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Regulation of immune response and cancer by mTOR: A review

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Abstract
mTOR or mammalian target of rapamycin is an evolutionarily conserved serine-threonine kinase and is composed of 2549 amino acids that acts as intracellular kinase and controls the production of proteins through effects on the machinery of mRNA translation and also plays a role in cell growth and metabolism. mTOR is a critical regulator of immune function due to its role in sensing and integrating cues from the immune microenvironment. mTOR plays a role in regulating diverse immune cells, including neutrophils, mast cells, natural killer cells, γδ T cells, macrophages, dendritic cells (DCs), T cells, and B cells. mTOR pathway is an important regulator of cell growth and proliferation and mTOR dysregulation can cause various cancers. mTOR's role as a regulator of many cell processes and its potential as a therapeutic target has opened up treatment possibilities in several types of cancer. An active mTOR coordinates a response in cell growth directly through its effects on cell cycle regulators and indirectly by sustaining nutrient supply into the cell through the production of nutrients.

Keywords: mTOR, Types, Signalling, Immune regulation, Cancer, Cancer therapy

Introduction

mTOR or mammalian target of rapamycin is an evolutionarily conserved serine-threonine kinase and is composed of 2549 amino acids that plays a central role in integrating environmental cues in the form of growth factors, amino acids, and energy (Powell *et al.,* 2012) [15]. The mTOR is an intracellular kinase that controls the production of proteins through effects on the machinery of mRNA translation and also plays a role in cell growth and metabolism (Bernstam and Angulo, 2009) [3]. mTOR is emerging as a critical regulator of immune function because of its role in sensing and integrating cues from the immune microenvironment (Delgoffe and Powell, 2009) [5]. mTOR plays a role in regulating diverse immune cells, including neutrophils, mast cells, natural killer cells (Fig.1), γδ T cells, macrophages, dendritic cells (DCs), T cells, and B cells (Powell *et al.*,2012) [15]. The mammalian target of rapamycin (mTOR) pathway is an important regulator of cell growth and proliferation and mTOR dysregulation can cause various cancers. The understanding of the science behind mTOR's role as a regulator of many cell processes and its potential as a therapeutic target has opened up treatment possibilities in several types of cancer (Yang *et al*., 2014) [22]. mTOR senses the growth conditions within the cell and help the cells to respond changes in this environment. An active mTOR coordinates a response in cell growth directly through its effects on cell cycle regulators and indirectly by sustaining nutrient supply into the cell through the production of nutrient. The activation of mTOR signifies a decision point that takes into account the availability of the basic materials required for cell growth (e.g., amino acids, glucose, energy) and the growth-regulating signals from other cells and tissues (e.g., hormones, growth factors) while monitoring conditions of cellular stress (e.g., hypoxia, DNA damage, heat shock, external pH, osmotic stress, oxidative stress) (Advani, 2010) [1].

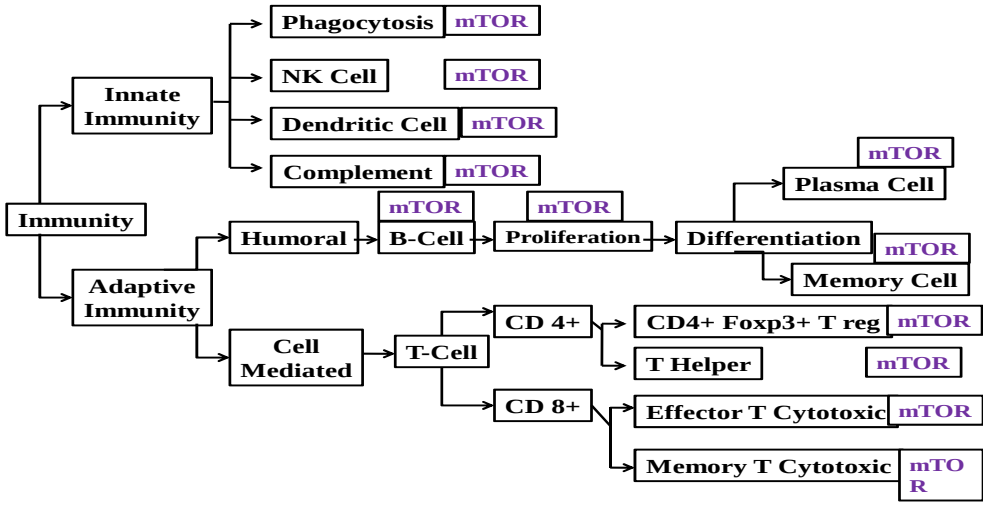

Fig 1: Reported sites of actions of mTOR on the immune system

Reasons behind its nomenclature

During identification of potential new antibiotics, scientists from pharmaceutical companies identified one compound from soil samples i.e., rapamycin, which was extracted from *Streptomyces hygroscopicus* found in soil from Easter Island (the local name for the island is *Rapa nui*). Rapamycin was found to inhibit the growth of yeast as well as to have immunosuppressive and antitumor properties (Powell *et al.,* 2012) [15]. The target of rapamycin (TOR) was found to be a 289-kDa serine/ threonine kinase. Subsequent studies led to the identification of the mammalian TOR (mTOR) also known as FKBP rapamycin associated protein (FRAP) or rapamycin- and FKBP12-associated protein (RAFT) (Bernstam and Angulo, 2009) [3].

Structure of the mTOR signaling complex

The N terminus of mTOR is characterized by a cluster of huntingtin, elongation factor 3, a subunit of protein phosphatase 2A, and TOR1 (HEAT) repeats that play a role in protein-protein interactions. Next, there is a FRAP, ATM, and TRRAP (FAT) domain, followed by the FKBP12 rapamycin–binding (FRB) domain. Adjacent to the FRB is the kinase domain responsible for mTOR's serine/threonine kinase activity and also the binding site for mTOR kinase–specific inhibitors. The C terminus consists of the FATC domain that is believed to play a role in maintaining structural integrity. In mammals, mTOR is encoded as a single gene product (Powell *et al.,* 2012) [15]. Downstream, mTOR signaling proceeds via two distinct complexes:

A) mTORC1

mTORC1 is composed of regulatory-associated protein of mTOR (RAPTOR), mammalian lethal with Sec13 protein 8 (mLST8), the proline-rich Akt substrate 40 kDa (PRAS40), and DEP domain–containing mTOR-interacting protein (DEPTOR).

B) mTORC2

mTORC2 is composed of mLST8 and DEPTOR in addition to the scaffolding protein RAPTOR-independent companion of TOR (RICTOR), mSIN1 proteins, and the protein observed with RICTOR (PROTOR) (Laplante and Sabatini, 2009) [11].

Upstream mTOR signaling

In this case Growth factor stimulation leads to the recruitment of PI3 kinase (PI3K), which phosphorylates phosphatidylinositol 4, 5-bisphosphate (PIP2) at the 3 position to generate Phosphatidylinositol 3, 4, 5-trisphosphate (PIP3). This leads to the recruitment of Akt to the membrane to be phosphorylated (at position T308) by PDK1. Activated Akt phosphorylates TSC2 in an inhibitory manner, yielding a separation of the TSC1/TSC2 complex and loss of GAP activity for RHEB-GTP. This leads to an accumulation of RHEB-GTP, which promotes mTORC1 function. Similarly, Erk can phosphorylate TSC2 in an inhibitory manner. Alternatively, AMPK (in response to low levels of energy), REDD1 (in response to low oxygen tension), and GSK3β (which is regulated by WNT and Akt) phosphorylate TSC2 in an activating way, thereby enhancing its GAP activity. In the presence of amino acids, Rag proteins bind to Raptor and promote the relocalization of mTORC1 with Rheb-GTP, leading to activation. Rapamycin bound to FKBP12 allosterically inhibits mTORC1 activity. Additionally, many immunologic inputs also play a role in regulating mTORC1 activity (Hay and Sonenberg, 2004) [10]. Positive costimulation (such as CD28 engagement) as well as cytokine signaling lead to recruitment of PI3K activity. Conversely, PD-1 ligation (co-inhibition) inhibits PI3K function. The upstream regulation of mTORC2 is poorly understood. Growth factor stimulation activates this complex, whereas high doses or prolonged exposure to rapamycin will disrupt the mTORC2 complex (Bernstam and Angulo, 2009) [3].

Downstream mTOR signalling

In this case upon activation of mTORC1, mTOR phosphorylates S6K1, leading to the Phosphorylation of ribosomal S6 protein, which allows for enhanced protein translation. Phosphorylation of 4E-BP1 by mTOR releases eIF-4E to participate in the translation-initiation complexes. Along with increasing protein translation, mTORC1 activity also upregulates gene expression programs necessary for glucose and lipid metabolism, mitochondrial biogenesis, and inhibition of autophagy. Immunologically, mTORC1 activity leads to the inhibition of SOCS3 and the increased activation of STAT4 and STAT3. This in turn leads to increases in T-bet and RORγt in response to IL-12 and IL-6, respectively, which promote Th1 and Th17 differentiation (Cobbold**,** 2013) [4].

mTORC2 activity leads to the phosphorylation of Akt (at position S473) and SGK1, leading to their activation and in turn resulting in the phosphorylation and sequestration of FOXO proteins in the cytoplasm. This prevents the FOXO proteins from activating the transcription of target genes such as Kruppel-like factor 2 (KLF2), which itself influences the expression of CD62L, CCR7, and S1P1. mTORC2 activity also inhibits SOCS5 expression, thereby enhancing STAT6 phosphorylation in response to IL-4 and subsequent GATA-3 expression and Th2 differentiation. In addition, mTORC2 signaling activates PKCθ, which in turn can also promote Th2 differentiation (Soliman, 2013) [17].

Different roles of mTOR

1. Role of mTOR In Cell Growth And Proliferation

mTOR senses the availability of nutrients [e.g., adenosine triphosphate (ATP), glucose, amino acids, cholesterol, and iron] and consolidates this information with growth factor signaling through the PI3K/Akt/tuberous sclerosis complex (TSC) pathway. An activated mTOR modulates the rate of protein synthesis for select mRNAs by activating the translational proteins S6 kinase (S6K) and 4E-binding protein 1 (4E-BP1). mTOR activation increases downstream effectors that stimulate cell growth and angiogenesis and regulate cellular metabolism. A primary way that mTOR exerts its regulatory effects on cell proliferation is by controlling the production of cyclin D1. Cyclins are proteins that regulate the activity of enzymes called cyclin-dependent kinases (CDKs) through the critical G1–S restriction point of the cell cycle, which in turn regulate the passage of cells. Recently, cyclin D1 has been shown to play a role in gene transcription, cell metabolism, and cell migration. Cyclin D1 overexpression had been associated with a number of cancers including breast cancer, colon cancer, prostate cancer, lymphoma, and melanoma (Advani, 2010) [1].

2. Role of mTOR in Angiogenesis

mTOR plays a key role in angiogenesis, i.e., the formation of new blood vessels to provide oxygen and nutrients to growing and dividing cells. mTOR increases the translation of hypoxia-inducible factor 1 (HIF-1)/hypoxia-inducible factor 2 (HIF- 2). The HIF transcription factors drive the expression of hypoxic stress response genes, including angiogenic growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor β (PDGF-β), and transforming growth factor-α(TGF-α). Increased levels of HIF-1α and HIF-1β have been shown to correlate with increased mortality in a number of tumor t ypes, including cervical cancer, breast cancer, lung cancer, ovarian cancer, head and neck cancer, and gastrointestinal stromal tumors (Advani, 2010) [1].

3. Role of mTOR in Cell Metabolism (Bioenergetics)

Bioenergetic research has shown that mTOR plays a keyrole in regulating cell metabolism. mTOR increases the surface expression of nutrient transporter proteins. An increase in these proteins results in greater uptake of amino acids and other nutrients by the cell, leading to adequate nutrient support to abnormal cell growth and survival. Additionally, abnormally activated mTOR may give cancer cells a competitive growth advantage by increasing production of the core enzymes necessary for glycolysis, which enables cancer cells to survive and grow even under hypoxic conditions (Advani, 2010) [1].

4. Role of mTOR in Cell Survival

There are several lines of evidence that mTOR activity plays a role in cell survival. Majority of this research has revealed that mTOR inhibition increases sensitivity to cell death pathways; however, there is also emerging evidence that mTOR activation may play a role in promoting cell survival through the activation of antiapoptotic proteins (Advani, 2010) [1].

Role of mTOR in immune-cell regulation

- **Regulation of T-Cells by mTOR**

The different sub-poppulations of T-cells are regulated in the following way-

- **Helper T-Cell (Th) Differentiation**

Investigators also found that mTOR was not required for CD4+ T cell proliferation, although the mTOR-deficient T cells proliferated more slowly than wild-type cells. Strikingly, the mTOR-deficient CD4+ Tcells failed to differentiate into Th1, Th17, or Th2 effector cells *in vitro* or *in vivo* under strongly polarizing conditions. Instead, stimulation of naive CD4+ mTOR-deficient T cells led to the generation of Foxp3+ T cells (Tregs) even under normally activating conditions. Mechanistically, the inability to become CD4+ effector cells was associated with a decrease in STAT4, STAT3, and STAT6 activation in response to the skewing cytokines IL-12, IL-6, and IL-4, respectively. This decrease in STAT activation was associated with decreased expression of the lineage-specific transcription factors Tbet, RORγt, and GATA-3. Consistent with their propensity to become Foxp3+ T cells, the mTOR-deficient T cells demonstrated hyperactive SMAD3 in the absence of transforming growth factor-β(TGF-β) (Powell *et al.,* 2012) [15].

- **In Cytotoxic T-Cell (Tc) Differentiation**

Activation of CD8+ cells also primarily depends on glycolysis but differentiation of effector CD8+ cells requires mTORC1-dependent T-bet expression. Most critically, mTOR is involved in the transition of effector to memory CD8+ T cells, and this appears to rely on conversion of T-bet to eomesodermin transcription factor expression (Araki *et al.,* 2010) [2]. Blocking mTOR with rapamycin has this exact effect, and therefore promotes the development and sustenance of memory T cells that transition, efficiently into effector cells highly capable of producing immune responses against tumours. Similar to Treg cells, memory CD8+ T cells depend on mitochondrial oxidative phosphorylation for energy (rather than glycolysis) and are driven by STAT5 signalling (Geissler, 2013) [8].

- **Regulatory T-Cell (Tregs) promotion**

Stimulation of naive CD4+ mTOR-deficient T cells led to the generation of Foxp3+ T cells (Tregs) even under normally activating conditions. One study reported that Foxp3+ T cells from humans display increased mTOR activity when compared with effector cells. In this study, transient inhibition of mTOR with rapamycin led to increased proliferation of the Tregs *in vitro*. However, addition of leptin to rapamycin-expanding Tregs inhibited their proliferation, thereby promoting Treg anergy (Yang *et al*., 2014) [22]. This correlatedwith enhanced mTOR activity in the leptin-treated cells. On the basis of these findings, the authors suggest that leptin keeps Treg proliferation in check by promoting mTOR activation, whereas loss of mTOR activation by rapamycin treatment enables Treg proliferation (Zeng and Chi, 2013) [24]. TGF-β signaling is contributing to the generation of Treg cells in the absence of mTOR. Under normal condition when mTOR is present, the low levels of TGF-β do not promote the generation of Treg cells, but in the absence of mTOR, Treg cells are hyperactive to this low concentration of TGF-β (Geissler, 2013) [8].

- **In B-cell development**

Data from the mTOR hypomorph mouse suggest that when mTOR levels becomes reduced, then B-cell development may be even more aff ected than T cells. In these mice, B-cell development in the bone marrow is partially inhibited, which was reflected by decreased B-cell proliferation in response to antigenic stimulation and reduced antibody production capability. Interestingly, mice with B cells that overexpress mTOR because of a TSC1 deletion (TSC1 normally inhibits mTOR) also demonstrate similar defects in B-cell differentiation and antibody production. Another indication for an mTOR role comes from the fact that activated B cells, like T cells, use glycolysis as a primary source of energy. These indications suggest that mTOR is likely to have a significant impact on B-cell activation, differentiation and function, but more in-depth studies are lacking to define the exact role of mTOR in B-cell mediated immunity (Geissler, 2013) [8].

- **In antigen-presenting cell(APC) development**

The mTOR pathway is also important for the differentiation and function of APCs. In particular, mTOR inhibition has a potent effect on the maturation of dendritic cells (DCs).

Differentiation into conventional, CD8+ and plasmacytoid DCs appears to depend on mTOR. Indeed, mice with uninhibited mTOR activity (via PTEN deletion) develop an abnormal highly expanded DC compartment suggesting that mTOR plays a critical role in maintaining APC homeostasis *in vivo*. Moreover, rapamycin treatment has a profound effect on APC function, in that co-stimulatory molecule expression is decreased, leading to an inhibited ability for APC to stimulate T-cell activation. Rapamycin-treated DCs are even known to induce tolerance in animal models, possibly through their ability to promote the development of Treg cells. In fact, researchers that are expanding Treg cells for the purpose of cell therapy often use rapamycin to produce a more stable Treg phenotype. Rapamycin has seemingly opposing effects on the early development of immune responses involving plasmacytoid DCs versus other DCs. While plasmacytoid DCs activated via toll-like receptors depend on mTOR to elicit type 1 IFN-based expression responses, lipopolysaccharide activation of monocytes and DCs leads to inhibition of a proinflammatory gene expression pattern through the mTOR pathway. mTOR inhibition could thus affect responses to bacterial challenges, especially in immunosuppressed transplant recipients. mTOR therefore has important eff ects on APC homeostasis and development (Geissler, 2013) [8].
Innate (e.g. TLRs) or adaptive signals (e.g. CD40) trigger the PI3 kinase-Akt-mTOR signaling cascade in the APCs. Activation of mTORc1 leads to the phosphorylation of 4E-BP1/2 and initiation of protein translation. Pathogenic virulence factors such as Gp63 and antibiotic rapamycin (RAPA) inhibit mTOR activation and hence downregulate translation of type I interferons and iNOS (inducible nitric oxide synthase). Inhibition of 4E-BP1/2 can selectively upregulate translation and hence may be an attractive drug target. mTOR activation can also upregulate anti-inflammatory molecule IL-10 and inhibits the proinflammatory molecules, such as IL-12. IL-10 may skew Th0 cells to the disease-promoting Th2/Treg cells, whereas IL-12 and other proinflammatory cytokines can enhance the Th1/Th17 axis. Activation of mTOR signaling by inhibition of TSC1/TSC2 (tuberous sclerosis complex) or inhibition of Rictor (rapamycin-insensitive companion of mTOR, an essential component of mTORc1 signaling), especially at the early stage of an infection, can boost the propensity of these cells to be skewed towards Th1 phenotype. mTOR inhibition of Treg cells by rapamycin can augment expansion of Treg cells with increased suppressive capacity. This can be prevented by the activation of mTOR by inhibiting TSC1/2 or PTEN (Phosphatase and TENsin homolog) and may be a lucrative drug target at the later stages of an infection. On the other hand, inhibition of mTOR signaling in memory cells can improve the memory cell differentiation. Blockade of mTOR by pharmacological and genetic ablation enhances the quality and quantity of surviving memory. Targeted inhibition of mTOR in memory cells can thus be an attractive drug target especially at the later stage of infection (Geissler, 2013) [8].

- **mTORC1 and mTORC2 together Regulate Helper T-Cell(Th) Development**

A series of genetic studies has revealed a central role for mTOR in regulating T helper cell differentiation. T cells lacking RHEB, and hence mTORC1 signaling, fail to differentiate into Th1 and Th17 cells under polarizing conditions. This failure to differentiate is associated with decreased STAT activation and decreased expression of lineage-specific transcription factors such as T-bet and RORγt (Valdes *et al*., 2011) [19]. Similarly, T cells lacking mTORC2 activity fail to differentiate into Th2 cells, due to decreased STAT6 activation, decreased PKC activity, and decreased GATA-3 expression under polarizing conditions. T cells lacking mTOR demonstrate increased SMAD3 activation and become Foxp3+ Tregs even under normally activating conditions (Powell *et al.*, 2012) [15].

mTOR and Cancer

The signaling pathways that activate mammalian target of rapamycin (mTOR) are altered and disregulated in many human cancers. The mammalian target of rapamycin (mTOR) and the phosphoinositide 3-kinase (PI3K) signaling pathways are commonly deregulated in cancers and promote cellular growth, proliferation, and survival.

mTOR Disregulation And Different Cacer Types

overactivation of mTOR due to dysregulation of upstream pathways, leading to abnormal activities in cell progression, angiogenesis, cell metabolism and apoptosis.

- **Renal Cell Carcinoma(RCC)**

mTOR controls production of HIF-1α, an important protein in RCC, where its unregulated activity is causally related to disease pathogenesis. mTOR regulates the production of several angiogenic growth factors in RCC (Martin *et al*., 2012) [13]. mTOR may control the ability of neovascular cells to respond to growth factors. mTOR controls cell growth and cell division in RCC and in cells of the tumor microvasculature, and is often dysregulated in renal cancer by signaling defects upstream of mTOR in the PI3K/Akt/mTOR pathway. mTOR regulates nutrient uptake and cell metabolism and contributes to the characteristic metabolic changes in RCC (Advani, 2010) [1].

- **Neuro Endocrine Tumor (NET)**

Several important molecular changes in NETs involve the mTOR pathway. Increased growth factor signaling, namely, epidermal growth factor (EGF) and insulin-like growth factor (IGF) signaling upstream of mTOR, has been observed frequently in NETs. Also, insulin secretion is believed to be involved in the autocrine activation of mTOR in pancreatic beta cell tumors. mTOR is activated by many gene mutations associated with NETs (germline deletion of the VHL gene). mTOR directs the supply of nutrients to cancer cells by regulating angiogenesis. NETs are highly vascular. VEGF expression has been observed in 80–86% of gastrointestinal carcinoid and pancreatic islet cell tumors (Advani, 2010) [1].

- **Gastric Cancer**

mTOR is activated in 60–80% of gastric adenocarcinomas and is expressed in early-stage and advanced-stage disease, in both diffuse and intestinal subtypes, and in tumor cells that invade lymphatic channels. The mTOR pathway is activated by multiple growth factor receptors, namely, epidermal growth factor receptor (EGFR), Human Epidermal growth factor Receptor 2 (HER2), insulinlike growth factor type 1 receptor (IGF-1R), that are overexpressed in many gastric tumors. mTOR regulates production of angiogenic factors

(VEGF/VEGFR) that promote new vessel formation and predict poor outcome in patients with gastric cancer. mTOR regulates nutrient uptake and cell metabolism and contributes to the characteristic metabolic changes in cancer. HIF-1α is expressed in most gastric cancers, and HIF-1α expression at the invading tumor edge is associated with advanced-stage disease, lymph node metastases, and poor survival (Advani, 2010) [1].

- **Breast Cancer**

mTOR signaling is critical in the pathogenesis of breast cancer. mTOR signaling may be related to estrogen receptor (ER) activation and adaptive estrogen hypersensitivity. mTOR pathway signaling is increased in HER2+ tumor cells resistant to endocrine therapy. mTOR activation predicts a worse clinical outcome for patients treated with endocrine therapy. mTOR controls the supply of nutrients to cancer cells by regulating nutrient uptake, cell metabolism, and angiogenesis (Seto, 2012) [16].

- **Hepato-Cellular Carcinoma (Hcc)**

mTOR-dependent signaling is active in 25–45% of hepatocellular carcinoma (HCC). Activation correlates with shorter overall survival; mTOR activation is an independent predictor of recurrence after surgery. mTOR regulates production of angiogenic factors. High VEGF levels have been associated with tumor cell proliferation, poor encapsulation of the tumor nodules, venous invasion, higher grade, and a poor prognosis following resection. mTOR activation through PI3K/ Akt pathway is associated with increased expression ofgrowth factors such as EGF, TGF-α, IGF, and hepatocyte growth factor (HGF) that promote HCC cell proliferation and survival (Seto, 2012) [16].

- **Lymphoma**

Approximately 85% of Non-Hodgkin lymphoma (NHL) s arise from cells of B-cell lineage.mTOR signaling is activated in Hodgkin lymphoma cell lines and primary tumors. Cyclin D1 overexpression is a characteristic feature of mantle cell lymphomas. PI3K and Akt overexpression is frequently observed in several B-cell lymphomas (Advani, 2010) [1].

Targeting of the mTOR Pathway by Cancerous cells:

Cancer cells require highly active metabolic processes to support rapid growth and proliferation, even in poor environmental conditions, such as hypoxia and energy depletion. Mammalian target of rapamycin (mTOR) and class I phosphoinositide 3-kinase (PI3K) are two major and inter-dependant oncogenic kinases that contribute to cancer biology through the synthesis of cellular components and the regulation of growth, proliferation, migration,survival, and angiogenesis (Tan and Yu, 2013) [18]. Aberrant activations of these pathways have been linked to cancer development and are frequently detected in malignancies (Seto, 2012) [16].

The recent development of new pharmacological compounds directed against the PI3K and mTOR kinases represents a major breakthrough in the field of targeted therapies. First-generation mTOR inhibitors, including rapamycin (sirolimus) and its derivatives (referred to as rapalogs: temsirolimus, everolimus, and ridaforolimus), are widely used in clinical trials as anticancer agents and some of them are already approved for the treatment of metastatic renal-cell carcinoma (temsirolimus, everolimus) and mantlecell lymphoma (temsirolimus). However, when used alone against other malignancies such as acute myeloid leukemia (AML) or breast cancer, these drugs have only modest *in vitro* and *in vivo* effects. Different mechanisms of resistance torapamycin have been recently identified, leading to the development of new agents. Among them, mTOR kinase inhibitors (TORK Inhibs) and dual PI3K/mTOR inhibitors have emerged (Willems *et al*., 2012) [21]. In simple words it can be said that cancerous cells target this mTOR pathways for growth, proliferation, etc., as these pathways are important in the regulation of growth, proliferation, migration, angiogenesis of cell, which are the characteristics of cancerous cellular biology (Easton and Houghton, 2006) [6].

How cancercerous cells target different mTOR signaling pathway complexes for growth and proliferation

In several ways cancerous cells target different mTOR signaling pathway complexes for growth and proliferation. These includes-

1. Suppression of PTEN function

PTEN is a tumor suppressor lipid phosphatase. PTEN function disrupted in cancerous condition. This loss of PTEN function in cancer can occur through mutation, deletion, or epigenetic silencing. Several studies have demonstrated a high frequency of PTEN mutations or deletions in a variety of human cancers, including brain, bladder, breast, prostate, and endometrial cancers. In tumor types where PTEN mutations are rare, such as lung cancer, epigenetic silencing may occur. Collectively, it can be said that the loss of PTEN is a common mechanism for activationof the PI3K/Akt/mTOR pathway and poor prognostic factor in human cancer (Lauring *et al*., 2013) [12].

2. Over-activation of PI3K

Over activation of PI3K has been described in human cancers. It can result from amplification, over-expression or from mutations in the p110 catalytic or p85 regulatory subunits of PI3K (Fregeau *et al*., 2011) [7]. It is usually caused by Amplification of the 3q26 chromosomal region, which contains the gene PIK3CA that encodes the p110 catalytic subunit of PI3K and mutations in the regulatory p85 subunit. Somatic mutations of this gene increases kinase activity of the mutant PI3K relative to wild-type PI3K (Willems *et al*., 2012) [21].

3. Inhibition of P70S6K protein downstream of mTORC1

Phosphorylated P70S6K protein(activated) downstream of mTORC1 exerts negative feedback on insulin and insulin-like growth factor 1 (IGF1) signaling through proteasomal degradation of IRS-1 and IRS-2, thus leading to PI3K/AKT down-regulation. But cancerous cells inhibit P70S6K that causes overactivation of PI3K through IRS/IGF1-R resulting in AKT overactivation and cancer progression. Because any of these alterations in individual components would result in activation of the pathway, these studies suggest that pathway activation is one of the most frequent molecular alterations in cancer (Willems *et al*., 2012) [21].

Cancer therapy by targetted inhibition of mTOR signaling complexes

The rationale for targeting the PI3K/Akt/mTOR pathway in

combination therapy is important in the cells that have developed resistance to conventional chemotherapy and radiation (Piccolo *et al*., 2007) [14] as well as to other targeted therapies such as EGFR antagonism. In these cases, combining chemotherapy or radiation with a pathway inhibitor can overcome these problems (Guertin and Sabatini, 2007) [9].

Cancer therapy by using mTOR pathway inhibitor

mTOR inhibitors have a broad therapeutic application across many tumor types. Combining pathway inhibitors with conventional chemotherapy and radiation cancerous cells can be eliminated. Several of these includes-

1. PI3K inhibitors

The main inhibitors of PI3K includes-

a) LY294002
b) Wortmannin

Targeting PI3 kinase, the most proximal pathway component, has advantages over targeting more distal components such as Akt and mTOR. Inhibitors of PI3K diminish signaling to Rac as well as Akt, providing a broader inhibition of downstream signaling than distal inhibition. The pharmacologic agents LY294002 and wortmannin both target the p110 catalytic subunit of PI3K. Although these commercially available inhibitors effectively inhibit PI3K, poor solubility and high toxicity have limited their clinical application. However, these compounds provide powerful preclinical tools to study the cellular consequences of pathway inhibition. Both of these inhibitors of PI3K sensitize cancer cells to various types of conventional chemotherapy. LY294002 increases cytotoxicity induced by antimicrotubule agent such as taxanes and vinca alkaloids in glioma, ovarian cancer, esophageal cancer, and lung cancer cells *in vitro* and *in vivo.* Wortmannin has also been shown to enhance apoptosis of several cell lines when used in combination with paclitaxel, cisplatin, gemcitabine, or 5-fluorouracil. Wortmannin can also increase the efficacy of chemotherapeutic agents *in vivo*. The treatment of human ovarian cancer xenografts with wortmannin plus paclitaxel increased apoptosis and decreased tumor burden compared to either agent alone (Piccolo *et al*., 2007) [14].

2. Akt inhibitors

There are several AKT inhibitors present. Among them

a) Perifosine

To date, the most developed inhibitor of Akt is perifosine, which is a lipid-based inhibitor *In vitro*, perifosine inhibits translocation of Akt to the cell membrane, and inhibits the growth of melanoma, lung, prostate, colon, and breast cancer cells in association with inhibition of Akt activity. Additional *in vitro* data demonstrates synergistic effects of perifosine and traditional chemotherapeutic agents such as etoposide in leukemia cells, doxorubicin in MM cells, and temozolomide in glioma cells. The combination of perifosine and temozolomide was more effective than temozolomide alone in inhibiting growth of glioma xenografts. Perifosine has also been found to sensitize cancer cells to apoptosis and cell cycle arrest induced by radiation *in vitro* and *in vivo* (Guertin and Sabatini, 2007) [9].

b) PIA

A relatively new group of lipid-based Akt inhibitors are the phosphatidylinositol ether lipid analogues (PIAs). PIAs were designed to interact with the PH domain of Akt and are structurally similar to the products of PI3 kinase. Although less clinically developed, PIAs are well characterized *in vitro* (Piccolo *et al*., 2007) [14].

c) Triciribine or API-2

Triciribine inhibits Akt2 phosporylation at both sites (T309 and S474) and inhibits EGF-induced phosphorylation of all three isoforms of Akt *in vitro*. *In vivo*, treatment with low doses of triciribine stimulated apoptosis in xenografts with constitutively activated Akt or PTEN mutations, but not in tumors with low Akt activity. Triciribine has not been preclinically combined with standard chemotherapies or radiation (Piccolo *et al*., 2007) [14].

3. mTOR inhibitors

The most important mTOR inhibitor includes

a) Rapamycin and its analogues

The first clinically relevant mTORC1 inhibitors were the rapalogs. These molecules specifically bind to FKBP-12 and then this complex associates to the FRB domain of mTOR and disrupts the raptor/mTOR association (Zaytseva *et al*., 2012) [23]. Their action is mainly restricted to mTORC1 although mTORC2 may be also inhibited with delay depending on the cell type and the concentration of the drug. Some of these first-generation mTOR inhibitors are already approved for the treatment of renal-cell carcinoma and mantle-cell lymphoma and multiple trials of these agents, alone or in association with chemotherapy, are currently underway (Willems *et al*., 2012) [21].

4. mTOR Kinase Inhibitors

ATPcompetitive inhibitors of mTOR named the TOR-Kinhibs (for mTOR kinase inhibitors), including Torin1, PP242, PP30, Ku-0063794, and AZD8055 (AstraZeneca), that have good affinity toward mTOR. TOR-Kinhibs prevent feedback-mediated AKT activation via direct inhibition of mTORC2 and cause more potent inhibition of mTORC1 activity than rapalogs, resulting in more effective inhibition of protein translation (Willems *et al*., 2012) [21].

5. mTOR/PI3K Dual Inhibitors

Dual PI3K/mTOR inhibitors are more effective but also more Toxic that rapamycin. However, they could more efficiently block AKT activity than pure ATP-competitive mTOR inhibitors. PI-103 and NVP-BEZ235 are two mTOR/PI3K dual inhibitors tested in preclinical studies in a wide range of tumors that efficiently inhibit AKT phosphorylation both on T308 and S473 (Piccolo *et al*., 2007) [14].

Clinical Relevance of mTOR Inhibition in Cancer Therapy

Because the number of potential defects that can cause inappropriate activation of mTOR is large and one or the other is common to most cancer cells, blocking their effect at the point of convergence is a rational approach. The mTOR protein itself is seldom altered, suggesting that mTOR is a stable target for influencing several important pathways in cancer. Furthermore, identifying these molecular defects in a tumor may provide the biomarkers that determine whether the cancer will be sensitive to mTOR inhibition and help select the most appropriate treatment strategy (Watanabe and Huang,

2011) [20]. Thus many clinical studies have supported preclinical findings regarding the importance of mTOR in cancer and validate mTOR inhibition as an effective cancer therapy. Given that the mTOR pathway is deregulated in a number of cancers, it is anticipated that mTOR inhibitors will have broad therapeutic application across many tumor types. It is possible that only a subset of cancer patients will have tumors sensitive to mTOR inhibitors as a monotherapy. Based on preclinical findings, mTOR inhibitors may be more efficacious when used in rational combination with other cancer regimens with activities supplemental to and/ or influenced by mTOR activity, such as DNA-damaging and hormonal agents, oncogene inhibitors, and other therapies. Thus, mTOR inhibitors may play an important role in the management of cancer. mTOR inhibition has been proven to result in reduced tumor cell growth and proliferation, decreased tumor angiogenesis and inhibition of cell metabolism (Watanabe and Huang, 2011) [20].

Conclusion

The most recent data suggest that mTOR acts as a central node for coordinating activities of the most important cells (T cells, B cells and APCs) forming the immune response to various challenges. Interestingly, some of these effects inhibit an immune response, and other effects actually promote immunity; the setting of the antigenic challenge appears to be crucial, since energy availability, signalling cues and cell activation all converge to at least some degree upon mTOR. Although early evidence suggests that mTOR inhibitors have the potential to promote an immune response against an infectious microorganism or tumour entity, and can paradoxically function to inhibit immunity against an organ allograft, further research is needed to untangle the operative mechanisms and to ultimately explore the full potential of mTOR inhibitors in the setting of organ transplantation.

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