby inhibiting cytotoxicity and IFN-γ production by NK cells.[90] Also, DCs inhibit NK cell activation in HCV infection.[91]

Moreover, HCV escape from adaptive immune response through several different mechanisms such as mutational escape and functional anergy of virus specific T cells.[85,92] Mutational changes in virus particle are due to lack of proofreading activity of RdRp and high replication rate such as 10^{12} virions per day.[24] Sequence changes in the hyper-variable region of the E2 envelope glycoprotein result in escape from B cell epitopes.[14] Naturally-acquired HCV-specific antibodies fail to neutralize HCV infection in human due

to the appearance of escape mutants that cannot be efficiently recognized and controlled by the immune system.[93] In addition, weak cellular immune responses in HCV infection such as $CD4^+$ T helper (Th) and $CD8^+$ cytotoxic T lymphocytes (CTLs) result in poor controlled viremia and HCV persistence.[94] In CHC patients, HCV-specific $CD4^+$ T cells are functionally impaired and their responses are weak, narrowly selected and short-lived, which is in clear contrast with resolved cases.[95] Also, HCV-specific $CD8^+$ CTLs may contribute to progressive liver disease with an inability to produce IFN-γ and to proliferate in response to HCV antigens in patients who have been chronically infected.[96] HCV mutations affect virus specific $CD8^+$ cell responses by decreasing binding affinity between epitope and major histocompatibility complex (MHC) molecule through decreasing T cell receptor (TCR) recognition and impairing proteosomal processing of HCV antigens.[92,97] Another important possible mechanism of immune evasion is functional anergy of virus-specific T cells. HCV-specific $CD8^+$ cells may be impaired in their proliferative capacity, cytotoxicity, and ability to secrete TNF-α and IFN-λ.[98] Moreover, the reduced DC numbers and activity might be responsible for the impaired Th1 polarization in chronic HCV infection.[99] A mutation of one amino acid within the epitope of the NS3 region recognized by Th1 cells results in a shift in cytokine secretion patterns from Th1 to Th2 cells leading to decreased antiviral responses.[100] Moreover, the HCV core-specific T regulatory (Treg) cells are induced in patients with CHC and IL-10 produced by these cells suppresses T-cell function directly leading to persistence of HCV infection.[101]

In chronic HCV infection, prolonged liver cell damage, fibrosis development, and impaired antiviral immunity can result in deregulation of multiple signaling pathways, which coordinate host cell environment for virus persistence and pathogenesis.[70]

TARGET OF RAPAMYCIN

Target of rapamycin (TOR) was identified in the mid-1990s shortly after the discovery of the two yeast genes, TOR1 and TOR2, in the budding yeast *Saccharomyces cerevisiae* during a screen for resistance to the immunosuppressant drug rapamycin, a TOR inhibitor.[102] Like its yeast counterpart, the TOR in mammalian cells has been identified and cloned.[103,104] The mammalian TOR (mTOR), was initially referred to as RAPT 1 (rapamycin target 1), RAFT (rapamycin and FKBP12 target), SEP (sirolimus effector protein), FRAP (FK506 binding protein 12-rapamycin associated protein) and recently described as the mechanistic TOR.[103-107]

Structure of mTOR:

The mTOR, a 289-kDa (2549 AA) evolutionarily conserved serine/threonine protein kinase, is a member the phosphoinositol 3-kinase (PI3K)-related kinase family and is encoded by *FRAP1* gene. Structurally, the domains found in mTOR, in order from the N to the C terminus of mTOR, compose the so-called HEAT repeats, the FAT domain, the FRB domain, the kinase domain, and the FATC domain (Figure 5).[108] The HEAT repeats occupy the N-terminal half of TOR and consist of ~20 HEAT motifs, each of which is ~40 amino acids that form a pair of interacting antiparallel α-helices and play a role in protein-protein interactions.[109] The central FAT domain (~500 residues) and the extreme C-terminal FATC domain (~35 residues) are always paired and modulate catalytic kinase activity.[110,111] Rapamycin, together with its cellular receptor, 12-kDa FK506-binding protein (FKBP12), binds to the FRB domain (FKBP12-rapamycin binding domain), ~100 residues, located between the FAT and kinase catalytic domains on mTOR and thus allosterically inhibits the activity of mTOR.[112]

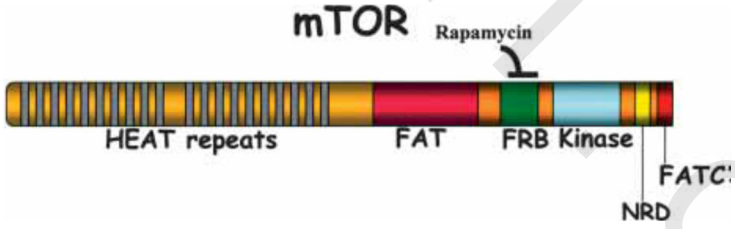

Figure 5*:* The primary structure of mammalian target of rapamycin (mTOR).[113]

Physically and functionally, mTOR forms two large distinct complexes, called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[114] Structurally, mTORC1 has five components: mTOR which is the catalytic subunit of the complex; the regulatory-associated protein of mTOR (Raptor); mammalian lethal with Sec13 protein 8 [mLST8, also known as G protein β-subunit-like protein (GβL)]; proline-rich AKT substrate of 40 kDa (PRAS40); and the DEP-domain-containing mTOR-interacting protein (Deptor).[115] On the other hand, the mTORC2 comprises six different proteins, several of which are common to the mTORC1 and mTORC2: mTOR; rapamycin-insensitive

companion of mTOR (Rictor); mammalian stress-activated protein kinase interacting protein; protein observed with Rictor 1 and 2 (Protor1/2); mLST8/GβL; and Deptor (Figure 6). There is some evidence that Rictor and mSIN1 stabilize each other, establishing the structural foundation of mTORC2.[116] Both mTORC1 and mTORC2, and most likely TORC1 and TORC2 in general, function as multimers. The structural components of both these TORCs are highly conserved from yeast to mammals.[114] TORC1 is concentrated on the limiting membrane of the cell vacuole; a major nutrient reservoir and TORC1 signaling is responsive to nutrient cues (see below).[117] On the other hand, TORC2 is located at or near the plasma membrane. A plasma membrane location is consistent with the role of TORC2 in controlling the actin cytoskeleton and endocytosis.[118] A prominent difference between these two complexes is their rapamycin sensitivity. TORC1 is sensitive to rapamycin treatment, whereas TORC2 is resistant to rapamycin treatment. However, mTORC2 is sensitive to long-term (>24 h) rapamycin treatment, and this sensitivity is most likely achieved through inhibition of nascent mTOR molecules from assembling with mTORC2 components such as Rictor and mSin1.[119]

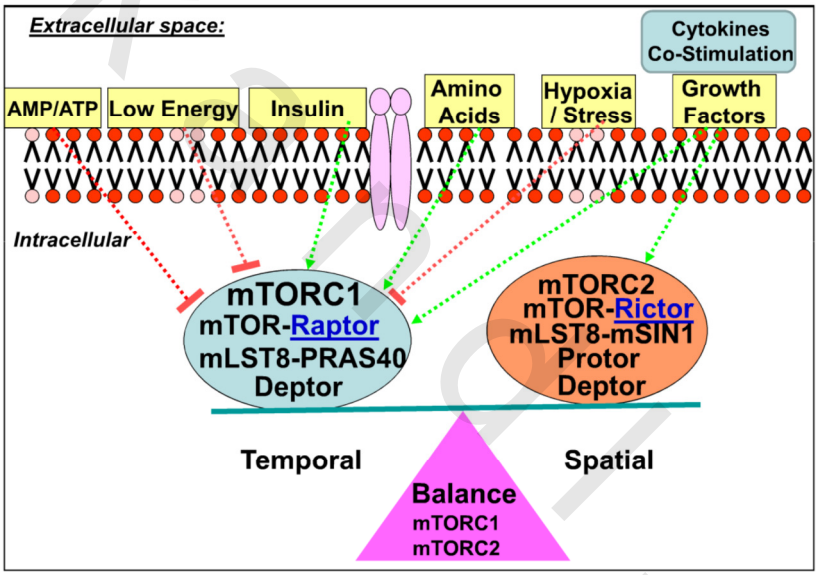

Figure 6: The mammalian target of rapamycin (mTOR) complexes, mTORC1 and mTORC2 and their signals from diverse extracellular inputs.[107]

The upstream of mTOR:

Because mTOR is involved in essentially all aspects of cellular activities owing to its fundamental ability to sense and respond to nutrients, the regulatory mechanisms of TOR must be tightly controlled by multiple mechanisms while allowing the necessary sharp and robust responses to even subtle or conflicting environmental conditions.[120] Intense efforts have revealed many mTOR regulatory proteins across a complex network of positive and negative regulatory mechanisms.[113,120] The mTOR pathway can be regulated by diverse exogenous stimuli, such as nutrients, energy, stress signals, growth factors, cytokines and signaling pathways, such as PI3K and AMPK (Adenosine-monophosphate-activated protein kinase), in order to regulate several physiological events (Figure 6).[107,120]

While mTORC1 is a nutrient-sensitive pathway, mTORC2 receives inputs only from growth factors, however mTORC2 upstream regulation is largely unknown.[107] The Tuberous Sclerosis Complexes (TSC1 and TSC2) have been identified as upstream negative regulators of mTOR. TSC1/TSC2 complex is negatively regulated by the serine/threonine kinase PKB/AKT downstream of the insulin-signaling pathway.[121] The mTOR can be auto-phosphorylated via a feedback loop from interaction with its substrates, 4EB and S6K.[197-199]

Control by nutrients:

The molecular mechanisms by which TOR proteins sense nutrient availability became more evident.[122] Raptor and mTOR are likely to comprise a nutrient-sensitive mTOR complex, whereby mLST8/GβL regulates the stability of the mTOR–Raptor association under different nutrient conditions. Upon nutrient deprivation, the mTOR–mLST8/GβL–Raptor complex precludes mTOR from access and/or binding avidly to its substrates. Conversely, in the presence of nutrients, a conformational change disrupts Raptor/ mLST8/GβL interaction and enables the accessibility of mTOR (or an associated kinase) to its targets, which are bound to Raptor (Figure 7).[113,123,124] Moreover, amino acids activate the Rag guanosine triphosphatases (GTPases), which interact with small complex proteins collectively known as "Ragulators" that facilitate docking of Rag to the lysosomal surface. This association in turn promotes the localization of mTOR and Rheb (Ras homolog enriched in the brain) to the lysosomes in response to amino acids.[125,126] Among environmental cues, nutrients, especially amino acids, are the most fundamental inputs affecting TORC1 activity.[122]

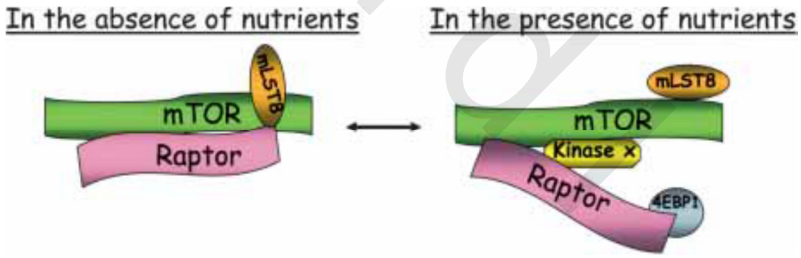

Figure 7: A model of how the mTOR–Raptor interaction may regulate mTOR activity in response to nutrients.[113]

Control by energy status and stress conditions:

The mTORC1 activity is inhibited under a broad array of stressful conditions including cellular energy depletion, deoxyribonucleic acid (DNA) damage and hypoxia.[127] In response to energy deprivation (low adenosine triphosphate (ATP) level), AMPK is activated and inhibits mTORC1 through the phosphorylation of TSC2 and via the association of Raptor with 14-3-3 protein and inactivation of mTORC1 kinase activity.[128] Also, DNA damage inhibits mTORC1 through upregulating negative regulators of mTORC1 including phosphatase and tensin homolog (PTEN), AMPK and TSC2 by p53.[129] Hypoxia has also an inhibitory effect on mTORC1 activity through hypoxia-inducible transcription factor-1 (HIF-1) or through the induction of energy stress.[130] In

mammalian cells, mTORC1 is also sequestered into oxidative stress- and osmotic stress-induced stress granules and inactivated.[131] It seems that the repression of mTORC1 under stressful conditions, is important to stop cell growth and to shift cell physiology towards inducing cytoprotective programs.[127]

Control by PI3K/Akt pathway:

It is well accepted that PI3K pathway is a genuine upstream positive regulator of mTOR.[132] Akt, a serine/threonine kinase, is the central mediator of the PI3K pathway with multiple downstream effectors that influence key cellular processes. Akt stimulates protein synthesis and cell growth by activating mTOR (as part of the mTOR-raptor or mTORC1 complex) through effects on the intermediary TSC1/TSC2 complex.[133] PTEN, a tumor suppressor gene and protein, is a phosphatidylinositol-3 phosphatase that counteracts PI3K activity by dephosphorylating phosphatidylinositol-3,4-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-triphosphate (PIP3) that are generated by PI3K, leading to negative impact on mTOR activity (Figure 8).[134]

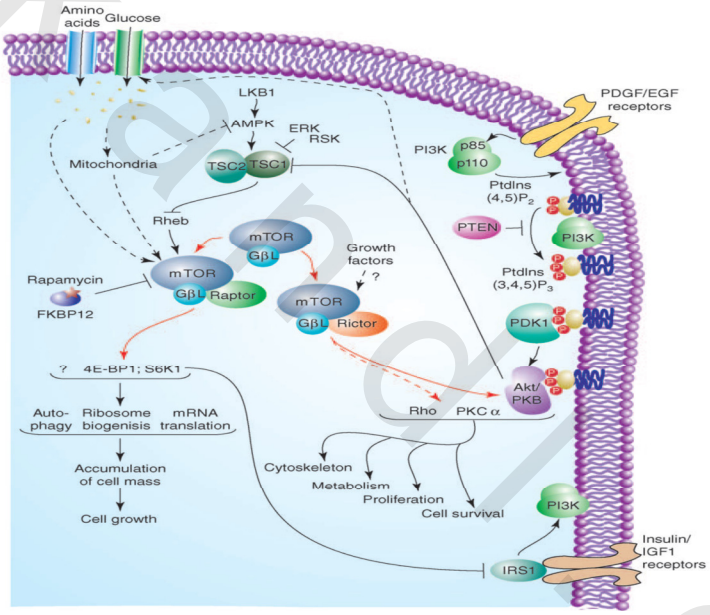

Figure 8*: A model of the upstream and downstream of mTOR.*[135]

Control by growth factors and cytokines:

The activity of mTOR is also regulated by growth factors such as insulin, insulin-like growth factor (IGF)-1 and platelet-derived growth factor (PDGF), that act in parallel or in concert with nutrients.[136] There are at least two mechanisms leading to activation of mTORC1 by growth factors: the TSC-dependent and TSC-independent pathways. Stimulation by insulin and other growth factors leads to activation of the PI3K-Akt pathway, and then activated Akt phosphorylates and inhibits TSC2, leading to activation of Rheb that acts as a scaffold and mediates the binding of mTORC1 to its substrates.[137] In the TSC1/2-independent pathway of mTORC1 activation, upon stimulation by growth

factors, Akt phosphorylates and inactivates PRAS40, the inhibitory component of mTORC1, leading to mTORC1 activation.[138] Although the mechanism of mTORC2 regulation is poorly defined, ribosome has been reported to be a direct activator of mTORC2 in response to insulin. Insulin promotes binding of the ribosome to mTORC2 and this interaction leads to activation of mTORC2. Because mTORC1 is a primary regulator of ribosome biogenesis, activation status of mTORC1 may coordinately control mTORC2 activation to achieve appropriate cell growth.[139]

Moreover, cytokines, such as TNF-α can also activate mTORC1. It has been described that IKKβ (inhibitor of nuclear factor κB kinase β), a major downstream kinase in the TNF-α signaling pathway, phosphorylates TSC1 leading to the inhibition of TSC1/TSC2 complex formation and mTORC1 activation.[140,141] Also, immunologically germane cytokines including CD28 and IL-2 and IL-4 have been shown to activate mTOR via PI3K/AKT upregulation.[142] Additionally, upon activation of mTOR, IL-12 and IFN-γ prolong this activation state in memory $CD8^+$ cells.[143]

The downstream of mTOR:

It is currently known that mTOR phosphorylates two well characterized downstream targets, S6 Kinase 1 (S6K1) and eukaryotic initiation factor 4E (eIF-4E)-binding protein 1 (4E-BP1), positive and negative regulators of protein synthesis respectively (Figure 8). The S6K1 or 4E-BP1 phosphorylation is often used as an in vivo readout of mTOR activity.[144] Raptor appears to serve as an adaptor protein that recruits mTOR substrates and is necessary for their phosphorylation.[145] It binds S6K1 and 4E-BP1 via a 5 amino acid motif termed TOS (TOR signaling) that is present in the N terminus of S6K1 and in the *C* terminus of 4E-BP1.[146] Rapamycin disrupts the mTOR–Raptor interaction, thereby preventing the ability of mTOR to phosphorylate S6K and 4E-BP1.[147]

The phosphorylation of S6K1 at Thr389 by mTOR is required for its activation.[144] Activated S6K1 then activates S6 (40S ribosomal protein S6), enhancing the translation of messenger ribonucleic acids (mRNAs).[148] The targets of S6K1 are ribosomal proteins, elongation factors, and IGF-2. A large body of evidence implicates S6K1 in the control of cell growth via increased mRNA translation.[149] On the other hand, the 4E-BP1 inhibits the process of protein translation by binding and inactivating eIF4E. The mTOR phosphorylates 4E-BP1 at multiple sites to promote the dissociation of eIF4E from 4E-BP1, relieving the inhibitory effect of 4E-BP1 on eIF4E-dependent translation initiation. Free eIF4E can form the multi-subunit eIF4F complex binding to eIF4G, eIF4A and eIF4B, enabling cap-dependent protein translation, and inducing increased translation of mRNAs with regulatory elements in the 5'-UTR of its downstream target genes (e.g., c-myc, ornithine decarboxylase and cyclin D1), which are required for G1-to-S phase transition.[150] Differently, in quiescent cells or under low growth factors levels, unphosphorylated 4E-BP1 binds to eIF4E, inhibiting the initiation of protein translation.[151] Interestingly, the mTOR can be auto-phosphorylated via a feedback loop from interaction with its substrates, S6K1 and 4E-BP1.[144] Moreover, the mTORC1 is also involved in the regulation of other proteins including cytoplasm linker protein-170, eukaryotic elongation factor 2 kinase, ornithine decarboxylase, glycogen synthase, HIF-1α, lipin, protein

phosphatase 2A, retinoblastoma protein, and signal transducer and activator of transcription 3 (STAT3).[152-160]

Additionally, mTORC2 phosphorylates several AGC family kinases on their hydrophobic motifs, including Akt, SGK1 and protein kinase C through which mTORC2 regulates a variety of cellular processes such as cell survival, proliferation and actin reorganization [161,162] The mTORC2 also regulates the activity of GTPases related to cell survival, migration and regulation of the actin cytoskeleton.[163] Moreover, mTORC2 activation inhibits two proteins namely, Forkhead Factor 1 and Kruppell-like factor 2, two transcription factors required for maintaining the T cell quiescent state. Hence, mTORC2 and mTORC1 have different physiological functions.[164]

Biological functions of the mTOR pathway:

The mTOR signaling pathway senses and integrates a variety of environmental cues to regulate many major cellular processes including cell growth, survival, and metabolism. When growth conditions are favorable, mTOR signaling is activated and controls transcription of many genes, some of which are involved in metabolic and biosynthetic pathways and regulates nutrient-responsive transcription programs.[165] On the other hand, nutrient deprivation, stress, or mTOR inhibition by rapamycin, lead to a starvation-like response; suggesting the biological role of mTOR in integrating nutritional signals.[122] Deregulation of the mTOR pathway has been implicated in an increasing number of pathological conditions, including cancer, obesity, type 2 diabetes, neurodegeneration, cardiovascular diseases, autosomal recessive polycystic kidney and transplantation.[166-174]

mTOR and cell metabolism:

Among the plethora of functions regulated by mTOR, a pivotal role is on cellular metabolism. It controls many aspects of cellular metabolism, such as amino acid biosynthesis and glucose homeostasis and lipid metabolism.[175] The mTOR has emerged as a key integrator of both *anabolic* and *catabolic* processes to make an appropriate decision of cell growth in response to environmental cues.[176] The mTOR proteins regulate the balance between protein synthesis and protein degradation. mTOR signaling is active in the presence of sufficient nutrients to fuel protein synthesis and allows for mRNA translation, ribosome biogenesis at multiple levels, including transcription, ribosomal ribonucleic acid (rRNA) processing, and translation, and the stabilization of high affinity amino acid permeases.[177,178] At the same time, TOR signaling destabilizes general amino acid permeases and represses the transcription of a subset of genes required for amino acid biosynthesis (Figure 9).[108,179] Also, mTOR activation suppresses the nutrient-recycling process known as autophagy as will be discussed later.[180] Moreover, mTOR serves as an integrative regulator of genes required for *de novo* biosynthesis of glutamine and glutamate.[181] mTOR controls protein synthesis through phosphorylation and activation of S6K1, which enhances the translation of mRNA and rRNA expression and through the direct phosphorylation and inactivation of 4E-BP1, the repressor of mRNA translation.[144]

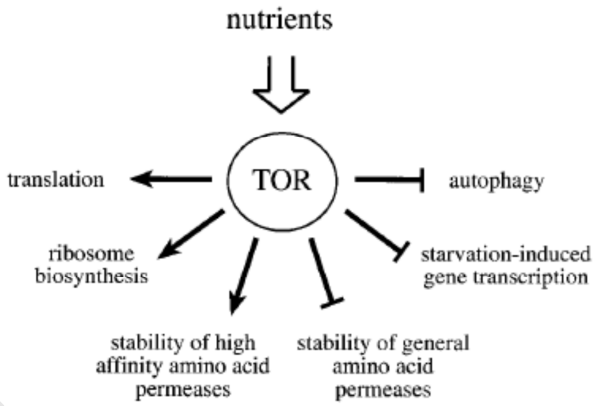

Figure 9: The mTOR proteins regulate the balance between protein synthesis and protein degradation.[108]

Although the biochemical link between mTOR signal transduction and glucose homeostasis remains unclear, it has been found that mTORC1 regulates the transcription of genes encoding glycolytic enzymes and glucose transporters through the translation of HIF1α, promoting glucose uptake and activation of glycolysis to generate energy.[182] It has been shown that mTOR inhibition with rapamycin induces a diabetes-like syndrome characterized by severe glucose intolerance, hyperinsulinemia and hypertriglyceridemia, which are attributed to increased hepatic glucose production as well as reduced skeletal muscle glucose uptake and adipose tissue peroxisome proliferator-activated receptor-γ (PPARγ) activity.[183] Also, mTOR interacts with glycogen synthase kinase to increase DNA synthesis [184] and regulate glucose-6-phosphate dehydrogenase (G6PDH) activity. G6PDH catalyzes the irreversible oxidation of G6P to 6-phosphogluconolactone, the rate-limiting reaction in the pentose phosphate pathway, which generates ribose for RNA and DNA synthesis as well as fatty acid synthesis.[185]

Also, mTOR plays an important role in lipids metabolism. It is believed that mTORC1 is an important novel controller of both anabolic and catabolic lipid metabolism by regulating lipogenesis and lipolysis, respectively.[186] The mTORC1 signaling induces adipogenic differentiation and maintains the adipogenic program by promoting the activation state of PPARγ, a nuclear hormone receptor that induces the expression of genes which promote fatty acid uptake, synthesis, esterification, and storage.[187] Moreover, mTORC1 activates the transcription factor sterol regulatory element binding protein-1 (SREBP-1), a major transcription factor that controls fatty acid, cholesterol, and triglyceride synthesis,[188] as well as activation of stearoyl CoA desaturase, a key enzyme in fatty acid metabolism required for double bond formation.[189] mTORC1 could promote SREBP-1c activation through S6K1 and phosphorylation of lipin 1, a phosphatidic acid phosphatase,[190] Additionally, mTORC1 signaling inhibits triacylglycerol lipolysis suggesting the importance of mTOR in blocking catabolic pathways.[187] Meanwhile. It has been suggested that mTORC2 appears to positively regulate lipogenesis, at least in part, through Akt1-mediated activation of SREBP-1c.[191] Also, mTORC2 may regulate lipolysis by inhibiting the activation of protein kinase A (PKA). In the absence of mTORC2, PKA is

activated and phosphorylates hormone-sensitive lipase with induction of its lipolytic activity.[186]

mTOR and cell growth/cell cycle:

Although distinct processes, cell growth and cell division are often intimately linked.[192] Ribosome biogenesis plays a major role in cell-size determination.[193] Environmental cues regulate the cell-size threshold via TORC1, i.e., that poor growth conditions reduce the activity of TORC1 and subsequently decrease ribosome biogenesis, which, in mysterious ways, would lower the cell size threshold required for cell division.[194] mTOR is known to increase cell growth and proliferation by activation of cell cycle progression.[195] It plays a critical role in the regulation of G1 phase of the cell cycle. Loss of mTOR function leads to G1 arrest along with a severe reduction in protein synthesis.[196] mTOR additionally regulates the transition through other phases of the cell cycle. Under conditions of DNA replication stress or DNA damage, mTORC1 promotes S phase by maintaining deoxynucleoside triphosphate pools which are the obligate building blocks for DNA synthesis and are necessary for error-prone translation DNA polymerases.[197] It also influences the G2/M transition via the Tap42-PPase branch. Specifically, mTORC1 regulates the subcellular localization of the polo-like cyclin-dependent kinase (Cdc)5. Cdc5 activity destabilizes Swe1, a kinase that phosphorylates and thus inactivates the mitotic Cdc28. Inhibition of mTORC1 mislocalizes Cdc5, causing an inappropriate stabilization of Swe1 and, consequently, inactivation of Cdc28 and prolonged G2/M.[198] In addition, mTORC1 promotes cell growth by suppressing a variety of stress-response programs, which are incompatible with rapid growth and result in cell death.[199] It also regulates transcriptional as well as post-transcriptional aspects of stress responses such as mRNA stability, protein trafficking, and the activities of metabolic enzymes.[200]

mTOR and aging:

The mTOR signaling alters whole body metabolism and causes aging and age-related disease.[201] Genetic or pharmacological inhibition of mTOR signaling delays the onset of age-related pathologies and extends lifespan in model organisms.[202] Also, dietary restriction through inhibition of mTOR, retards the onset of age-related disease in mammals.[203] mTOR appears to control aging via its substrates S6K and 4E-BP, positive and negative regulators of mRNA translation and protein synthesis.[204] Indeed, experimental studies have demonstrated that knockdown of S6K results in increased life span whereas 4E-BP deletion blocks the life-extending effects of caloric restriction.[204,205] A reduction in mRNA translation could attenuate age-associated pathologies by allowing endogenous protein repair and degradation machinery to better maintain protein homeostasis in the face of protein aggregation and oxidative damage.[206] Also, activation of 4E-BP leads to activation of stress responsive genes and genes encoding the mitochondrial electron transport chain.[207] Upregulation of stress genes exerts a positive effect on lifespan by protecting cells and tissues from age-related damage.[208] Moreover, mTOR modulates aging through inhibition of autophagy.[209] This leads to an accumulation of damage, such as protein aggregates and degenerate mitochondria, that contribute to age-related cellular dysfunction.[210] In addition, inflammation associated with hyperactivation

of mTOR is another mechanism which could promote age-related pathologies.[211] Furthermore, there is increasing evidence that mTOR causes age-related pathology through a decline in stem-cell function [212] and that inhibition of mTOR can preserve, and perhaps even rejuvenate, stem-cell function in a variety of tissues.[213]

mTOR and immune responses:

Attention has been paid to the importance of mTOR in innate and adaptive immunity.[107] In DC cells, mTOR is necessary to promote differentiation, antigen uptake, maturation, and migration through enhancement of up-regulation of cell surface class II MHC and T-cell co-stimulatory molecules (CD40, CD80, CD86).[214] Moreover, mTOR activation inhibits the production of IL-12, IL-1β and TNF while enhances the production IL-10 by myeloid DCs [215] and type I IFN production in plasmacytoid DC in response to viral infection.[216] In addition, mTOR plays an important role in regulation of other components of the innate immunity including macrophages, neutrophils and NK cells.[217] mTORC1 activation critically modulates macrophage polarization with M2 to M1 phenotype switch [218] and promotes the expression of proinflammatory cytokines and nitric oxide in response to TLR stimulation.[219] Also, mTORC1 activation is essential in TLR2- and TLR4-induced neutrophil activation and regulates neutrophil extracellular traps formation.[220,221] Likewise, the mTOR pathway is required for enhanced NK and NKT cell effector functions thereby coupling the metabolic sensor mTOR to NK cell anti-viral responses.[222,223]

Furthermore, mTOR plays an important role in the adaptive immune responses. The mTOR pathway controls the development and maturation of T cells under steady state and their subsequent activation and differentiation upon antigen recognition.[224] Moreover, mTOR regulates T-cell trafficking by altering the expression of cell surface receptors that are important for migration into lymphoid organs.[225] Also, it prevents T cell "unresponsiveness or anergy" through prevention of calcium-induced up-regulation of anergy-inducing genes.[226] In addition, mTOR signaling determines T cell fate decisions during the adaptive immune response.[227] mTOR has been reported to serve as a controller to balance between naïve $CD8^+$ T cell differentiation to effector cells versus memory cell formation. Once the $CD8^+$ T cell recognizes their cognate antigen, these cells activate mTOR pathway to switch to the anabolic state. The switch between the $CD8^+$ effector cells and $CD8^+$ memory cells is associated with metabolic switch to catabolism.[228] Also, mTOR activation induces the differentiation and the development of $CD4^+$ Th cells into Th1, Th2, and Th17 subsets under appropriate skewing conditions.[229,230] In contrast to effector CD4 lineages, mTOR antagonizes differentiation of naïve $CD4^+$ cell to forkhead box P3 $(Foxp3)^+$ Treg cells.[231] In B cells, mTOR signaling is required for B cell development, maturation, proliferation and immune responses.[232]

***mTOR and cancer*:**

Several observations support the importance of mTOR pathway in cancer.[233] Many components of the upstream of mTOR signaling (PI3K,, PTEN and AKT) and its downstream effectors S6K1, 4EBP1 and eIF4E are dysregulated in human cancers.[234-236]

Additionally, several tumor suppressor genes are involved in the upstream inhibition of mTOR signaling whereas oncogene proteins are known that activate mTOR. The loss of p53, a very common event in cancer, promotes mTORC1 activation.[237] Meanwhile, the inflammatory cytokines, important factors in tumorigenesis, have roles in regulating the mTOR pathway. It has been found that the TNF-α/IKKb signaling pathway could activate the mTOR pathway by TSC1 phosphorylation.[141]

The oncogenic activation of mTOR signaling induces many processes required for cancer cell growth, survival, and proliferation [196,197] by up-regulating anabolic processes such as protein, glycogen, lipid, and organelle synthesis and down-regulating catabolic processes such as autophagy.[122,176] Also, mTOR as a survival pathway has been suggested to modulate apoptosis through eIF4E by upregulating the translation of anti-apoptotic mRNAs, such as Bcl-2, Bcl-xL, and Mcl-1.[238] Moreover, mTOR activation enhances vascular endothelial growth factor (VEGF) production, angiogenesis, and tumorigenesis.[239] mTORC1 activates the early hypoxic response by enhancing translation and stabilization of HIF-1 itself, as well as by enhancing translation of mRNAs encoding a subset of HIF-1 target genes that include VEGF.[182] It is important to understand that cancer is an age-related disease and mTOR can enhance aging leading to an accumulation of damage products, such as protein aggregates and mitochondrial degeneration, that contribute to age-related cellular damage.[201,205,240]

Pharmacological inhibition of mTOR:

The impact of mTOR on cellular metabolism, the immune microenvironment and cell proliferation and differentiation, provides an attractive therapeutic target for solid organ transplantation as well as metabolic diseases and cancer.[241] Historically, the mTOR inhibitor, rapamycin (also known as sirolimus), has been used for its immunosuppressive and antiproliferative properties.[242] However, the clinical applications of rapamycin were hampered by unfavorable pharmacokinetic properties and the development of adverse effects.[243] The relatively recent development of rapamycin analogs (rapalogs), (everolimus, temsirolimus and ridaforolimus) endowed with a more favorable pharmacokinetic profile, opened up the present era of mTOR inhibitors.[245] Everolimus is an orally available mTOR inhibitor that is usually administered on a continuous daily schedule.[246] Temsirolimus is a pro-drug whose primary active metabolite is rapamycin and is administered intravenously on a once- weekly schedule.[247] Ridaforolimus is not a pro-drug, but like temsirolimus, it was originally administered intravenously on an intermittent schedule, while an oral formulation has also been subsequently developed.[248,249] All these agents have similar structure and mechanism of action, and function intracellularly, forming a complex with the FKBP-12 that is then recognized by mTOR. The resulting complex prevents mTOR activity, leading to inhibition of cell cycle progression, survival, and angiogenesis and activation of autophagy.[112] Notably, rapamycin and its analogs, are similar in that they affect only mTORC1, and not mTORC2.[241]

A second generation of mTOR inhibitors, mTOR kinase inhibitors (TORKinibs), has recently emerged.[250] They are ATP-competitive inhibitors and thus target the kinase domain of mTOR, repressing both mTORC1 and mTORC2 activity and their downstream

effectors in the hope of developing novel mTOR inhibitors with better therapeutic efficacy than rapalogs.[251,252] Generally, these inhibitors are more potent suppressors of protein synthesis and 4E-BP1 phosphorylation, and strongly promote autophagy.[252] Several TORKinibs have been developed and are in early clinical trials.[253,254]

AUTOPHAGY

Autophagy constitutes one of the four mechanisms of cell death, which totally are the following: necrosis, apoptosis (programmed cell death (PCD) type I), autophagy (PCD type II) and mitotic catastrophe.[255] Autophagy "self-eating" is the basic catabolic process by which cells degrade unnecessary or dysfunctional cellular components through the lysosomal machinery.[256] There are three different variants of autophagy: *macroautophagy*, *microautophagy*, and *chaperone-mediated autophagy* (CMA). All three pathways share the same mode of degradation via the lysosome, but are mechanistically distinct from each other. Macroautophagy (hereafter referred to as autophagy) is the main pathway, occurring mainly to eradicate damaged cell organelles or unused proteins.[257] By contrast, micro-autophagy involves the direct engulfment of cytoplasm at the lysosome surface, whereas CMA degrades only soluble proteins, albeit in a selective manner.[255] Macroautophagy, the most extensively studied form, is the focus of the present review.

Autophagy molecular machinery:

Autophagy begins with the formation of a small crescent-shaped structure called isolation membrane or phagophore. In mammalian cells, phagophore membranes initiates primarily from the ER in dynamic equilibrium with other cytosolic membrane structures, such as the trans-Golgi and late endosomes and possibly even derive membrane from the nuclear envelope under restricted conditions.[258] This phagophore elongates into a double-membraned vesicle called autophagosome through non-specific encircling of the bulk cytoplasm or a selective process to engulf and sequester the intra-cellular cargo, such as protein aggregates, organelles and ribosomes.[259] The loaded autophagosome matures through fusion with the lysosome to form an autolysosome, thus, promoting the degradation of autophagosomal contents by lysosomal acid proteases. Lysosomal permeases and transporters export amino acids and other by-products of degradation back out to the cytoplasm, where they can be re-used for building macromolecules and for metabolism.[260] Thus, autophagy may be thought of as a cellular 'recycling factory' that also promotes energy efficiency through ATP generation and mediates damage control by removing non-functional proteins and organelles.[261] Autophagy and autophagy-related processes are dynamic and can be broken down into several steps including induction, cargo selection and packaging, nucleation of vesicle formation, vesicle expansion and completion, retrieval, targeting, docking and fusion of the completed vesicle with the lysosome/vacuole; and lastly, the breakdown of the intra-luminal vesicle (Figure 10).[262]

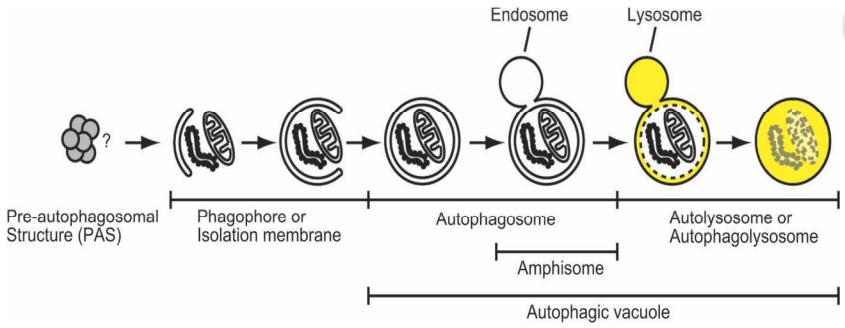

Figure 10: The process of autophagy in mammalian cells. [261]

The central machinery of autophagy includes a series of complexes composed of autophagy-related proteins (Atg) that assemble autophagosomes.[263] To date, 32 *ATG* genes, that are involved in autophagy, have been identified in mammals (Table I) and many of them gather at a site that can be identified by fluorescence microscopy as a punctate spot very close to the vacuolar membrane.[263,264]

Table I: Identification of autophagy-related proteins (Atg) genes and their functions.[263]

Autophagy-related gene (Atg) - products	
Mammalian Atg gene	Functions
ULK1, ULK2	Protein Kinase: Atg1-Atg13-Atg17-Atg29 complex
Atg2	Atg9/Atg2-Atg18 complex
Atg3	E2-like enzyme for Atg8s-lipidation
Atg4A,B,C,D	Cysteine protease: Atg8s-activation and delipidation
Atg5	Atg12-Atg5 conjugate: E3-like activity for Atg8s-lipidation
Beclin-1	Subunit of Vps34 PI3K complex
Atg7	E1-like enzyme for Atg12-and LC3-conjugation
LC3, GATE-16, GABARAP	Modifier: Conjugates to PE to localize to Autophagosome
Atg9L1, L2	Atg9 interacts Atg2-Atg18 complex: membrane-bound
Atg10	E2-like enzyme for Atg12-conjugation
Atg12	Modifier: Conjugates to Atg5
Atg13	mTor signaling: Atg1-Atg13-Atg17-Atg29 complex
Atg14	Subunit of Vps34 PI3K complex
Atg16L	Complex between Atg16 and Atg12-Atg5 conjugate
FIP200	Atg1-Atg13-Atg17-Atg29 complex
WIPI-1,2,3,4	Atg9/Atg2-Atg18 complex

Autophagy starts by activation of the UNC51-like kinase 1 (ULK1), (a mammalian homologue of Atg1) in a complex with Atg13, focal adhesion kinase family interacting protein of 200 kDa (FIP200; a mammalian homologue of Atg 17) and Atg 101 that is required for phagophore formation, possibly by regulating the recruitment of the trans-membrane protein Atg9 that may act by promoting lipid recruitment to the expanding phagophore.[264] Initiation of autophagosome formation requires Bcl-2-interacting myosin-like coiled-coil protein (Beclin 1), (the mammalian homolog of yeast Atg6), which forms a complex with the class-III PI3K (Vps34), p150 (Vps15 in yeast) and Atg14L or with Vps34, Vps15, and ultraviolet radiation resistance-associated gene (UVRAG).[265] After the dissociation of the Beclin-1 complex from Bcl-2 in autophagy-inducing conditions such as nutrient deprivation, Vps34 is activated and produces phosphatidylinositol-3-phosphate (PI3P). PI3P then recruits Atg proteins, which play crucial roles in the formation of the autophagosome cradle.[266,267] The subsequent elongation of autophagosomes requires the activation of two ubiquitin-like conjugation systems.[268] The first consists of a covalent conjugation of Atg12 as a ubiquitin-like protein to Atg5 through the concerted action of Atg7, an E1-like enzyme, and Atg10, an E2-like enzyme. The Atg5–Atg12 complex in turn associates with Atg16L (the mammalian homolog of yeast Atg16).[269] The second ubiquitin-like protein conjugation system is the conversion of microtubule-associated protein light chain 3 (LC3) (a mammalian homolog of Atg8) to LC3-I immediately after synthesis by Atg4. LC3-I is conjugated to its lipid target, phosphatidylethanolamine (PE), through Atg7, Atg3 and Atg12-Atg5-Atg16L1 complex. This process leads to the conversion of LC3-I (unconjugated form) to LC3-II (lipidated form) which associates with newly forming autophagosome membranes. LC3-II remains on mature autophagosomes until its fusion with lysosomes. The conversion of LC3 to LC3-II is thus well-known as a marker of autophagy-induction.[270] In the final stages of autophagy, the autophagosome

matures and fuses with the lysosome, where encapsulated cargoes are digested by resident hydrolase activities. LC3 is a receptor for p62. Hence, unwanted ubiquitinated proteins can be selectively eliminated by binding to p62 and becoming conjugated to LC3-II of autophagosomes (Figure 11).[271]

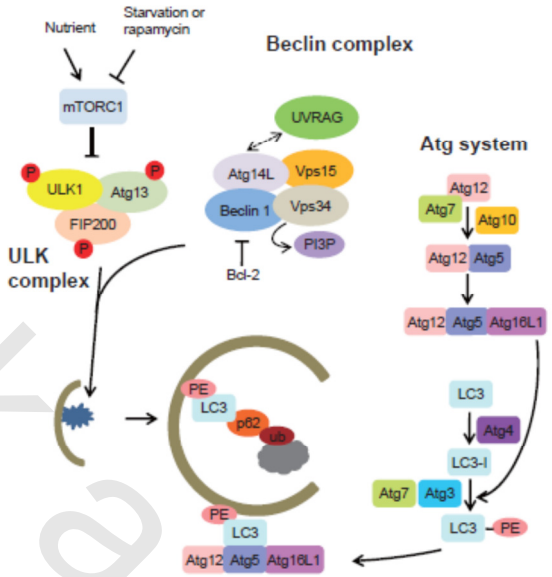

Figure 11: Steps of autophagy induction and autophagosome formation. mTORC1, Mammalian target of rapamycin complex 1; ULK1, UNC51-like kinase 1; Atg, Autophagy-related gene; Beclin 1, Bcl-2-interacting myosin-like coiled-coil protein; UVRAG, ultraviolet radiation resistance-associated gene; PI3P, Phosphatidylinositol-3-phosphate; LC3, light chain 3; PE, phosphatidylethanolamine).[272]

Autophagy regulation:

Eukaryotic cells have developed a mechanism through which autophagy induction is tightly coupled to the regulation of cell growth. The molecular machinery of autophagy is under the control of diverse signaling pathways. Nutrient, oxidative and energetic stresses result in a marked induction of autophagy in most cell types, and this plays a key role in cell survival under stressful conditions.[273] Under growing and high-nutrient conditions, autophagy is suppressed while nutrient depletion leads to autophagy induction to compensate for the loss of external nutrient supply by increased production of nutrients from intracellular stores.[274] In addition to nutrient signaling, hormones, growth factors and many other factors, including Bcl-2, reactive oxygen species, calcium, BNIP3, p19ARF, DRAM, calpain, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), FADD and myo-inositol-1,4,5-triphosphate (IP3), have also been reported to regulate autophagy.[266,275-283] In addition, autophagy is regulated by several protein kinases. Increased levels of Ras/PKA signaling activity results in a complete block to autophagy.[284] The SNF1 kinase, a key factor in glucose sensing, has also been implicated in autophagy, since deletion of this gene completely blocked autophagy induced by nitrogen starvation.[285] Finally, the general control nonderepressible (GCN)2 kinase, which responds to amino acid availability, appears to regulate autophagy via the GCN4 transcription factor.[286]

Role of mTOR in autophagy regulation:

The mTOR has been identified as down-regulator of autophagy in response to diverse inputs. Thus, inactivation of mTOR is essential for the induction of autophagy.[180,287] The stimulatory effect of the mTOR inhibitor, rapamycin on autophagy has been confirmed.[288] Under nutrient starvation, decreased TOR activity results in induction of autophagy to compensate for the loss of external nutrient supply by an increased production of nutrients from intracellular stores.[122,289] As mTOR is a suppressor of autophagy, signaling pathways that activate TOR also inhibit autophagy, whereas signaling pathways that inhibit TOR stimulate autophagy.[120] In that manner, the inducers of autophagy in mTOR signaling pathways includes serine/threonine kinase 11 (STK11/LKB1), AMPK, TSC1/TSC2 complex, and PTEN,[127-129,134] while the suppressors of autophagy in the same pathways are Akt and Rheb.[133,137] Moreover, nuclear p53 induces autophagy through activation of TSC2 and AMPK activators Sestrins 1 and 2 which subsequently down-regulates the mTOR pathway.[290] Control of autophagy by mTOR occurs primarily at the induction step by regulating the activities of the ULK1 kinase complex (ULK1-Atg13-FIP200-Atg101). mTOR activation contributes to hyperphosphorylation of Atg13 at multiple residues causing a reduced binding affinity between of Atg13 and ULK1 and thus inhibition of the membrane targeting of the ULK1 kinase complex and repression of autophagy.[291] During rapamycin treatment or nutrient starvation, the inactivated mTOR causes rapid dephosphorylation of Atg13, which increases the affinity of this protein for ULK1 and enhances ULK1 kinase activity; an essential for autophagosome formation.[264,291] Also, mTOR inhibits p73, a member of p53 family, which regulates the expression of several Atg genes including Atg5 in response to different cellular stresses.[292]

Autophagy in health and disease:

Autophagy is now recognized to play multifunctional roles in the maintenance of cellular homeostasis.[255] Added to the well-characterized role in quality control, autophagy has proved to be important in the maintenance of cellular energetic balance, in cellular and tissue remodeling, and cellular defense against extracellular insults and pathogens.[272] It is not a surprise that, in light of this growing number of physiological functions, connections between autophagic malfunction and human pathologies have also been strengthened. Defective autophagy has been implicated in certain diseases, including cancer, neurodegenerative diseases, cardiomyopathy, liver and immune disease, metabolic syndrome and infectious diseases.[293] In most of these situations, autophagy has both beneficial and harmful effects.[294] Elucidating the factors that determine the switch between these dual functions of autophagy has become a priority when considering the potential therapeutic modulation of autophagy in many of these pathological conditions.[295]

Autophagy and cell survival during starvation:

The occurrence of autophagy in response to environmental stress, most notably starvation, is generally regarded as a cell survival mechanism.[261] During periods of nutrient shortage, autophagy provides the constituents required to maintain metabolism and are essential for cell survival until nutrients become available again.[255] Stimulating

autophagy contributes to the lysosomal recycling of nutrients to replenish pools of precursor molecules. Free amino acids (especially branched-chain amino acids) released by autophagic proteolysis of intracellular proteins and organelles, are used to maintain protein synthesis and glucose synthesis.[296] Autophagy in the liver converts this organ into a main source of amino acids that are then delivered to other organs through the blood stream during starvation.[297]

***Autophagy and cellular quality control*:**

Autophagy is active at basal levels in most of the cells in the body, where it plays a housekeeping role in maintaining the integrity of intracellular organelles and proteins.[298] Basal autophagy, often referred to as 'quality control autophagy', is integral to the cellular surveillance machinery responsible for recognition and removal of damaged/old organelles as well as denatured proteins and protein aggregates, and thus maintains quality control of essential cellular components.[299] Exposure to stressors, such as oxidative stress, ER stress or other conditions resulting in massive amounts of unfolded proteins and organelle damage, elicits activation of inducible forms of autophagy.[300] In this context, autophagy facilitates the clearance of protein aggregates and whole organelles and also the in-bulk sequestration of 'still-soluble' forms of the pathogenic proteins before they aggregate and, thus, ameliorates proteotoxicity.[301,302] Autophagy is also important to restore organelle homeostasis, to eliminate damaged organelles after stress and to assist cells to adapt their organelle content to the changing environmental conditions.[302] Autophagy-mediated control of protein and organelle quality, as well as organelle number, is essential for the maintenance of cellular homeostasis and to guarantee cellular survival during stress.[298]

Autophagy and mitophagy:

Autophagy performs a cardinal homeostatic function in the removal of damaged or dysfunctional mitochondria, in a selective process referred to as mitophagy, which plays an important role in erythrocyte maturation and the maintenance of cellular homeostasis. The increased turnover of mitochondria by mitophagy may occur as a result of chemical or physical stress (e.g., hypoxia).[303] Loss of mitochondrial membrane potential and the increased production of mitochondrial reactive oxygen species (ROS) may provide initiating signals for mitophagy. Mitophagy can regulate mitochondrial number to match metabolic requirements. Damaged or dysfunctional mitochondria are recruited to the autophagosome for removal by mitophagy through a process regulated by PTEN-induced putative kinase 1 and Parkinson protein-2.[304]

Autophagy and cellular energetic balance:

Autophagy provides substrates for energy generation and biosynthesis. Amino acids resulting from autophagic breakdown could be utilized for the production of cellular ATP through direct oxidation, or by fuelling the tricarboxylic acid (TCA) cycle and gluconeogenesis with intermediates such as oxaloacetate.[305] Moreover, an array of energy stores mobilized by autophagy is now recognized to include more energetically efficient molecules, such as lipids, glycogen and nucleic acids. The hydrolytic products of these

molecules, free fatty acids, glucose and nucleotides, can be funnelled into the TCA cycle, gluconeogenesis or glycolysis to produce ATP.[306] This capability of autophagy to maintain ATP production and support macromolecular synthesis makes it a pro-survival pathway of particular importance in organs with high energetic requirements, such as the heart or skeletal muscles. Alterations of this specific autophagic function also constitute the basis of some common metabolic disorders.[293]

Autophagy and cellular death:

Despite a widely accepted role for autophagy in cellular survival, autophagy has also been suspected of being involved in type-2 PCD or autophagic cell death (as distinct from type-1 PCD or apoptosis). Cells can be killed by autophagy when apoptosis is inhibited. [307] Increased autophagosome formation is often coincident in cells that are dying and results from a compromise in their clearance by lysosomes and, consequently, from impaired rather than enhanced autophagy. Thus, autophagy may prevent death under milder conditions while excess activation of autophagy may represent a failed adaptive mechanism and may contribute to cell death through unchecked degradative processes.[308] Moreover, it has been shown that autophagy contributes with apoptotic signals to the killing of cells and elimination of apoptotic cells by phagocytic cells.[309]

Autophagy and immune responses:

In addition to maintaining cellular homeostasis, autophagy can also serve as an effector arm of the immune system and a mechanism contributing to the immune response toward pathogens.[310] In innate immunity, autophagy works downstream of pattern recognition receptors including TLRs where it facilitates a number of effector responses, including cytokine production and phagocytosis.[311] Autophagy is also able to intersect pathways of innate and adaptive immunity through its potential to deliver antigens for antigen presentation.[312] Autophagy provides a substantial source of antigens for loading onto MHC class II molecules [313] and it may be important in DCs for cross-priming to $CD8^+$ T cells.[314] In lymphocytes, autophagy is essential for cell survival and homeostasis, particularly in T cells. Autophagy has recently been found to regulate energy metabolism in T cells.[315] Interestingly, a role for autophagy has recently been described in inducible NKT development and in $Foxp3^+$ Treg cell homeostasis and function.[316] Moreover, autophagy is needed for B-cell development at specific stages in the bone marrow.[317]

Autophagy and cancer:

Recently, accumulating evidence suggest the existence of strong links between autophagy and cancer. Autophagy is considered a process that suppresses tumor development.[318] Several tumor suppressor genes as p53 and PTEN stimulate autophagy, whereas oncogenes like Bcl-2 are known to inhibit autophagy.[319,320] Also, activation of the PI3K/Akt pathway leads to decreased autophagy in many settings largely through mTOR activation.[287] Autophagy may function as a tumor suppression mechanism by removing damaged organelles/proteins by shuttling them via autophagosomes to the lysosome for degradation.[321] For tumor cells, autophagy genes are frequently mono-allelically deleted,

silenced, or mutated in human tumors, resulting in an environment of increased oxidative stress that is conducive to DNA damage, genomic instability, and tumor progression.[322-324] Alternatively, autophagy-induced cell death may kill developing tumor cells and prevent tumor initiation.[325] In addition, it has been found that the autophagy gene Beclin-1 is a haplo-insufficient tumor suppressor [326] and Beclin $1^{+/-}$ mice were shown to be tumor prone.[327] Moreover, autophagy may also protect against tumorigenesis by limiting necrosis and chronic inflammation, which are associated with the release of proinflammatory cytokines, such as high mobility group 1 (HMGB1) protein leading to increased tumor growth.[328] Also, autophagy has tumor-suppressive functions through its role in promoting Ras oncogene-induced senescence, which represents a state of cell cycle arrest maintained by the expression of cell cycle inhibitors ($p16^{Ink4a}$, $p21^{Cip1}$, and $p27^{Kip1}$) in metabolically viable cells and is thought to a mechanism for autophagy-mediated tumor dormancy.[329] Also, autophagy prevents the accumulation of p62, which was shown to be important in the promotion of tumorigenesis through deregulation of nuclear factor κB (NFκB) signaling, accumulation of ROS, and increased DNA damage.[330] Other mechanisms for autophagy-mediated tumor suppression, include autophagy-regulated immunosurveillance against tumor cells by increasing tumor antigen presentation [331] and autophagy-inhibited angiogenesis through degradation of neuropilin 1, a positive regulator of VEGF signaling.[332] On the other hand, there is indirect evidence of a potential cancer-promoting effect of autophagy. The cytoprotective function of autophagy in cells subjected to starvation might enhance the prolonged survival of tumor cells exposed to metabolic stresses due to limited angiogenesis leading to nutrient deprivation and hypoxia.[333] Remarkably, constitutive activation of autophagy is critical for continued growth of some tumors, serving to inhibit apoptosis, reduce oxidative stress and provide key intermediates to sustain cell metabolism.[334] There are considerable data indicating that tumor cells use autophagy for survival in response to cytotoxic agents.[335] These findings strongly suggest that autophagy may play dual roles in cancer progression, which is likely dependent on tumor type, stage, and genetic context. It seems clear that modulation of autophagy will be an attractive avenue for future cancer therapeutic approaches.[336]