mTOR Signalling: Jack-of-All-Trades

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Summary

The mechanistic target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that senses and integrates environmental information into cellular regulation and homeostasis. Accumulating evidence has suggested a master role of mTOR signaling in many fundamental aspects of cell biology and organismal development. mTOR deregulation is implicated in a broad range of pathological conditions, including diabetes, cancer, neurodegenerative diseases, myopathies, inflammatory, infectious and autoimmune conditions. Here, we review recent advances in our knowledge of mTOR signaling in mammalian physiology. We also discuss the impact of mTOR alteration in human diseases and how targeting mTOR function can treat human diseases.

Résumé

Mechanistic target of rapamycin (mTOR) est une serine/thréonine kinase qui joue un rôle essentiel dans l'homéostasie cellulaire en réponse aux instructions environnementales. En effet, au cours des deux dernières décennies, plusieurs études ont clairement montré le rôle de mTOR dans plusieurs fonctions cellulaires impliquées dans la régulation cellulaire (par exemple : la synthèse des protéines et des lipides) et de l'organisme (par exemple : système musculaire et immunitaire). Cependant, quand dérèglementée, mTOR est à nouveau impliquée dans plusieurs maladies cliniques (par exemple : le diabète et le cancer). Dans cette revue, nous exposant la structure fonctionnel de mTOR ainsi que les mécanismes d'activation de ses voies de signalisation, nous discutant les fonctions physiologiques mammaires régulées par

34 mTOR, et nous résumant l'actualité sur les avantages et les désavantages de la
35 pharmacologie des thérapies innovante ciblant la fonction de mTOR.

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I. Introduction

An important homeostatic mechanism in the growth and development of all life forms is the cell's ability to sense and respond to surrounding nutrient supplies (Efeyan et al. 2015; Laplante and Sabatini 2012a). Nutrient sensing pathways promote anabolism and storage of biomolecules in the presence of an abundance of nutrients. However, during nutrient starvation, cells engage in catabolic processes to deplete nutrient storage. Starvation also initiates autophagy to regenerate nutrients when nutrient storage is depleted. Maintaining the balance between anabolic and catabolic processes in response to environmental cues is crucial in regulating the cell's decision to switch from proliferation to apoptosis and from hormone secretion to cell migration (Dibble and Manning 2013; Hatzoglou et al. 2014). Therefore, it is not surprising that alteration in the mechanism responsible for integrating extracellular and intracellular information about nutrients is often associated with the incidence of many diseases, including cancer (Laplante and Sabatini 2012b; Saxton and Sabatini 2017).

One of the most important nutrient sensors is the target of rapamycin (TOR) kinase, more commonly known as the mechanistic TOR (mTOR) (Brown et al. 1994; Sabatini et al. 1994; Sabers et al. 1995). As its name implies, this kinase is the physical target of rapamycin, a molecule which is now considered to be a key player in cell growth and a therapeutic drug in the treatment of cancer (Babcock and Quilliam 2011; Fasolo and Sessa 2008; Liu et al. 2017; Rubio-Viqueira and Hidalgo 2006; Wu and Liu 2013). Identified first in the *Saccharomyces cerevisiae* (Sehgal et al. 1975; Vezina et al. 1975) and then found in mammalian cells (Brown et al. 1994; Sabatini et al. 1994; Sabers et al. 1995), mTOR is a highly conserved serine/threonine kinase that functions as a

master regulator of cell growth and development by sensing and integrating diverse nutritional and environmental cues, such as amino acids, energy levels, and growth factors. In this review, we provide a summary of our current understanding of the mTOR pathway and its role in cell physiology, particularly in cancer development.

II. mTOR structure and cascade pathways

Early work investigating the mTOR signalling network relied on pharmacological observations to decipher the intricacies of this pathway. By using rapamycin, the direct inhibitor of mTOR function, mTOR was first described as having only one component composed of 5 domains: 1) the Huntingtin, Elongation factor 3, PR65/A subunit of protein phosphatase 2A, TOR (HEAT) repeats (HR) domain at the N-terminal involved in protein-protein interactions, 2) the FKBP12-rapamycin-associated protein/TOR (FRAP), ATM, TRRAP (FAT) domain, 3) the FKBP12 rapamycin-binding (FRB) domain, 4) the kinase domain that mediates mTOR activity, and 5) the FATC domain found at the C-terminal, which is important in the structural stability of the mTOR protein (Figure 1A) (Yang et al. 2013; Yang and Guan 2007). However, with the introduction of RNA interference technology, scientists have discovered that mTOR exists in two different multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Figure 1B and 1C) (Sarbasov et al. 2005). mTORC1 is composed of three principal proteins: 1) mTOR, 2) a regulatory protein associated with mTOR (Raptor), and 3) mammalian lethal with Sec13 protein 8 (mLST8), also called G protein β -subunit-like protein (G β L) (Hara et al. 2002; Kim et al. 2002; Kim et al. 2003; Nojima et al. 2003; Yang et al. 2016). Raptor functions as a docking protein facilitating the binding with two

regulatory associated proteins: the proline-rich Akt substrate (PRAS40) and the DEP domain containing the mTOR interaction protein (DEPTOR), while mLST8 binds to and stabilizes the catalytic domain of mTOR (Yang et al. 2013). Similarly, mTORC2 also consists of three main proteins: 1) mTOR, 2) rapamycin insensitive companion of mTOR (Rictor) and 3) mLST8. Like Raptor in mTORC1, Rictor facilitates the binding and interaction of regulatory proteins on mTORC2. These regulatory proteins include DEPTOR (Peterson et al. 2009), protein observed with Rictor-1 and -2 (Protor1/2) (Pearce et al. 2007; Pearce et al. 2011; Thedieck et al. 2007) and the mammalian stress-activated protein kinase-interacting protein 1 (mSin1) (Frias et al. 2006; Lu et al. 2011; Yao et al. 2017). With the exception of Rictor, all components of both mTORC1 and mTORC2 are highly conserved in all eukaryotes.

In addition to the structural difference between mTOR complexes, they are also functionally distinct, as reflected by differences in their pharmacology, the upstream signals they integrate, the substrates they regulate, and the biological processes they control (Caron et al. 2010). For example, mTORC1 is sensitive to rapamycin whereas mTORC2 is not (Jacinto et al. 2004; Sarbassov et al. 2004); yet, sustained rapamycin treatment can also inhibit mTORC2 through the impairment of the complex stability (Sarbassov et al. 2006). Additionally, while mTORC1 mediates a wide variety of cellular processes, such as protein synthesis, autophagy, cell growth, and cell proliferation, mTORC2 regulates the activity of the AGC (protein kinase A, PKG, PKC) kinase family and controls the actin cytoskeleton dynamics (Cargnello et al. 2015; Huang and Fingar 2014; Julien and Roux 2010; Saxton and Sabatini 2017). However, many aspects of

104 the function and regulation of the components forming the mTOR complexes remain
105 unknown (Figure 1B and 1C).

106 mTORC1 signaling plays a central role in cell growth through the regulation of the
107 activity of many effectors (Saxton and Sabatini 2017). Major downstream targets of
108 mTORC1 are the ribosomal protein p70 S6 kinase 1 (S6K1) (Hannan et al. 2003; Holz
109 et al. 2005; Nemazanyy et al. 2004) and the Eukaryotic Initiation Factor 4E (eIF4E)
110 Binding Protein 1 (4EBP1) (Fingar et al. 2002; Hsieh et al. 2010; Livingstone et al.
111 2009). When activated, mTORC1 phosphorylates S6K1 (threonine 389) and 4EBP1
112 (multiple sites). This further activates downstream substrates that trigger mRNA
113 translation (Choo et al. 2008), subsequently leading to protein synthesis (Dorrello et al.
114 2006; Ma et al. 2008). mTORC1 also regulates protein recycling and turnover by
115 controlling the activity of the transcription factor EB (TFEB) and the extracellular signal
116 regulated kinase 5 (ERK5) (Rousseau and Bertolotti 2016), both of which are important
117 in the regulation of the autophagy machinery (Martin et al. 2012; Zhao et al. 2015). In
118 addition, mTORC1 facilitates the activity of Hypoxia-Inducible Factor 1(HIF-1) through
119 increasing its translation (Hudson et al. 2002) and the production of Lipin1, a
120 phosphatidic acid phosphatase (Peterson et al. 2011), driving the expression of many
121 glycolytic enzymes essential in the generation of metabolites that impact cell growth
122 (Duvel et al. 2010).

123 mTORC1 is also subjected to many upstream regulators (Corradetti and Guan 2006;
124 Saxton and Sabatini 2017). For example, activation of the phosphatidylinositol 3-kinase
125 (PI3K)-Akt (also called PKB) pathway stimulates mTORC1 through regulating a
126 signaling cascade. A small G protein Ras homolog enriched in brain (Rheb) is

considered as the direct upstream of mTOR. It stimulates mTOR through an unknown mechanism. Rheb is regulated by the tumour suppressor gene tuberous sclerosis complex 1/2 (TSC1/2) that functions as a guanine triphosphate (GTP)ase activating protein (GAP) for Rheb (Dibble et al. 2012; Inoki et al. 2005; Inoki et al. 2003a; Li et al. 2004; Santiago Lima et al. 2014; Shah et al. 2004; Tee et al. 2003; Yang et al. 2006). When active, mTORC1 can also suppress PI3K activity by a negative feedback loop that is at least in part mediated by S6K1. In addition, amino acids regulate mTORC1 activity independently of TSC1/2 by a mechanism involving the Ragulator and Rag GTPases (Bar-Peled et al. 2012; Efeyan et al. 2013). Furthermore, TSC represents a crossroad for mTORC1 signaling in response to other cellular processes. For example, glucose deprivation activates the 5' adenosine monophosphate-activated protein kinase (AMPK) due to an increase in AMP/ATP ratio. This in turn inhibits mTORC1 through TSC2 activation (Gwinn et al. 2008).

mTORC2 signaling represents a major signal network essential for cell survival, mainly through the phosphorylation of several members of the AGC protein kinase family, such as PKB/Akt, PKC, and serum- and glucocorticoid-regulated kinase (SGK) (Cameron et al. 2011; Heikamp et al. 2014). PKB/Akt is considered as the most important component in mTORC2 signaling (Guertin et al. 2006; Polak and Hall 2006). Once activated, PKB/Akt promotes and inhibits the expression of many substrates including TSC2 (Guertin et al. 2006). On the other hand, PKC activation by mTORC2 plays a central role in cell differentiation and insulin secretion (Xie et al. 2017; Yamada et al. 2010), as well as in maintaining many aspects of the cytoskeleton dynamic and regulating cell migration (Gan et al. 2012; Jacinto et al. 2004). Finally, mTORC2 controls the activity of

SGK1, an important regulator of sodium homeostasis and cell survival (Garcia-Martinez and Alessi 2008; Lang and Pearce 2016; Yan et al. 2008). mTORC2 is also activated by growth factors through PI3K signaling. However, the mechanism by which mTORC2 activity is regulated in response to PI3K signaling is currently unclear.

III. Major cellular processes controlled by mTOR

Due to its ability to integrate environmental cues and coordinate the subsequent metabolic changes within the cell, mTOR signalling is central in the regulation of many cellular processes and, to an extent, acts as a determinant of cell survival. As such, this section of the review aims to summarize current knowledge about the major physiological roles of mTOR.

Neuronal function: Although neurons are postmitotic (non-proliferative), their proper function often requires a significant change in size and shape, both of which are controlled by mTOR complexes (Kwon et al. 2003; Lipton and Sahin 2014). Numerous studies have demonstrated the crucial role of mTOR in dendrite or spine development and circuit establishment. Pharmacological (rapamycin treatment) or genetic (RNA interference) inhibition of mTOR causes a decrease in the total number, volume and expansion of dendritic tree (Jaworski et al. 2005a; Jaworski et al. 2005b). Rapamycin treatment also results in a decrease in both filopodia and spine numbers (Kumar et al. 2005). In addition to its role in neuron morphology, mTOR has also emerged as an essential regulator of many neurological functions including synaptic plasticity (Tang et al. 2002), learning and memory formation (Jobim et al. 2012a; Jobim et al. 2012b; Qi et al. 2010; Tischmeyer et al. 2003), and neuronal control of nutrient uptake (Cota et al.

2006; Morrison et al. 2007). On the one hand, mTOR inhibition has been shown to prevent the formation of fear memories and impair synaptic plasticity in mouse models (Parsons et al. 2006), as well as reduce food intake and body weight in rats (Guzman-Quevedo et al. 2013; Rovira et al. 2008). On the other hand, hyperactive mTOR is often associated with a range of debilitating neurological disorders including autism, epilepsy, Parkinson and Alzheimer diseases (Swiech et al. 2008). In line with this, accumulating evidence indicates that mTOR inhibition has beneficial effects on mouse models of Alzheimer disease (Pei and Hugon 2008; Spilman et al. 2010; Wang et al. 2014). Given that autophagy is important for the clearance of aggregate-prone proteins and damaged organelles that exist in many neurodegenerative disorders (Frake et al. 2015; Nixon 2013), by facilitating autophagy, mTOR inhibition might be beneficial for common neurodegenerative disorders.

Immune function: In addition to its role in cell proliferation, rapamycin has also been found to have potent immunosuppressive activity (Delgoffe and Powell 2009; Powell et al. 2012). The immunosuppressive effect of rapamycin is mediated by its ability to inhibit the maturation, function and activation of T cells, as well as interfering with the differentiation and expansion of CD4⁺FoxP3⁺ Regulatory T cells and CD8⁺ memory T cells (Araki et al. 2009; Araki et al. 2010; Haxhinasto et al. 2008). It is presumed that mTOR senses the immune microenvironment to facilitate the switch between anabolism and catabolism, thereby regulating the differentiation, function and activation of immune cells (Mills and Jameson 2009; Weichhart et al. 2015; Weichhart and Saemann 2009). Currently, some mTOR inhibitors have been approved in the treatment of kidney transplant patients (Buchler 2009; McMahon et al. 2011; Pape and Ahlenstiel 2014;

Yost et al. 2011) and for cancer (Andrassy et al. 2005; Basu et al. 2012; Geissler et al. 2008). Thus, a comprehensive understanding of mTOR signalling in the immune system is key for the development of new strategies to improve transplantation protocols, promote vaccine response and enhance the efficacy of cancer therapies.

Skeletal muscle function: Aside from its involvement in modulating neuronal activity and immune functions, early studies revealed a role of mTOR in promoting muscle growth (Esser 2008; Hornberger et al. 2006; Lawrence 2001). For example, it has been shown that mTOR activation in response to growth factors or amino acids (e.g. leucine) enhances muscle hypertrophy in both *in vitro* and *in vivo* models (Chen et al. 2011; Murgas Torrazza et al. 2010). Conversely, the deletion of Raptor resulted in the severe loss of body weight due to muscle atrophy in mice. A similar phenotype is also observed in muscle-specific mTOR knockout mice but not in Rictor-deficient mice, which suggests a critical role of mTORC1 in muscle function (Bentzinger et al. 2008; Lopez et al. 2015). Additionally, accumulating evidence suggests that muscle contraction can also activate mTOR (Baar and Esser 1999; Baar et al. 2002). While the mechanism is not clear, studies have demonstrated that mechanical stimuli modulate the mTOR signaling pathway by inducing multisite phosphorylation of Raptor (Frey et al. 2014). Because skeletal muscles play an important role in locomotion and whole-body metabolism, and because the loss of skeletal muscle mass is associated with a wide array of diseases including myopathies, cancer, AIDS, liver cirrhosis, kidney failure, heart failure, sepsis, obesity, aging, lysosomal storage diseases and diabetes (Dudgeon et al. 2006; Fearon et al. 2013; Lim et al. 2017; Moylan and Reid 2007; Risson et al. 2009; Yoon 2017), understanding how mTOR senses and processes distinct cues in muscles (e.g. growth

factor, amino acids) and how muscles translate this information into muscle growth and function may benefit patients with these conditions.

Glucose homeostasis: Glucose deprivation serves as the trigger for the switch from anabolism to compensatory cellular processes to maintain whole-body homeostasis. These processes involve the induction of glycogenolysis, autophagy and the production of alternative energy sources, such as ketone bodies that can be used as substrates for anabolism (e.g. the synthesis of lipids such as cholesterol), for peripheral tissues. It is suggested that glucose deprivation results in a reduction of the production of cellular ATP, which leads to an increase in the AMP/ATP ratio and subsequent activation of the metabolic regulator AMPK pathway (Carling et al. 2011; Gowans and Hardie 2014; Hardie 2011; Hue and Rider 2007). Once activated, AMPK inhibits mTOR directly via the phosphorylation of Raptor and indirectly through the activation of TSC2 (Dong et al. 2013; Kudchodkar et al. 2007; Shi et al. 2017). Accumulating evidence has demonstrated the key role of mTOR complexes in the generation of ketone bodies, which serve as an alternative energy source during glucose deprivation (Havel 2001; Laplante and Sabatini 2012b; Miniaci et al. 2015) by activating the master transcriptional activator of ketogenic genes peroxisome proliferator activated receptor α (PPAR α) – also known as the fasting modulator gene (Sengupta et al. 2010). For instance, Sengupta et al. have shown that liver-specific loss of TSC1 undergo sustained mTOR activation causing a decreased PPAR α and subsequent reduction in the production of ketone bodies under fasting conditions (Inoki et al. 2003b; Sengupta et al. 2010). In addition to its role of providing alternative sources of energy to cells, mTOR plays a crucial role in glucose homeostasis by directly controlling pancreatic β -

cell function (Bussiere et al. 2006; Cai et al. 2008; Gleason et al. 2007). Indeed, published literature revealed that TSC2 deletion is associated with the hyperactivation of mTOR, which leads to an increase in β -cell mass and insulin levels and improved glucose tolerance (Mori et al. 2009). In contrast, aberrant mTOR activity can reduce β -cell mass, lower insulin levels and trigger hyperglycemia in adult TSC2 depleted mice (Koyanagi et al. 2011; Shigeyama et al. 2008). Thus, discovering the best strategy to manipulate mTOR activity might open the door for a relatively new area of research to improve β -cell function in pathological conditions such as those associated with diabetes.

Lipid homeostasis: In recent years, mTOR has emerged as the principal regulator of various aspects of lipid metabolism, including lipogenesis and lipolysis in response to nutrition fluctuation (Caron et al. 2015; Lamming and Sabatini 2013; Laplante and Sabatini 2009; Soliman 2011). The sterol regulatory element-binding protein (SREBP) is considered to be the main transcription factor involved in lipid synthesis. Under normal conditions, SREBP resides in the endoplasmic reticulum membrane in its inactivate form. In response to starvation, SREBP is cleaved to its mature form, mSREBP, which translocates into nuclei and triggers lipogenic gene induction (Horton et al. 2002a, b). Accumulative evidence suggests that mTOR regulates the translocation and activation of SREBP, potentially through lipin1 (Horton et al. 2002a, b; Peterson et al. 2011; Quinn and Birnbaum 2012). mTORC1 activation phosphorylates cytoplasmic lipin1, leading to nuclear SREBP binding to lipogenic target genes to increase lipogenesis. In contrast, mTORC1 inactivation results in nuclear entry of lipin1, inhibiting SREBP binding to target genes and thus suppressing lipogenesis. Consistently, rapamycin treatment

prevents nuclear accumulation of SREBP and inhibits the expression of lipogenic SREBP target genes in both cultured cells and mice (Bentzinger et al. 2008). This result strengthens the findings showing that Raptor deletion is associated with a decrease in SREBP activation and a reduction in the expression of lipogenic genes (Porstmann et al. 2008; Porstmann et al. 2009).

IV. The role of mTOR in cancer and its potential as a cancer therapeutic

In normal cells, mTOR can translate environmental instructions into a balance between proliferation and apoptosis. However, in cancer cells, mTOR does not rely on environmental instructions, and it is often constitutively activated to promote uncontrolled tumour growth and proliferation (Saxton and Sabatini 2017; Weichhart 2017; Zarogoulidis et al. 2014). The observed mTOR hyperactivity in cancer cells is likely due to many deregulated oncogenic pathways, such as PI3K/Akt, and mutated tumour suppressors, such as TSC1/2 (Habib and Liang 2014; Taneike et al. 2016; Yan et al. 2006; Zoncu et al. 2011). The conjunction of these components maintains high mTOR activity that is necessary to promote the synthesis of proteins and lipids required for the high rate of tumour growth. Emerging evidence further suggests that a diverse set of cancer-associated mutations in mTOR itself also contributes to the constitutive activation of mTOR observed in many cancers (English et al. 2013; Ghosh et al. 2015; McCubrey et al. 2012; Murugan et al. 2013; Rejto and Abraham 2014; Striano and Zara 2012). So far, 33 mTOR activating mutations have been identified. The activating mTOR mutations can be in one amino acid (e.g. E1799K) or in many different residues (e.g. S2215F/P/T/Y). Different mTOR mutations can also be particularly prevalent

288 depending on cancer types. For instance, 31% of mTOR mutations are found in the
289 C1483 cluster in renal cancers and 22% of mTOR mutations are found in the S2215
290 cluster in colorectal cancers (Grabiner et al. 2014). Some mutations alter protein-protein
291 interactions between mTOR and its signalling partners; thus, shaping the outcome of
292 the mTOR signalling cascade (Cerami et al. 2012; Gao et al. 2013). For example,
293 mutations in A1459P and C1483Y activate the FAT domain of mTOR but strongly
294 reduce the interaction between mTOR and DEPTOR (Grabiner et al. 2014). Some other
295 mTOR mutations alter the sensitivity of mTOR signalling to amino acids and glucose
296 deprivation (Grabiner et al. 2014; Sato et al. 2010; Yamaguchi et al. 2015).

297 Because of the importance of mTOR in many aspects of cancer development and
298 progression, the therapeutic potential of mTOR-dependent signalling in cancer
299 therapies has been suggested (Ciuffreda et al. 2010; Houghton and Huang 2004;
300 Huang and Houghton 2003; Hudes 2007; Petroulakis et al. 2006; Xie et al. 2016). Given
301 its direct and specific inhibitory effect on mTOR, rapamycin was first proposed as a
302 promising anticancer drug. However, its poor solubility and low bioavailability renders it
303 inefficient for the treatment of cancer and leads to the development of numerous
304 rapamycin derivatives known as rapalogs, two of which have already been approved by
305 the FDA for the use in cancer therapy (temsirolimus and everolimus since 2007 and
306 2009, respectively)(Dufour et al. 2011; Hudes et al. 2007; Motzer et al. 2008; Waldner
307 et al. 2016). For example, everolimus was approved for patients with progressive
308 neuroendocrine tumors of pancreatic origin (Feldmann et al. 2012; Thompson et al.
309 2012; Vidal et al. 2017; Yao et al. 2013; Yao et al. 2011). This rapalog has also shown
310 potential in treating patients with advanced gastric cancer (Cejka et al. 2008; Doi et al.

2010; Ohtsu et al. 2013; Taguchi et al. 2011) and advanced non-small cell lung cancer (Besse et al. 2014; Dong et al. 2012; Singhal et al. 2015). Another example is temsirolimus, which was first approved for advanced renal cell carcinoma (Kwitkowski et al. 2010), and then for many types of advanced recurrent endometrial cancer (Emons et al. 2016; Fury et al. 2012; Galanis et al. 2005; Goodwin et al. 2013; Oza et al. 2011). Rapalogs inhibit the development of tumors associated with not only mTOR mutations but also mutations in mTOR upstream regulators. For example, breast cancer cells harboring PI3K mutations are sensitive to everolimus (Di Nicolantonio et al. 2010; Meric-Bernstam et al. 2012; Weigelt et al. 2011). Temsirolimus has also been found to preferentially block the progression of cancer cells with mutations in Phosphatase and tensin homolog (PTEN), the negative regulator of PI3K (Blando et al. 2009; Motzer et al. 2008).

However, the beneficial effects of rapalogs in pre-clinical models have been translated to only a few benign and malignant cancers. Even in the case of a positive impact on tumor growth, the tumors returned to their original states upon the cessation of the rapalogs' treatment (Bissler et al. 2013). The modest effectiveness of rapalogs in the clinic may be explained by several factors. First, rapalogs do not completely suppress mTOR substrates (Choo et al. 2008; Feldman et al. 2009; Thoreen et al. 2009; Thoreen and Sabatini 2009). For example, the protein synthesis regulator 4EBP1 is largely insensitive to rapamycin (Livingstone and Bidinosti 2012). Secondly, the ability of rapalogs to induce autophagy contributes to the maintenance of cancer survival under nutrition scarcity in tumour environments (Chagin 2016; Palm et al. 2015; Ye et al. 2015). In order to overcome some of these limitations, combinations of rapalogs with

334 autophagy inhibitors and/or chemotherapeutic agents have been proposed as a
335 potential strategy to improve the efficacy of rapalogs in cancer therapies (Lei et al.
336 2008; Mondesire et al. 2004; Rangwala et al. 2014; Shigematsu et al. 2010; Yardley
337 2013). For example, everolimus in combination with the aromatase inhibitor,
338 exemestane, is approved by the FDA for the treatment of patients with advanced breast
339 cancer (Ballatore et al. 2016; Dhillon 2013; Piccart et al. 2014; Yardley et al. 2013).
340 There are also phase I clinical trials using temsirolimus and an autophagy inhibitor,
341 hydroxychloroquine, in patients with advanced solid tumors and melanoma (Rangwala
342 et al. 2014). (Rangwala et al. 2014; Vogl et al. 2014). In addition, many combination
343 therapies with rapalogs and several chemotherapeutic agents, including paclitaxel
344 (Shafer et al. 2010; Yardley et al. 2015), doxorubicin (Dai et al. 2014; Numakura et al.
345 2014) and capecitabine (Kordes et al. 2015; Vidal et al. 2017), are currently undergoing
346 clinical trials for use in the treatment of various cancers. Unfortunately, rapalog-based
347 therapies have been associated with many substantial side-effects (Li et al. 2014).
348 These include frequent gastrointestinal effects, such as vomiting, nausea, and diarrhea
349 (Sasongko et al. 2015; Sasongko et al. 2016), impaired wound healing (Nashan and
350 Citterio 2012; Weinreich et al. 2011), and altered insulin signaling and glucose uptake
351 (Pereira et al. 2012). The lipid profile alteration (hyperlipidemia) (Waldner et al. 2016)
352 and the induction of auto-immune diseases (thrombocytopenia) (Lamming et al. 2013)
353 are also frequently seen in rapalog-based therapies. To circumvent the side-effects of
354 mTOR-targeted therapies and to promote their effectiveness, second- and third-
355 generation rapalogs have been developed (Renna 2016; Rodrik-Outmezguine et al.
356 2016). The second generation of rapalogs consists of a number of ATP-competitive

mTOR inhibitors that are now under clinical trial. The third-generation rapalogs are also ATP-competitive mTOR inhibitors and are chemically linked to rapamycin, so called “RapaLink”. Unlike rapamycin, which specifically inhibits mTORC1, the new generation of rapalogs and Rapalink fully suppress both mTORC1 and mTORC2. This offers larger, more durable and continued mTOR inhibition, and, therefore, a better chance to improve cancer therapies.

V. Concluding remarks

After more than two decades of extensive research, it is now well recognized that mTOR is the master regulator of many cell functions, in particular, their response to environmental stimuli. Thus, understanding the intricacies of mTOR signaling is essential for the development of effective treatment strategies for mTOR-related diseases such as cancer. Although research has taken a big leap forward in terms of our understanding of the underlying mechanism of mTOR signaling and its function at both the physiological and pathophysiological levels, a gap still exists between these molecular insights and efficient mTOR-based therapies. In spite of the limitations of mTOR-targeted therapies, rapalog remains one of the drugs currently used for the treatment of many diseases. Due to the ubiquitous expression of mTOR in human tissues and many downstream targets, inhibition of this protein by rapalog leads to numerous off target effects. Therefore, research efforts should focus on the development of new drugs against proteins that are specifically expressed in cancer cells or activated in tumor microenvironments. Such approaches will likely pave the way to harvest the full therapeutic potential of this major signalling pathway.

380

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Figure legends

Figure 1. Schematic presentation of mTOR structure and functions. (A) The domain structure of mTOR is composed of HEAT (Huntington, EF3A, ATM, TOR) repeats (HR) and FAT domain (named after FRAP, ATM and TRRAP) followed by FRB (a unique feature of mTOR that serves as the binding site for the inhibitory FKBP12 rapamycin complex), a kinase domain and the FATC (FAT C-terminus) domain. (B, C) Composition of mTORC1 (B) and mTORC2 (C), major cellular downstream and their roles in mRNA translation, metabolic homeostasis, protein turnover, apoptosis, glucose metabolism and cell migration. mTORC1 is composed of mTOR and Raptor (regulatory-associated protein of mTOR), which mediates physical links of mTOR to its binding substrates and negative regulators including DEPTOR (DEP-domain-containing mTOR-interacting protein) and PRAS40 (proline-rich AKT substrate 40 kDa, also known as AKT1S1). mLST8 (mammalian lethal with SEC13 protein 8) binds to the mTOR kinase domain in both complexes, with a crucial role in protein-protein interaction. mTORC1 promotes cell growth through a broad range of mitotic signaling pathways including S6K1 (ribosomal protein p70 S6 kinase 1), Eukaryotic Initiation Factor 4E (eIF4E) Binding Protein 1, Lipin 1, Hypoxia-Inducible Factor 1(HIF-1), the transcription factor EB (TFEB), and the Extracellular-signal-regulated kinase 5 (ERK5). mTORC2 is composed of mTOR and Rictor (rapamycin-insensitive companion of mTOR), which functions as a docking protein important for mTOR binding with its regulators. These regulators include the inhibitory regulator DEPTOR and the stimulatory regulators Protor1 (protein observed with Rictor-1 and -2) and mSIN1 (mammalian stress-activated protein kinase

1108 interacting protein). mTORC2 promotes cell survival primarily through phosphorylating
1109 several members of the AGC (protein kinase A, PKG, PKC) family of protein kinases.

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