The Expanding Role of the mammalian Target of Rapamycin Complex 2 (mTORC2) in Cellular Metabolism

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Abstract

The mammalian Target of Rapamycin (mTOR) nutrient-sensing pathway plays a fundamental role in cell growth, and proliferation, as well as in tumor metabolism. mTOR is the catalytic subunit that nucleates two functionally and structurally distinct complexes namely; mTORC1 (mammalian Target of Rapamycin Complex 1) and mTORC2 (mammalian Target of Rapamycin Complex 2).

mTORC1 integrates inputs from nutrients, growth factors, and environmental cues; and transduces signals to downstream targets to promote anabolism and decrease catabolism. As such, mTORC1 is considered a master regulator of metabolism, cell growth, and proliferation. mTORC1 is activated by PI3K (phosphoinositide 3-kinases)-Akt/PKB (protein kinase B) and Ras-ERK (extracellular signal-regulated kinase) pathways, both of which are the most common oncogenic signaling pathways activated in cancer. Recently, mTORC2 emerged as an important contributor to cellular and tumor metabolism. mTORC2 receives inputs mainly from growth factors and regulates the organization of actin cytoskeleton, cell survival, and cellular metabolism. This review focuses on the role of mTORC2 in cellular metabolism and cancer metabolism.

Understanding the underpinning of mTORC2 signaling and targeting both mTORC1 and mTORC2 complexes, holds promise for regimens in cancer early detection, monitoring, and treatment.

Keywords: mTOR, mTORC1, mTORC2, metabolism, signal transduction, metabolic reprogramming, upstream signals, downstream targets

Introduction

The conserved evolutionary Target of Rapamycin (TOR) protein was initially discovered in the budding yeast, *Saccharomyces cerevisiae* [1,2]. Subsequently, orthologs were identified in a variety of organisms including Drosophila (dTOR) [3], Zebrafish [4], and higher eukaryotes [5]. The mammalian Target of Rapamycin (mTOR) is a conserved serine/threonine protein kinase that functions as a key integrator of cell growth and metabolism (reviewed in [6-9]). mTOR signaling pathway receives inputs from nutrients, growth factors, immune cells, and energy; and integrate signals that drive cell growth and metabolism (reviewed in [10]). Additional level of complexity of mTOR regulation stems from the negative [11] and positive feedback [12] from insulin signaling pathway; as well as the cross-talk with several oncogenic; and tumor suppressor pathways [13,14].

mTOR is the catalytic submit of two functionally and structurally distinct complexes namely; mTORC1 (mammalian Target of Rapamycin Complex 1) and mTORC2 (mammalian Target of Rapamycin Complex 2) **(Figure 1)** [15]. mTOR kinase nucleates
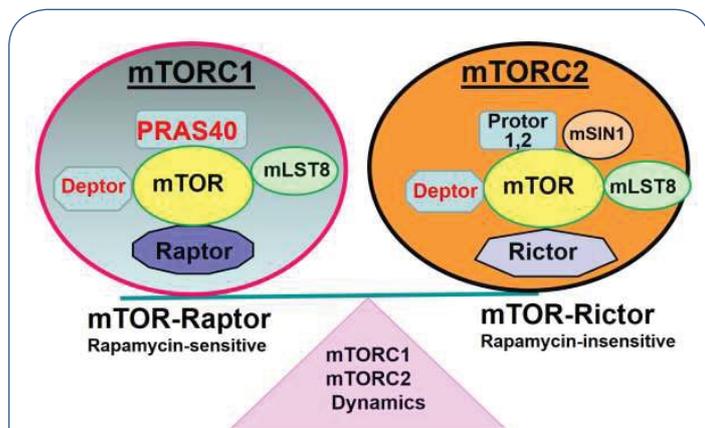
Figure 1 Components of the Mammalian Target of Rapamycin (mTOR) complexes. The mTOR serine/threonine protein kinase catalytic subunit enucleates the center of both mTOR complexes. mTOR complexes have some unique partners and some shared partners between the two complexes. mTORC1 consists of mTOR catalytic subunit exclusively contains Raptor (regulatory associated protein of mTOR) and); PRAS 40 (AKT/PKA substrate 40 kDa protein; and shares mLST/GβL (mammalian lethal with sec13 protein 8/G-protein β-protein subunit likeDeptor (DEP-domain-containing mTOR-interacting protein.

mTORC1 complex. As such, mTOR binds exclusively to the rapamycin-sensitive regulatory-associated protein of TOR (Raptor) in the mTORC1 complex. The complex also binds other proteins including the DEP domain-containing mTOR-interacting protein (Deptor), the mammalian lethal with Sec13 protein 8 (mLST8, also known as GβL), and PRAS 40 as shown in **Figure 1.** By contrast, mTORC2 complex exclusively binds the rapamycin-insensitive companion of TOR (Rictor), mammalian stress-activated map kinase-interacting protein 1 (mSIN1), Protor1/2 (protein observed with rictor, also called PRR5), and shares mLST/GβL, and Deptor subunits (**Figure 1**). Furthermore, eIF4E-Binding Protein 1 (4E-BP1), a downstream effector of mTORC1, is also not inhibited by rapamycin suggesting the presence of rapamycin-resistant mTORC1 functions [6,16].

The prototype and most studied inhibitor of mTOR is the lipophilic macrolide drug rapamycin that has antifungal properties (also known as sirolimus). Subsequently, the immunosuppressive properties of rapamycin were discovered, and thus its use as an antifungal was discontinued. Rapamycin was originally extracted in 1975 from a soil bacterium termed *Streptomyces hygroscopica* in the Eastern Island, Rapa Nui, hence the name Rapamycin (RAPA). RAPA and its analogs, referred to as rapalogs (everolimus, temsirolimus, deforolimus or ridaforolimus, etc.) are currently assessed as cancer treatment candidates in several clinical trials including Phase I [17], II [18], and III [19] trials. The FDA has approved the use of everolimus in treating pancreatic neuroendocrine tumors [20]. However, the results of the clinical trials vary across the cancer spectrum. This is attributable, at least in part, to the discovery of mTORC2 complex, rapamycin-resistant functions of mTOR [21]; the unfolding intricacies of mTOR signaling and crosstalk [22]; as well as mTOR tissue-specificity [23].

Although rapamycin and rapalogs are considered the prototypes of mTOR inhibitors; several investigators documented that rapamycin mainly blocks mTORC1 functions but not mTORC2, at least, during acute treatment [6, 24,25]. However, prolonged treatment with rapamycin inhibits mTORC2 functions secondary to blocking mTORC2 assembly in some cell lines [26]. As mentioned earlier, 4E-BP1, a downstream effector of mTORC1, is not inhibited by rapamycin suggesting the presence of rapamycin-resistant mTORC1 functions [6,16]. These data partially explain the less enthusiastic and perhaps conflicting results of mTOR inhibition by rapamycin in clinical trials [27]. Recently, a newly developed class of mTOR inhibitors, termed the ATP-competitive mTOR kinase inhibitors, have been shown to inhibit both mTORC1 and mTORC2 complexes [28,29]. These mTOR kinase inhibitors are currently under investigation in clinical trials for their use as anticancer agents either alone or in a combination with other drugs.

mTORC2 and Amino Acid Metabolism

In yeast, it has been well documented that amino acids are critical regulators of TOR signaling [30]. mTORC1 controls cell growth, ribosome biogenesis, mRNA translation, autophagy, and metabolism [31-35]. On the other hand, mTORC2 control cell proliferation, survival, and organization of actin cytoskeleton [36,37]. mTORC1 is regulated independently by insulin, growth factors and amino acids. Also, active mTORC1 phosphorylates 4E-BP1 to release the translation initiation factor 4E (initiation factor eIF4E) and maintain protein translation. Recent studies show that mTORC2 associates with ribosomes and thus may be required for protein synthesis as well [38]. Using a genetic screen in yeast, Zinzalla and colleagues [38] identified the interaction between mTORC2 and the ribosome. As such, mTORC2-ribosome binding is suggested to be an important factor in driving the oncogenic PI3K signaling in cancer. Compelling evidence revealed that the dysregulation of mTOR is linked not only to the development of chronic diseases [13,14,39], but also to the progression of several types of cancers [40,41].

In higher eukaryotes, mTORC1 is regulated by nutrients such as amino acids. In this manner, amino acids activate S6K1 (p70S6k1), downstream of mTORC1, to drive cellular anabolism; and inhibits autophagy [42]. Ben-Sahra et al. (2013) reported that mTORC1 regulates metabolic flux by controlling de novo synthesis of pyrimidine that can lead to increased DNA synthesis required for tumor growth [43]. Significant progress in the last few years identified the signaling molecules implicated in amino acid sensing by mTORC1. These signaling molecules include Rag GTPases [44,45], v-ATPase (vacuolar H+-adenosine triphosphate) [46] and the Ragulator complex [10]. Until recently, it has been assumed that mTORC2 is largely resistant to regulation by nutrients and is activated by growth factors only. However, Tato and colleagues suggested that amino acids may activate mTORC2 via Class I PI3 kinase [47], which in turn, phosphorylates the Forkhead transcription factor (FOXO3a) to promote cell survival and proliferation. As a result, phosphorylation of FOXO3a on Thr-24 leads to the exclusion of FOXO3 from the nucleus and thus preventing the activation of its proapoptotic targets [47]. Using mTOR and Rictor siRNA knockdown, and in vitro kinase assays in

Hela cells, the authors show that upon stimulation with amino acids, mTORC2 failed to stimulate Serine 473 phosphorylation of Akt/PKB. Thus, it appears that amino acid homeostasis is mediated via both TORC1 and TORC2. The authors showed that isp7 (+) regulates amino acid uptake and that isp7 (+) transcript is positively activated by TORC2 and negatively regulated by TORC1 [48].

Branched chain amino acids (BCAA) particularly leucine, has been reported to facilitate insulin-mediated Akt phosphorylation and this effect was blocked by siRNA against mTORC2 components [49-51]. Additionally, it has been shown that leucine is transported intracellularly in a glutamine-dependent manner [52] via a mechanism that involves the heterodimeric Solute Carrier Family, SLC7A7, and SLC3A2 [15]. Another amino acid, glutamine, is known to stimulate small intestinal mucosa growth and constitutes a major fuel for the small intestine together with aspartate and glutamate [53]. The Glutamate Transporter 1 (GLT1) upregulation is currently thought to be mediated via an mTOR-Akt-NF-kB mechanism [54]. In this manner, Ji and colleagues reported that GLT-1 is regulated by the oxygen-glucose deprivation, via a mechanism that involves mTOR/Akt/NF-kB signaling. Additionally, the authors' findings indicate that both mTORC1 and mTORC2 are involved in this process in the astrocytes [54]. Recently, it has been shown that the glutaminolysis pathway, a pathway that catabolizes the conversion of glutamine into glutamate and ketoglutarate, is activated in cancer and that mTOR complexes may regulate this pathway; [55]. In addition to glycolysis, the glutaminolysis pathway is upregulated in cancer via c-myc and provides TCA cycle intermediates to fuel the tumor accelerated growth and promote its survival [56,57].

mTORC2 and Glucose Metabolism

mTORC1 senses the nutrients and energy level and is activated by glucose and the glycolytic intermediates [58]. It has been well documented that increased glucose uptake leads to increased mTORC1 activity [59]. In turn, increased mTORC1 activity increases the expression of glucose transporters that will further promote glucose uptake. Studies in the skeletal muscles and liver in rodents revealed that mTORC1 signaling regulates both insulin sensitivity and insulin signaling, via a negative feedback loop [11,60]. We have shown in guinea pigs that rapamycin administration led to increased plasma glucose and insulin resistance as determined by the Homeostatic Model Assessment (HOMA) analysis [46]. Furthermore, Um and Colleagues discovered that the global knockout of S6K1 in mice (a downstream target of mTORC1) enhanced insulin sensitivity due to loss of S6K-mediated feedback inhibition of IRS1 (Insulin receptor substrate 1) [11]. Whereas, deletion of Rictor, which phosphorylates Akt-Ser [473] upstream of mTORC1 [46], led to glucose intolerance due to the discordant beta cell proliferation and cell size [61].

sesn3, is a member of the stress-induced protein implicated in the resistance to oxidative and genotoxic stress. Using Sestrin 3 (sesn 3) liver-specific knockout and transgenic mice models, Tao and colleagues demonstrated that sesn3 activates Akt via mTORC2 to regulate glucose metabolism [62]. In this capacity, sesn3 interacts with mTORC2 directly and as a result mTORC2 activates and phosphorylates Akt at Ser 473. Interestingly, mTORC2 increases glycolysis via Akt activation in the skeletal muscle [63] and liver [64], and regulates IRS-1 degradation which may lead to insulin resistance [65]. Tissue-specific rictor knockout mice studies provided a significant insight into the role of mTORC2 signaling in pancreatic glucose regulation [66]. Also, knockout experiments in mice revealed that mTORC2 core component, rictor, has been shown to activate GLUT 4 (glucose transporter 4), to induce glucose transport in skeletal muscles [67]. These studies demonstrated that deletion of rictor results in decreased both beta cell mass and beta cell proliferation that together led to decreased insulin synthesis. As a results, these animals exhibited glucose intolerance and hyperglycemia.

Additional level of regulation stems from the emerging role of mTORC2 in regulating a multifunctional kinase GSK-3B (Glycogen Synthase Kinase) via Akt [68-70]. GSK protein phosphorylates glycogen synthase, and the dysregulation of GSK is implicated in the pathogenesis of chronic diseases such as diabetes and cancer. Interestingly, GSK3 was also found to phosphorylate rictor at Thr-1695 site in a process mediated by FBXW7 (F-Box and WD Repeat Domain Containing 7, E3 Ubiquitin Protein Ligase) and this leads to rictor ubiquitin degradation [71]. Furthermore, Akt activity and activation of eIF2 α, [72] a key regulator of cell oxidative stress response, are both required during cell proliferation and survival under stress. To maintain homeostasis, GSK-3β has been shown to negatively regulate mTORC2 by phosphorylating rictor at S1235 [73]. This S1235 inhibitory phosphorylation impairs the ability of mTORC2 to phosphorylate and active Akt at Ser 473 [69]. Ramakrishnan and colleagues reported that Sirtuin 2 (Silent mating-type information regulation 2), a cytoplasmic NAD+ dependent deacetylase involved in the cell cycle regulation, is necessary for optimal Akt activation in cell culture [74]. These findings suggest that Akt is regulated at multiple levels that have significant impact on glucose metabolic homeostasis. Thus it appears that the coordinated signaling of mTORC1 and mTORC2 is required to maintain cell proliferation and metabolism.

Treins et al (2012) showed that in mice, the double mutant deletion of both S6K1, downstream of mTORC1, and Akt2, downstream of mTORC2, led to impaired glycemic control [75]. Currently, the phosphoproteomic profiling and genomic analysis studies suggest that hepatic mTORC2 is involved in several steps of the intermediary glucose and lipid metabolism [76]. Along the same line, fat-tissue specific rictor knockout studies in mice suggest that mTORC2 is important in whole body glucose and lipid metabolism [77].

mTORC2 and Fatty Acid Metabolism

Lipogenesis, the process by which acetyl-CoA is converted to fatty acid is mainly under the control of mTORC1 and other anabolic signals (reviewed in [78,79]). mTORC1 is activated during the nutrient abundance to promote synthesis, cell growth, and proliferation. As a result, mTORC1 activates the expression of the lipogenic genes [22], and downregulates the lipid catabolic processes such as lipolysis, [46, 80], β-oxidation, and autophagy [81, 82]. The integral role of mTORC1 in lipid metabolism has been documented by several laboratories (reviewed in [82-86]. On the other hand, less is known about the role of mTORC2 in lipid metabolism.

The role of mTORC2 in lipogenesis is just beginning to unravel. Studies in liver-specific rictor knockout mice revealed that these mice exhibited decreased SREBP1c (sterol regulatory element binding protein 1c) activity. SREBP1c is a transcription factor required for sterol biosynthesis and denovo lipogenesis. Furthermore, the reduction in SREBP1 activity was coupled with decreased phosphorylation of Akt serine 473 on the hydrophobic domain, a site that is phosphorylated by rictor [64]. Along the same line, Yuan and colleagues reported decreased expression of SREBP1 and other genes involved in cholesterol and fatty acid synthesis in the rictor-liver specific knockout mice [87]. In cancer cells, mTORC2 regulated lipogenesis by inhibiting GSK3/FBXW7 dependent degradation of SREBP1 [88]. In a goose model of hepatocytes, Han et al. reported that Sirt 1, the NAD-dependent deacetylase regulates mTOR complexes signaling in hepatocyte lipid metabolism.

Surprizinglying, studies in adipose tissue specific rictor-knockout mice had no defect in adipogenesis [89]. Adipogenesis is the process by which preadipocytes differentiate into adipocytes. The adipocytes in turn store fatty acid energy as triglycerides. Yoa et al. reported that mTORC2 activates adipocyte differentiation and early adipogenesis via a mechanism that involves interaction of Akt1 with BTSA (BTSA domain-containing Signal transducer and Akt interactor). Akt-BSTA interaction was a crucial step prior to mTORC2-mediated phosphorylation of Akt at Serine 473 site [90]. The authors show that mTORC2 facilitated BSTA and Akt1 interactions, and as a result, mTORC2 phosphorylated Akt1 at Ser 473 and promoted the adipogenesis process [81, 90]. Full activation of Akt requires the phosphorylation on two sites located on the hydrophobic domain, namely Thr 308 (which is are phosphorylated by PDK1 3 phosphoinositide-dependent-kinase1 and Ser 473 (which is phosphorylated by rictor). Therefore, it is possible that both phosphorylation sites are required for optimal phosphorylation of Akt. Akt could retain partial activity with the initial phosphorylation at Thr 308 [77, 89, 91] . Therefore, the discrepancy between both studies might reflect differences in the partial reduction of Akt activity as a result of rictor deletion. Alternatively, it could be argued that mTORC2 is essential in early adipogenesis but dispensable during late adipogenesis and terminal differentiation [92].

Lysophosphatidic acid is converted to phosphatic acid (PA) by the enzyme Lysophosphatidic acid acyltransferase (LPAAT-β), a step that is required for triglyceride synthesis. Blaskvich and colleagues found that PA produced LPAAT- β regulates mTORC2 signaling. Therefore, it appears that mTORC2 may play a role in triglyceride synthesis [93] in addition to the documented role of mTORC1 on lipogenesis [82]. Additionally, PA is formed from phosphatidylcholine in a reaction that is catalyzed by phospholipase D. Recently; Lyo et al. demonstrated that mTORC2 is required for PLD-mediated survival signaling. In this manner, PLA stabilizes MDM2 (Murine Double Minutes 2), the major regulator of tumor suppressor gene p53 suggesting that mTORC2 provide growth advantage in cancer.

Lipolysis, the process of breaking down triglyceride to release free fatty acids, is increased during nutrient deprivation to provide FFA to produce energy. It has been shown that mTORC1 inhibits lipolysis in 3T3-L1 adipocytes [80]. Additionally, ablation of rictor in adipose tissue-specific mice was unable to inhibit lipolysis even in the presence of insulin. Other investigators have shown that mTORC2 inhibits lipolysis by blocking the activation of PKA (Protein Kinase A) [77]. Taken together, it appears that both mTORC1 and mTORC2 are required as negative regulators of lipolysis in the adipose tissue.

Another component of mTORC2 is Protor (**Figure 1**). Studies using single and double mutant Protor isoforms Protor-1 and Protor-2, demonstrated that protor did not affect the ability of mTORC2 components to assemble into complex. However, -1 was needed for SGK-1 phosphorylation in the kidney, and thus contributes to cell proliferation [94, 95].

mTORC2 and Cancer Metabolism

As discussed earlier, mTOR is a central regulator of cellular metabolism, growth, and proliferation, cell cycle progression, as well as cancer metabolism [15,79,96]. Targeting the cancer metabolic and bioenergetic pathways affords great promise in the field of cancer therapeuticsCancer metabolic reprogramming is a hallmark of cancer and allows the cells to rewire their metabolism and energy sources to favor tumor survival [97]. Increased glycolysis and preference of lactate production even in the presence of oxygen commonly known as the Warburg effect [30, 98], is a metabolic characteristic of cancer; however its significance remains elusive. This altered homeostatic state allows for an anabolic state to drive the nutrients to increase their cellular growth capacity [38], and to promote rapid proliferation, invasion, metastasis and resistance to cancer therapy. One such anabolic pathway, is mTOR nutrient-sensing pathway, that provides energy and vital metabolites to favor tumorigenesis.

Furthermore, mTOR lies downstream of several oncogenic and tumor suppressor pathways and is a critical player in the tumor microenvironment network [99]. Current studies have shown that the tumor microenvironment mediates mTOR-targeted tumor resistance to therapy [99]. The tumor microenvironment is not static entity but rather functions as a sophisticated network between tumor cells, stromal, immune cells, acellular matrix, soluble cytokines, proteases as well as components of the extracellular matrix. This complex interaction is a key player in cancer metabolism, tumor growth, progression, and metabolism and in acquiring drugresistance [100]. The tumor microenvironment can influence the tumor immune-editing process [101], tumor heterogeneity [102], and thus may lead to therapeutic resistance [99]. Understanding cancer metabolism will enable developing effective cancer treatments. As such, targeting metabolic reprogramming is a promising direction for cancer therapeutics; and pathways that contribute to bioenergetics, and metabolic reprogramming can provide significant insight into optimal personalized therapeutic interventions.

mTORC 2 consists of mTOR catalytic subunit, exclusively Rictor (rapamycin-insensitive companion of mTOR), mSIn1 (mammalian stress-activated protein kinase interacting protein 1) and Protor1/2 (protein observed with rictor, also called PRR5), and shares mLST/GβL, and Deptor subunits.

Acknowledgement

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