The Role of mTORC1 in Autophagy as it Relates to Cancer

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Abstract

The mammalian target of rapamycin complex 1, mTORC1, is composed of several subunit proteins with many cellular responsibilities including participation in a complex cell signaling cascade leading to autophagy, which is the regulated degradation of cell components. mTORC1 is frequently mutated or dysregulated within human cancer. Normally, mTORC1 functions to provide efficient regulation of autophagy according to intracellular levels of growth factors, amino acids, nutrients, oxygen levels, and more that can either inhibit mTORC1 and upregulate autophagy or activate mTORC1 and downregulate autophagy. A better understanding of mTORC1 is imperative to preparing cancer therapy treatments. Various cancerous tissue types require specific mTORC1 inhibitors based on the area of dysregulation in the autophagy cell signaling pathway.

mTORC1's Path to Cancer

Introduction

The mammalian target of rapamycin complex 1, or mTORC1, is a cell signaling protein complex including the protein mTOR and other subunits involved in a cellular cascade that when activated inhibits autophagy and when deactivated allows autophagy to occur. Autophagy, the process by which a cell can digest and remove worn out organelles, will be activated in low nutrient conditions to preserve energy and breakdown necessary molecules as they are needed.

Cancer occurs when cells rapidly grow and divide without regulation. Studies have shown that at least 30% of cancers contain a genetic mutation related to the mTORC1 protein complex that hinders the ability of mTORC1 to regulate autophagy in a cell (Tian et al., 2019). Cancerous tissues grow rapidly when they are no longer inhibited by cell death through autophagy that would normally be able to sense dysregulation within a cell pathway. Cancer therapy utilizes mTOR protein inhibitors that is designed to allow autophagy to resume, thereby removing cancerous cells, at least in theory (Tian et al., 2019). Studying the mechanisms of mTORC1 interactions within a cell is critical to understand what genetic mutations of mTORC1 may lead to specific types of cancer and their eventual treatments.

mTORC1 and Autophagy

The life span of a cell varies among tissues and relies on molecular signaling to cause the cell to proliferate or perform autophagy of certain cellular components. Autophagy is activated in starved and stressful conditions where the catabolism of cell parts may be beneficial as nutrients and energy to the cell (Zhou et al., 2013). mTORC1 is an integral component in the negative regulation of autophagy (Zhou et al., 2013). The mTOR protein, a single subunit of mTORC1, is

produced by the mTOR gene that also provides the subunit mTOR for the mTORC2 complex (Tian et al., 2019). A cell depends on the mTORC1 signaling cascade for proliferation, growth, and termination according to the metabolic situation.

mTORC1 belongs to the phosphoinositide 3-kinase family that are defined as serine/ threonine kinases (Rabanal-Ruiz et al., 2017). mTORC1 has been identified through cryo-electron microscopy that displays the obligate dimer rhomboid shape of the protein with a central cavity (Yip et al., 2010). The proteins of mTORC1 are mTOR, raptor (regulatory associated protein of mTOR), PRAS40 (proline-rich AKT substrate 40 kDa), and mLST8 (mammalian lethal with sec-13) (Yip et al., 2010). Each mTORC1 protein complex subunit has a specific role in regulating autophagy. Raptor proteins join the subunits of mTORC1 as a scaffold so that the protein mTOR that possesses serine/threonine kinase activity can be activated by the Rheb GTPase protein (Yip et al., 2010). PRAS40 proteins negatively regulates the mTORC1 path inhibiting activation of the complex when necessary and the mLST8 protein has an unknown purpose as the disappearance of the subunit does not interfere with any pathways (Yip et al., 2010). The activation of the mTOR protein involves a variety of intracellular actions that are stimulated by the Rheb GTPase leading to mTORC1 activation.

mTORC1 relies on a cascade of protein interactions to become activated and produce the necessary repression required to inhibit the autophagy pathway. Reducing the level of autophagy in a cell corresponds with a healthy and well-fed state. When a cell is distressed or nearing senescence, autophagy is important to terminate unnecessary systems until favorable conditions can be met. mTORC1 possesses the capability to respond to signaling molecules or slight deviations in intra- and extracellular fluid such as growth factors, amino acids, nutrients, and

oxygen levels that indicate if a cell is capable of normal function (Rabanal-Ruiz et al., 2017). The ability for mTORC1 to detect these variations in cell signals leads to the regulation of autophagy.

Growth Factors

Growth factor stimulation of mTORC1 is crucial for the inhibition of autophagy in a healthy cell. The presence of growth factors increases in cells proliferating and maintaining energetically favorable states. Research has demonstrated that growth factor hormones activate both MAP kinase and PI3-kinase (PI3K) activity which activate mTORC1 through regulation of tuberous sclerosis complex or TSC1/2 (Hayashi and Proud., 2007). The activation of mTORC1 inhibits autophagy and monitors cell stability to ensure cell synthesis and cell division can occur. The mTOR protein activates ribosomal S6 kinases, a component of the 40S ribosomal subunit, and the eukaryotic elongation factor 2 (eEF2), but inhibits eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (Hayashi and Proud., 2007). 4E-BP1 and eEF2 are critical components of translation that can completely inhibit or allow protein synthesis according to whether mTORC1 is activated or deactivated. The activation of mTORC1 in response to the growth hormones occurs directly through the GTPase Rheb that is located at lysosomal surfaces and positively regulates mTORC1 by binding the protein at the catalytic domain of the amino-terminal lobe (Paquette et al., 2018). Rheb is regulated by TSC2 that binds lysosomal membranes near mTORC1 during low levels of growth factors to inhibit the complex and is removed from the membranes in elevated levels of growth factor to allow activation of the complex (Paquette et al., 2018). Figure 1 indicates the pathway when activated TSC1/2 inactivates the Rheb GTPase protein, leading to activation of mTORC1. TSC1 and TSC2 bind tightly allowing for the GAP

activity of TSC2 to activate the intrinsic GTPase activity of Rheb (Paquette et al., 2018). Intrinsic GTPase activity is the ability of Rheb to hydrolyze active GTP into the inactive GDP form (Paquette et al., 2018). The mTORC1 pathway of TSC1/2 and Rheb are imperative to regulating mTORC1 and respond to many cell signaling molecules.

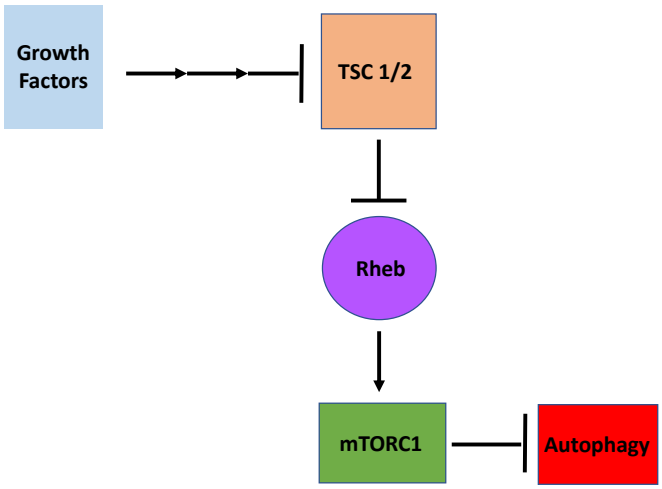

Figure 1. Growth factors activate the TSC1/2 complex to inactivate GAP activity. Rheb GTPase activity is inhibited by TSC1/2 and mTORC1 is activated. Activated mTORC1 then inhibits autophagy.

PI3K is an important regulator of mTORC1 through a signaling cascade based on growth factors present in the cellular environment. The lipid kinase PI3K is a heterodimer of catalytic and regulatory subunits that is activated when growth factor binding of a tyrosine kinase receptor or G-protein-coupled receptor at the cell surface triggers a signaling cascade (Dibble and Cantley, 2015). PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) on the cytoplasmic face of the phospholipid membrane to produce the binding site phosphatidylinositol-3,4,5-bisphosphate (PIP3) (Dibble and Cantley, 2015). PIP3 serves as a binding site for proteins

that possess pleckstrin homology domains, such as protein kinase AKT and 3-phosphoinositide-dependent kinase 1 (PDK1) (Dibble and Cantley, 2015). Once at the membrane PDK1 phosphorylates and activates AKT (Dibble and Cantley, 2015). AKT inhibits the binding of the TSC1/TSC2 complex through the phosphorylation of TSC2 on multiple sites so that it cannot function as a GTPase activating protein for Rheb (Dibble and Cantley, 2015). Rheb remains attached to GTP and can activate mTORC1 if AKT and mTORC1 phosphorylate the inhibitory 40-kDa proline rich AKT substrate (PRAS40) attached to mTORC1 as shown in Figure 2 (Dibble and Cantley, 2015). Activated mTORC1 can then induce autophagy according to the presence of growth factors in the cell.

Amino Acids

mTORC1 has been referred to as a nutrient sensor due to its increased activity in the presence of amino acids. Cells require amino acids for a variety of functions including gene expression, signal transduction, protein production, cell metabolism, and other important roles in cell homeostasis and proliferation. mTORC1 is inhibited in response to low levels of amino acids allowing autophagy to occur, which recycles the amino acids. The amino acids leucine, glutamine, and arginine are the primary activators of mTORC1, and in their absence the protein complex is inhibited (Rabanal-Ruiz et al., 2017). Rag, a Ras-related GTPase, has A, B, C, and D protein types that create the heterodimers A/B and C/D to bind mTORC1 at lysosomal sites for amino acid sensing (Paquette et al., 2018). A complex called Ragulator contains GTP-bound RagA/RagB and GDP-bound RagC/RagD, as well as the lysosomal adaptor and mTOR activator (LAMTOR) subunits that function as guanine exchange factors and interact with the vacuolar H-adenosine triphosphatase ATPase complex (v-ATPase) (Paquette et al., 2018). The v-ATPase

complex identifies the presence of adequate amounts of amino acids for mTORC1 activation (Paquette et al., 2018). Proton-assisted amino acid transporter 1 (PAT1), provides transportation of amino acids into the lysosomal lumen and collaborates with Rag GTPases to properly activate mTORC1 (Goberdhan et al., 2016). Sufficient levels of amino acids activate mTORC1 and maintain autophagy inhibition to provide stability for normal cell processes and proliferation.

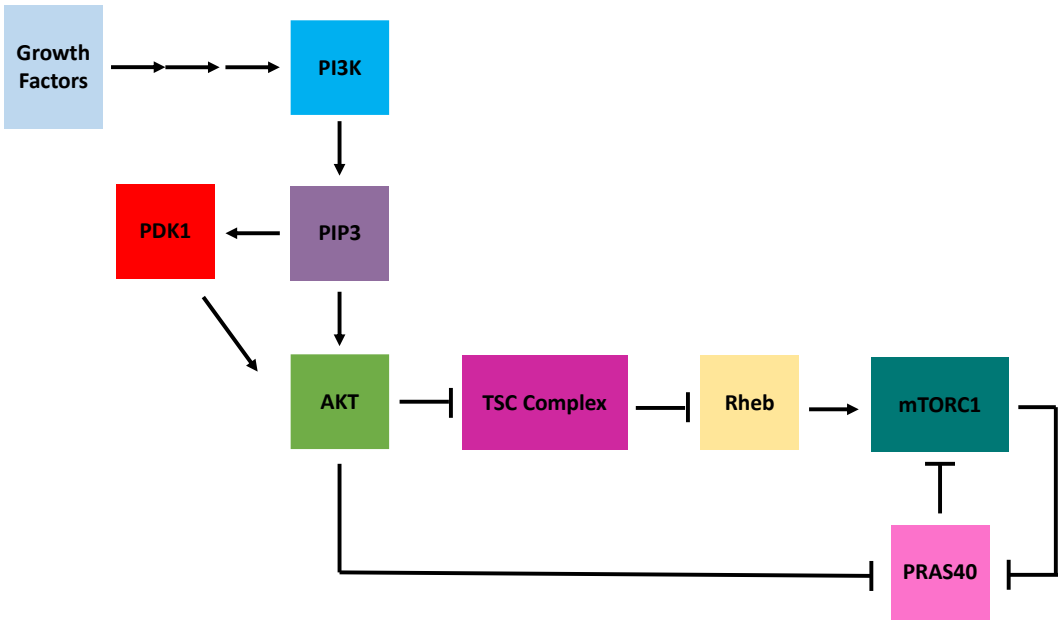

Figure 2. The pathway of activated PI3K through growth factor signaling. Growth factors detected through extracellular receptors activate PI3K that produces PIP3 for both PDK1 and AKT to bind. PDK1 also activates AKT that can then phosphorylate and deactivate the TSC1/2 complex, so no GAP activity is present to inactivate Rheb. Active Rheb binds mTORC1 and allows the protein complex to activate autophagy. PRAS40 is inactivated by AKT and mTORC1 to maintain activation of mTORC1.

Nutrition

Nutritional dependence of mTORC1 activation and autophagy inhibition relies on adenosine monophosphate-activated protein kinase (AMPK) and the presence of glucose within a cell. Glucose is the primary source of energy in a cell that is catabolically processed through the mitochondria and provides the ATP a cell needs to survive and function. AMPK monitors the levels of ATP, AMP, and ADP in a cell to deactivate mTORC1 downstream in instances of low glucose (Leprivier and Rotblat, 2020). Diminished levels of glucose, ATP, and the glucose metabolite fructose-1,6-bisphosphate lead to the synthesis of a protein complex within the lysosomal membrane that triggers phosphorylation and activation of AMPK due to the upstream serine/threonine kinase 11 (LKB1) (Sanguesa et al., 2019). AMPK phosphorylates the previously discussed TSC2 to inhibit mTORC1 (Paquette et al., 2018). Once TSC1/2 has been phosphorylated the two subunits bind tightly allowing TSC2 to function as a GAP which results in the Rheb-GTP protein hydrolyzing itself to Rheb-GDP (Paquette et al., 2018). mTORC1 is inactive without the presence of Rheb-GTP and autophagy is activated (Paquette et al., 2019). Research has also determined hexokinase 2 functions as a glucose sensor where glucose adheres to hexokinase 1/2 upon admittance into the cell and is phosphorylated (Leprivier and Rotblat, 2020). When hexokinase 2 does not have ample glucose to bind it will bind and deactivate mTORC1 allowing autophagy to occur (Leprivier and Rotblat, 2020). Without proper energy and nutrition within a cell autophagy is activated due to active AMPK, hexokinase 2, and possibly other undetermined signals. The complex cascade of protein interactions leading to the activation of autophagy through the AMPK protein that responds to low levels of energy is depicted in Figure 3.

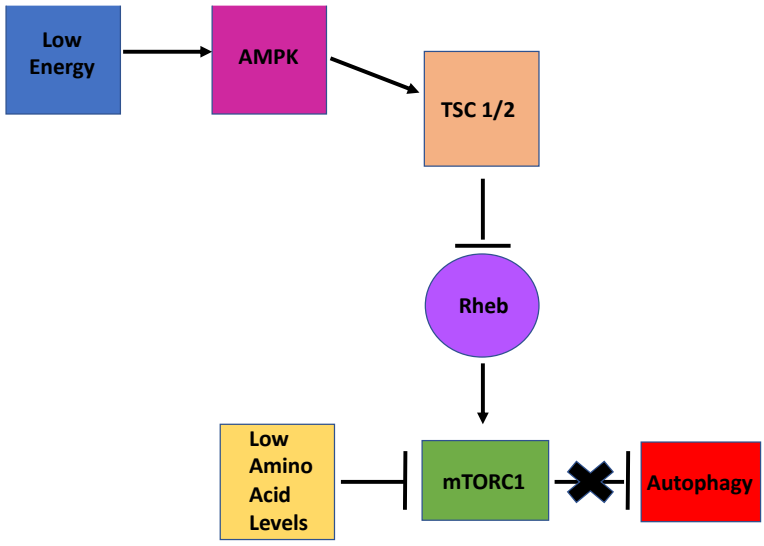

Figure 3. The pathway of activated AMPK, through low levels of energy. Once low levels of energy are detected by the AMPK protein it phosphorylates and activates the TSC1/2 protein. The TSC1/2 protein binds its two subunits tightly to produce GAP activity that inactivates Rheb and therefore TSC1/2 inactivates mTORC1.

Hypoxia

Other factors such as hypoxia, a low concentration of oxygen within a cell, leads to mTORC1 inhibition (Vadysirisack and Ellisen, 2014). When a cell reaches critical levels of oxygen the least important cellular functions are suppressed to prioritize energy retention. The hypoxia induced protein REDD1 (regulated in development and DNA damage responses 1) is an upstream inhibitor of mTORC1 through the activation of TSC2 that further down-regulates mTORC1 and upregulates autophagy processes (Vadysirisack and Ellisen, 2014). REDD1 detaches 14-3-3 proteins bound to the TSC2 that inhibit the function of the protein through competitive pathways (Vadysirisack and Ellisen, 2014). Phosphorylation of the Ser939 position of TSC2 by the upstream inhibitor protein kinase B (AKT) creates the binding attraction for 14-

3-3 proteins (Pozuelo-Rubio, 2012). Once 14-3-3 proteins are detached from TSC2 they bind other phosphorylated proteins such as FOXO that transcribe genes in the nucleus. Research has identified autophagy as a final resort for hypoxic cells and allows recycling of the intracellular organelles and molecules before apoptosis occurs (Tan et al., 2016). Intracellular components with hypoxia rely on the ability of mTORC1 to be deactivated according to cell sensing pathways to increase autophagy and extend cell life.

mTORC1 Activation of Autophagy

Once mTORC1 has been deactivated the autophagy pathway is regulated via a variety of molecular interactions. Activated mTORC1 phosphorylates proteins of the autophagy process during induction, nucleation, elongation, and maturation of the autophagy pathway through ULK1, Atg13, Atg 14, AMBRA1, NRBF2, WIPI2, P300, UVRAG, and Pacer substrates (Dossou and Basu, 2019). The ULK1 serine/threonine kinase and the autophagy-related protein 13 are induction substrates that are key in the interaction between mTORC1 and autophagy. The phosphorylation sites on ULK1 include Ser637/638 that regulates kinase activity of ULK1 and Ser757/758 that modulates the phosphorylation between ULK1 and AMPK (Dossou and Basu, 2019). The phosphorylation site on Atg13 is Ser25 which inhibits ULK1 kinase activity and eliminates autophagy processes (Dossou and Basu, 2019). When the metabolic needs of the cell are met mTORC1 phosphorylates the ULK1 and Atg13 at specific sites to inhibit the kinase activity of the ULK1 complex as seen in Figure 4 (Dossou and Basu, 2019). Dephosphorylation of ULK1 occurs though protein phosphatase 2A and protein phosphatase 1D magnesium-dependent delta isoform while the Ptc2 and Ptc3 PP2C phosphatases dephosphorylate the substrate Atg13 when mTORC1 is deactivated (Dossou and Basu, 2019). ULK1 is then activated

via autophosphorylation at the Thr180 site and Atg13, FIP200, and ATG101 are consecutively activated as a result before the ULK1 complex binds the isolation membrane of the endoplasmic reticulum to trigger autophagy (Dossou and Basu, 2019). mTORC1 regulates autophagy primarily through the two induction substrates that inhibit the process early but has links to the other substrates to conduct autophagy according to the specific metabolic needs of a cell.

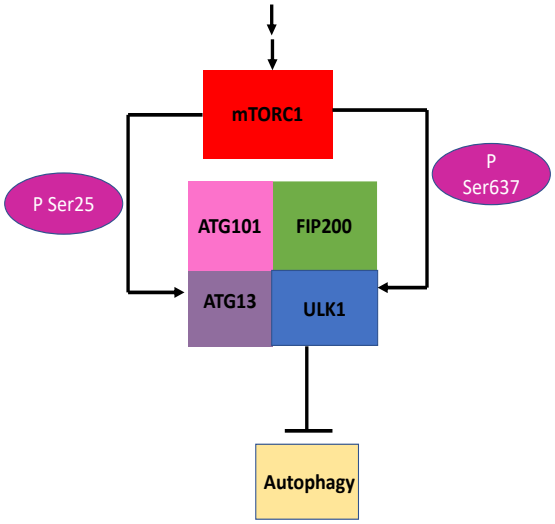

Figure 4. The activation of mTORC1 when the metabolic needs of a cell are met stimulate mTORC1 to phosphorylate ULK1 at Ser637 and ATG13 at Ser25. Phosphorylation of the protein subunits eliminates kinase activity of ULK1 that inhibits autophagy. FIP200 and ATG101 are subunits that function in the activation of autophagy and are complexed with ULK1 and ATG13.

Cancer and mTOR Signaling

The most common genetic mutation within 70% of cancer results in a hyperactive mTOR protein that increases tumorigenesis (Tian et al., 2019). Autophagy induced through mTORC1 has been identified to both negatively and positively impact cancer cells. Highly metabolic cancer cells induce autophagy promoted by cellular stress to stimulate pathways related to cell

survival, tumorigenesis, or anticancer treatment (Paquette et al., 2018). The autophagy pathway is tightly regulated to avoid unnecessary cell death. There are three known cell signaling processes involving mTOR proteins that lead to cancer including gene mutations of mTOR that create overactive mTORC1, gene mutations of the specific mTORC1 and mTORC2 proteins that dysregulate cell signaling, and upstream gene mutations creating loss-of-function or gain-of-function irregularities in suppressor proteins and oncogenes (Tian et al., 2019). Without the ability to turn the process of autophagy on or off cells are unable to regulate the availability of nutrients and energy within a cell contributing to cancer.

mTORC1 Gene Mutations

Excessive activation of mTORC1 results in cancer due to reduced autophagy which would normally remove cancerous cells. Thirty-three total mutations of mTOR between mTORC1 and mTORC2 have been found that are the source of hyperactive mTOR proteins in cancerous tumors (Tian et al., 2019). Ten genetic mutations of mTOR were identified from an examination of 750 cancer samples across the human body (Sato et al., 2010). The genetic mutations included two silent mutations that did not alter protein formation, addition of a stop codon resulting in an mTOR protein without a kinase domain, and point mutations altering the protein that primarily clustered in the c-terminal kinase domain region (Sato et al., 2010). Cell signaling, cell cycle progression, cell size, and sensitivity to the mTOR inhibitor rapamycin were affected in various ways due to the type of mutation, with either an increase or decrease in mTORC1 signaling (Sato et al., 2010). According to close examination single amino-acid mutations S2215Y and R2505P involve nutrient-independent signaling, S2215Y mutations increase S phase of the cell cycle, and any mutant variation of mTOR fails to reduce cell size in

low nutrient environments (Sato et al., 2010). Gene mutations present on the mTOR protein itself yield a varying array of signal cascades allowing cancer growth through an irregular cell signal.

Dysregulated Cell Signaling

Downstream of mTORC1 the deregulation of 4E-BP1/eIF4E is significant in tumor formation (Laplante and Sabatini, 2012). 4E-BP1/eIF4E contributes to oncogenic AKT signaling during mRNA translation, cell growth, and tumor progression in a manner yet to be determined (Laplante and Sabatini, 2012). eIF4E is part of a complex stimulating translation of pro-oncogenic proteins involved in cell survival and growth, angiogenesis, metabolism, and metastasis (Tian et al., 2019). mTORC1 phosphorylates 4E-BP1 so that it can no longer bind and inhibit eIF4E from initiating protein synthesis (Tian et al., 2019). Although researchers have deemed the cancer promoting pathway of 4E-BP1/eIF4E unclear, it is understood that translation of pro-oncogenic mRNAs provides stimulation for cancerous cells when 4E-BP1/eIF4E is dysregulated (Laplante and Sabatini, 2012). Researchers hypothesize that 4EBP1 and eIF4E expression can predict metastasis in certain cancer types as patients with overexpressed 4EBP1 proteins produce few metastatic cancer cells (Easton and Houghton, 2006). Downstream disruptions in the normal activation of translation factors such as 4E-BP1/eIF4E can fail to inactivate mTORC1 and therefore not induce autophagy through deactivation of the signaling protein AKT.

Upstream Genetic Mutations

Proteins upstream from mTORC1 that promote cancer due to genetic mutations include TSC1, TSC2, PTEN, PIK3CA, and serine/threonine kinase 11 that are all involved in the intracellular signaling cascade (Laplante and Sabatini, 2012). These mutated proteins can no

longer sustain tumor suppressor functions to activate mTORC1. Mutated and inactive TSC1/2 that normally inhibits mTORC1 in response to hypoxic and low energy conditions results in benign tumorigenesis, tuberous sclerosis, bladder cancer, urothelial carcinoma, clear cell renal carcinoma, and pancreatic neuroendocrine tumors (Tian et al., 2019). Overactivation of TSC1 and TSC2, the tumor suppressor proteins, results in mTORC1 hyperactivation through upstream signaling based on a mutation of either of the two proteins that form the complex (Johnson et al., 2015). In a normal regulation TSC1 and TSC2 bind with TBC1D7, a protein that can sense cell growth conditions and has enzymatic activity, to perform a GTPase activating function for Rheb (Johnson et al., 2015). When the GTPase activating function is not present Rheb is functional and can activate mTORC1. A loss-of-function mutation in TSC1 or TSC2 produces irregular signal cascades that no longer allows inhibition of mTORC1 in the instance of poor cellular conditions or cellular signals related to cancer (Johnson et al., 2015).

Phosphatase and tensin homolog (PTEN) protein mutations result in cancers located in plasma cells, breast tissue, and the endometrium after downregulation of the gene through mechanisms such as mutation, methylation, protein volatility, and delocalization (Tian et al., 2019). PTEN is a lipid phosphatase tumor suppressor that hydrolyzes PIP3 to PIP2 at the intracellular membrane where cell signaling cascades initiate (Hopkins et al., 2014). The protein is an inhibitor of PI3K signaling through inactivation of AKT suppressing cell growth, proliferation, migration, protein synthesis, and cell cycle progression that all promote apoptosis (Hopkins et al., 2014). Loss-of -function mutations of PTEN result in irregular signaling for PI3K and AKT activation (Hopkins et al., 2014). Tumor development has been correlated with loss of PTEN where mTORC1 cannot be activated to stimulate autophagy when necessary

(Hopkins et al., 2014). Protein-protein interactions with scaffold proteins that regulate PTEN activity in non-cancerous cells are typically not present when loss-of-function mutations occur and effect localization, protein stability and conformation, and phosphatase activity (Hopkins et al., 2014).

PI3K, upstream of mTORC1, commonly has genetic mutations that result in increased activation of AKT, growth factor receptors, and insulin growth factor receptors so that the transduced signal is atypical and tumor forming (Tian et al., 2019). PIK3CA mutations are gain-of-function mutations that have a transforming capacity (Mukohara, 2015). PIK3CA genes code for p110α, the catalytic subunit of PI3K, important in upstream regulation of mTORC1 (Kawano et al., 2006). The genetic mutations are in exon 9 and exon 20 that effect the lipid kinase activity of the protein (Kawano et al., 2006). With increased lipid kinase activity growth factors are not necessary to initiate the signaling cascade activating AKT (Kawano et al., 2006). The presence of the mutation correlates with elevated phosphorylation levels of downstream proteins in the signaling cascade including mTOR which allows tumorigenesis to occur by bypassing inhibiting proteins (Mukohara, 2015).

Serine/threonine kinase 11 (STK11) inactivating mutations cause mTOR to fail to respond to a low energy environment (Easton and Houghton, 2006). SK11 activates AMPK in response to low energy levels to inhibit mTORC1 and apoptosis normally but when SK11 is mutated then mTORC1 is not inhibited. The protein is important for cell cycle progression, metabolism, differentiation, polarity, and tumor suppression (Kwon et al., 2020). When STK11 is mutated, the protein is unable to regulate programmed cell death 1 (PD-1) inhibitors and has shown immune suppression characterized by decreased T cell infiltration and increased immune

suppressive neutrophils in the location of the tumor cells (Kwon et al., 2020). Tumor growth progresses quickly in the absence of tumor-infiltrating lymphocytes and immune checkpoint inhibitors (Kwon et al., 2020). STK11 inactivation leads to pro-inflammatory cytokines reducing the expression of T cell infiltration and programmed death ligand 1 (PD-L1) on tumor cells (Kwon et al., 2020). Inactive STK11 promotes tumorigenesis as cellular signals no longer activate apoptosis stimulating signaling cascades and the protein fails to initiate a proper immune response through T cell lymphocytes.

Cancer Therapy

mTOR inhibitors are useful for cancer treatment but are inhibited by biological prevention mechanisms located in signaling pathways (Tian et al., 2019). The current proposed treatment in mTOR cancer therapy is inhibition of mTORC1 through rapamycin derived drugs (Guertin and Sabatini, 2007). The cancer therapy of rapamycin is used to inhibit mTORC1 which should increase autophagy since it would no longer be inhibited. Rapamycin derived drugs attach to the FK506 Binding Protein 12 (FKB12) on mTORC1 inactivating the protein and therefore activating autophagy (Guertin and Sabatini, 2007). Rapamycin FKB12 inhibits kinase activity of mTORC1 through competitive allosteric interactions (Kim and Guan, 2015). Without the binding of raptor proteins mTORC1 is inhibited and S6K1 and 4E-BP1 downstream are no longer phosphorylated (Tian et al., 2019). This in turn leads to a failure to induce cell growth and protein synthesis (Tian et al., 2019). Additionally, other important proteins such as vascular endothelial growth factor, platelet-derived growth factor, and basic fibroblast growth factor are suppressed through rapamycin derived drugs (Tian et al., 2019). Research has determined that only particular cancers respond to the drugs administered, which include mantle cell lymphoma,

endometrial cancer, and renal cell carcinoma (Guertin and Sabatini, 2007). The immunosuppressive behavior of rapamycin prompted the formation of rapalogs, or derivative drugs, named RAD001, CCI-779, and AP2357 that were produced to eliminate immune system inhibition (Kim and Guan, 2015). Rapalogs developed in clinical trials include temsirolimus (intravenous), everolimus (oral), ridaforolimus (oral), and ABI-009 (intravenous) (Tian et al., 2019). Analogs of rapamycin are most efficient when applied to specific cell and tissue types (Paquette et al., 2018). The mTOR inhibiting or regulating drug must be prepared differently depending on what mTOR genetic mutation is present in the particular cancer (Guertin and Sabatini, 2007).

Positive and negative feedback loops of mTORC1 are inhibited by rapamycin drugs so that suppression of dysregulated autophagy pathways in cancer treatment will not occur (Zou et al., 2020). mTORC1 negatively regulates itself by activating S6K1 as well as phosphorylating the insulin receptor substrate-1 (IRS1), which leads to its degradation (Laplante and Sabatini, 2012). Inhibition of growth factors upstream of receptor tyrosine kinase (RTK) also occurs via mTORC1 negative regulation (Laplante and Sabatini, 2012). Without growth factors PI3K is inactive and the phosphorylation of phosphatidylinositol (4,5)- bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3) that activates AKT will not occur (Zou et al., 2020). Another negative feedback loop induced via mTORC1 occurs when IRS1 and the RTK inhibitor growth factor receptor-bound protein 10 (Grb10) are phosphorylated which reduces growth factor stimuli and inactivates mTORC1 (Laplante and Sabatini, 2012). Positive feedback mechanisms are present when the PI3K/AKT pathway activates the inhibitor of nuclear factor κB (NF-κB) (Zou et al., 2020). mTORC1 signaling is promoted when the NF-κB inhibitor halts

PTEN gene production and dephosphorylation of PIP3 and AKT (Zou et al., 2020). Rapamycin prevents S6K1 from inhibiting IRS allowing the PI3K/AKT pathway to occur (Zou et al., 2020). Rapamycin is then unable to inhibit mTORC1 activation due to AKT activation produced by the effects of S6K1 on IRS (Zou et al., 2020). Other than the feedback loops, research regarding rapamycin derived drugs does not indicate that 4E-BP1 phosphorylation is completely inhibited allowing for an abundance of cancer cells (Laplante and Sabatini, 2012).

Several other drug models have been examined for effectiveness against cancer due to irregular mTOR pathways. The Halitulin analog ICSN3250 is an mTORC1 inhibitor that replaces phosphatidic acid located in the FKBP-rapamycin-binding domain and could potentially eliminate tumor cells (Zou et al., 2020). The drug LY3023414 eliminates the PI3K/mTOR/DNA dependent protein kinase pathways present in tumor cells that have phosphoinositide-3-kinase regulatory subunit 1, phosphate, and PTEN mutations (Zou et al., 2020). Similarly, the drug OSU-53 has experimentally been shown to deplete cancer cell growth using AMPK as an inhibitor of mTORC1 to promote apoptosis in tumor cells (Zou et al., 2020). Autophagy inducing drugs such as metformin activate AMPK indirectly through the mitochondrial respiratory chain complex I that when inhibited concentrates AMP and ATP in the cell activating autophagy (Paquette et al., 2018). Metformin also inhibits mTORC1 independently of AMPK through Raf GTPases of REDD1 producing autophagy (Kim and Guan, 2015). The availability of diverse mTOR regulating drugs provides a broader treatment for mTORC1 related tumorigenesis throughout the body based on the type of pathway irregularities occurring.

Cancer Caused by the mTORC1 Pathway

Cancer that is induced through errors in the mTORC1 and autophagy pathway is

prevalent throughout the entire human body. Any metabolic tissue can be affected through irregularities in the autophagy pathway. Further research for reversing or eliminating mutated or dysregulated autophagy induced cancer cells is a current field of study as the protein cascade and cellular signaling mechanisms are still not completely elucidated. Cancer present in the lungs, gastric region, colon, renal organs, urinary bladder, prostate, breasts, head, and neck are currently being researched to determine what genetic mutations of mTORC1 are present leading to aberrant tissue as well as what inhibitors may be used for patient therapy and treatment.

Lung Cancer

Non-small cell lung carcinomas (NSCLC) have dysregulated PI3K/AKT signals that activate the mTORC1 pathway upstream in 47% of squamous cancers (Tian et al., 2019). The PI3K negative phosphatase regulator PTEN is mutated, silenced, or deleted creating a dysfunction that leads to a continuously active mTORC1 (Easton and Houghton, 2006). Research has not determined the specific role of mTORC1 in NSCLC (Tian et al., 2019). Evidence has been found that show mTOR inhibitors, rapamycin derived drugs, affect the mutated tissue of NSCLC by slowing tumorigenesis (Tian et al., 2019). Constitutively activating mutations in the mTORC1 pathway of NSCLC creates drug and therapy resistance to mTORC1 in recent experiments and requires further study (Gremke et al., 2020). Rapamycin derived drugs reduce cell death related to hypoxic conditions from cancerous tissue and promote increased levels of ATP and glucose in cancerous lung tissue (Easton and Houghton, 2006). The mTOR inhibitor everolimus paired with chemotherapy treatment produced positive results in 47.1% of patients (Tian et al., 2019). NSCLC patients have nearly a 50% chance of recovery using mTOR

inhibiting therapies and provide researchers with further opportunity to study the effects of the drugs in the human body and how they might be improved.

Gastric Cancer

Cancerous gastric cell lines typically display overexpression of PIK3CA, PIK3CB, AKT1, and mTOR proteins (Tian et al., 2019). The PIK3CA, PIK3CB, and AKT proteins are prominent proteins in PI3K/AKT mTORC1 signaling pathways (Tian et al., 2019). The PIK3CA gene expresses the p110α catalytic subunit of the PI3K heterodimer and can be mutated at a rate of up to 18% in gastric cancers (Campbell et al., 2004). The protein PTEN that inhibits the PI3K pathway is a known tumor suppressor that is deleted, mutated, or amplified in 0.3%, 3.1%, and 4% of gastric cancer cases respectively (Tian et al., 2019). The rate of alteration of the PI3K pathway proteins as well as the PTEN proteins that affect mTORC1 activation in gastric cancer is also dependent on ethnicity and is compared between Asian and Caucasian races in Table 1 (Tian et al., 2019). Recent research recognized that overexpression of a phosphorylated mTOR protein in gastric cancer tissue results in a detrimental prognosis when coupled with the downregulation of TSC1 proteins (Zou et al., 2020). Suppressing tumor initiation and growth through mTOR inhibiting drugs has shown promising results in patients with gastric cancer but has not yet been able to effectively eliminate the tumorigenesis (Tian et al., 2019).

Table 1. **The rate of alteration of PI3K and PTEN proteins in mTORC1 pathways of Asian versus Caucasian gastric cancer patients**

Ethnicity	**PI3K Mutation Rate**	**PTEN Deletion Rate**	**PTEN Losses**
Asian	7%	21%	47%
Caucasian	15%	4%	78%

Colorectal Cancer

Colorectal cancer cell (CRC) mutations affect PI3K and PTEN signaling pathways (Tian et al., 2019). 15% of metastatic CRC patients have PI3KCA mutations while 20% to 40% show a PTEN loss mutation (Tian et al., 2019). Advanced CRC tumors present overexpression of the PI3K subunit p85 as well as AKT and an increase in phosphorylation of mTOR and the S6K1 protein (Tian et al., 2019). Mutation of the gene p53 inactivates mTORC1 activity through AMPK-B1, an isoform of AMPK, and TSC2 proteins which are prevalent in the activation process that allows tumor initiation (Tian et al., 2019). The target gene, REDD1, with the p53 protein regulate mTORC1 through hypoxia pathways utilizing TSC1/2 (Tian et al., 2019). Immunohistochemical studies established that early mTORC1 signaling is essential for tumorigenesis as well as the development from a normal colorectal cell to a neoplastic cell in adenomas and cancers (Tian et al., 2019). mTOR inhibiting drugs have only shown limited clinical success in suppressing mTOR signal cascades through 4E-BP1 kinase responsible for resistance in CRC (Tian et al., 2019). Future research of combining mTOR inhibiting drugs with other cancer therapies and inhibitors has the most promising options for CRC patients (Tian et al., 2019).

Renal Cancer

Renal cell carcinoma (RCC) is an extremely lethal cancer type as minimal therapies and knowledge of biomarkers exist (Tian et al., 2019). mTOR proteins regulate cell metabolism and a patient with RCC will have a dysregulated metabolism (Tian et al., 2019). Genetic alterations in every protein of the PI3K/AKT signaling pathways and various mTOR protein mutations have demonstrated mTOR hyperactivation of RCC in patients (Tian et al., 2019). Most notably the

amplification of the protein GNB2L1, amplification of PIK3CA, deletion of PTEN, and mutations of mTOR affect the PI3K/AKT pathways (Tian et al., 2019). Sustained activation of PI3K/AKT pathways occurs through positive feedback mechanisms between VHL/HIF tumor suppressing pathways and PI3K/AKT pathways in RCC (Tian et al., 2019). Mutations of the TSC1/TSC2 complex in RCC causes tumorigenesis due to the loss of an important upstream negative regulator of mTORC1 (Kim and Guan, 2015). Different subtypes of RCC have various US Food and Drug Administration (FDA) authorized rapamycin derived drugs used for mTOR inhibition including temsirolimus and everolimus (Tian et al., 2019). mTOR inhibiting drugs have the best results in metastatic RCC in patients with mutations of mTOR, TSC1, or TSC2 proteins (Tian et al., 2019).

Urinary Bladder Cancer

The ninth most prevalent cancer, urinary bladder cancer (UBC), has an mTOR pathway genetic alteration in 40% of patients (Tian et al., 2019). PTEN, TSC1, TSC2, PI3KCA, and AKT1 are the most mutated and amplified proteins involved in the alterations of mTOR pathways in UBC (Tian et al., 2019). Higher rates of mortality and progression of tumorigenesis are linked with mTOR dysregulate ion than any other irregularity resulting in UBC (Tian et al., 2019). Non-muscle-invasive (NMIUBC) and muscle-invasive (MIUBC) UBC differ in the loss of PTEN proteins and expression of mTOR proteins that lead to tumorigenesis (Tian et al., 2019). Treatment of UBC using mTOR inhibitors is not clinically as effective as in other cancers and presents minimal tumorigenesis results (Tian et al., 2019). Clinical trials of mTOR inhibitors paired with EGFR/HER2 inhibitors provide synergistic results that are positive in treating both NMIUBC and MIUBC (Tian et al., 2019). Future research regarding the difference in drug

combinations for NMIUBC and MIUBC is necessary as the drug response differs according to the phosphorylation of mTOR in MIUBC but not in NMIUBC (Tian et al., 2019).

Prostate Cancer

Research has found mTOR pathway aberrations prevalent in prostate cancer (PCa) as well (Tian et al., 2019). 30 to 50% of PCa tissues show PI3K/AKT pathway dysregulation leading to tumorigenesis (Tian et al., 2019). PTEN activity is an early prognostic marker for PCa as the loss of the protein correlates with a higher risk of abnormal and cancerous tissue growth (Tian et al., 2019). Risk of death in PCa patients can be attributed to mTORC1 downstream regulation of 4E-BP1 and eIF-4E that aid in determining prognosis (Tian et al., 2019). The PI3K/AKT/mTORC1 pathway in PCa tissue is involved with cancer cell proliferation, intracellular hypoxia, and radio resistance significant to tumor growth (Tian et al., 2019). mTOR inhibiting drugs do not have promising clinical results in slowing tumor growth (Tian et al., 2019). Combination therapies of rapamycin derived drugs with other mTOR inhibitors is under further investigation to eliminate PCa cell proliferation and growth (Tian et al., 2019). The other inhibitors involved in research include PI3K pathway inhibitors and ATP competitive mTOR inhibitors that in specific experiments were enhanced when combined with the loss of PTEN (Tian et al., 2019). mTOR therapies that produce results in one mTOR related cancerous tissue of the body may not elicit the same results in another area of the body emphasizing how various genetic mutations of the apoptotic pathways are still undefined.

Breast Cancer

Breast cancer pathway alterations are upstream of mTORC1 stimulating the complex with a hyperactive signal (Tian et al., 2019). PIK3CA is mutated in 20 to 30% of breast cancer

patients while the loss of PTEN can be found in 30% of breast cancer patients (Tian et al., 2019). Mutations of mTORC1 have been identified and located to the FAT and FATC domains responsible for structure as well as its kinase domains (Tian et al., 2019). The cancer inducing mutations of mTOR in breast cancer result in a negative prognosis for patients (Tian et al., 2019). mTOR inhibitors such as everolimus have been approved by the FDA for specific types of breast cancer treatment as well as with other cancer drugs for a combinatorial drug therapy (Tian et al., 2019). HR positive and HER2 negative breast cancer are named depending on whether hormone receptors are present. The two breast cancers show improved results when using the rapamycin analog, everolimus, with another drug, exemestane, allowing patients longer life expectancies (Tian et al., 2019). Newer research is being developed using combination therapies of ATP competitive inhibitors with PI3K/mTOR inhibitors that produce anti-proliferative responses with undesired negative side effects that scientists are working to reduce (Tian et al., 2019).

Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinomas (HNSCC) are 90% of the cancers identified in the human head and neck regions (Tian et al., 2019). Using whole-exome sequencing, researchers found that the PI3K pathway is mutated in 30% of HNSCC (Tian et al., 2019). Genetic mutations causing HNSCC are identified to be in the PIK3CA, PIK3CD, PTEN, PDK1, AKT, RICTOR, RAPTOR, TSC1, TSC2, and mTOR genes (Tian et al., 2019). PI3KCA protein amplification determined that the PI3K pathway is involved in HNSCC oncogenic development (Tian et al., 2019). Progression of HNSCC has been hypothesized to be a result of mutations of PIK3CA and mTOR or mutations of PIK3CA and PTEN (Tian et al., 2019). HNSCC biological

markers are downstream effectors of mTOR including eIF-4E, 4EBP1, and S6K1 that gives a prognosis for patients based on their presence in a dysregulated autophagy pathway (Tian et al., 2019). mTOR inhibiting drugs such as temsirolimus and everolimus have shown positive clinical application in reducing angiogenesis and lymphomagenesis while also acting synergistically with radiation treatment in HNSCC (Tian et al., 2019). Combination therapy using mTOR inhibitors with PI3K inhibitors are slated to be included in future clinical trials to provide treatment for HNSCC (Tian et al., 2019).

Future Research

Autophagy in cancer both acts as a tumor suppressor and a reducer of cancer cell proliferation. Research determining the effects of enhancing autophagy and decreasing autophagy in cancer cells results in conflicting information that correlates with higher rates of tumors in either scenario (Laplante and Sabatini, 2012). Ideally autophagy mediated by healthy mTORC1 would impede tumorigenesis but would be available for cell apoptosis if cancer within the cells prevails (Laplante and Sabatini, 2012). Autophagy may allow survival of tissue where tumor initiation is prevalent through apoptosis or by promoting tumor survival. Tumor survival is promoted when the cells are supplied with nutrients in a limited environment via apoptosis of surrounding cells. Mice who had their ability to perform autophagy inhibited developed tumors more rapidly due to increased levels of impaired mitochondria, reactive oxygen species, and protein aggregates (Laplante and Sabatini, 2012). Conflicting evidence demonstrates that in situations of repressed autophagy TSC2 and Lkb1 null cells are hypersensitive and become more beneficial in apoptosis of cancer cells (Laplante and Sabatini, 2012). Two hypotheses exist regarding the action of autophagy in cancer tissue. The first hypothesis states that autophagy

activity within a cancerous cell is dependent on the stage of tumor development (Wang et al., 2011). Early stages of tumor growth are hindered by autophagy, but later stage tumor cell survival is promoted because cancer is already present (Wang et al., 2011). The second hypothesis suggests that autophagy regulation in cancer is tissue-specific and depends on expression of tumor suppressor genes like Beclin 1, ultraviolet irradiation resistance-associated gene, Bax-interacting factor-1, and p53 (Wang et al., 2011). Therapy providing autophagy regulation in cancerous cells would be a future outcome of mTORC1 cancer research in drug treatments (Paquette et al., 2018). Unique alterations of the mTORC1 pathway to inhibit or activate the protein according to the specific tissue and stage of cancer is necessary to effectively prevent cancer growth through cellular apoptosis mechanisms.

To treat different forms of autophagy related cancer the genetic mutations of mTORC1 must be known so the altered pathway can be corrected. Without a clear understanding of how rapamycin induced cancer treatment affects select patients, doctors are unable to provide care to many suffering from mTORC1 related cancer. Research limitations of mTORC1 inhibitors include the effectiveness and safety of coupling inhibitors for cancers depending on tissue locations and the ability to determine and anticipate treatment responses in patients (Tian et al., 2019). Negative patient responses from using rapamycin as an mTORC1 inhibitor could be derived from a loss of the negative feedback loop between S6K1 phosphorylation and AKT signaling (Guertin and Sabatini, 2007). The loss of the negative feedback loop may contribute to uncontrolled cell proliferation and possibly chemoresistance (Guertin and Sabatini, 2007). Additionally, the drugs that block kinase activity available to promote mTORC1 inactivation in cancer therapy can be cytotoxic due to inhibition of mTORC2 in multiple pathways (Kim and

Guan, 2015). The rapamycin derived drugs used to block mTORC1 activity have been experimentally shown to be cytostatic inducing fewer toxic effects (Kim and Guan, 2015). Rapamycin derived drugs as well as kinase inhibiting drugs both produce immunosuppressive effects that should continue to be studied to reduce negative effects according to dosages of the drugs (Kim and Guan, 2015). mTORC1 inhibition drug therapy is a promising cancer treatment dependent on the specific genetic mutations present in a cancerous cell.

Cancer formation due to dysregulated mTORC1 signaling, which is required to regulate autophagy, is a prominent research area in cancer treatment. The normal cascade of protein interactions involving mTORC1 relies on cellular signaling according to stress and metabolic signals that may become aberrant due to a genetic mutation among the interacting proteins. Inhibiting tumorigenesis using rapamycin derived drugs is the key goal in future research designed to correct dysregulated signaling in mTORC1 related autophagy pathways. In light of the current review, it is seen that interactions of rapamycin drugs with cell signaling pathways have promising results for inhibiting mTORC1.

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