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The "other" mTOR complex: new insights into mTORC2 immunobiology and their implications

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Abstract

A central role of the mechanistic target of rapamycin (mTOR) in regulation of fundamental cell processes is well-recognized. mTOR functions in two distinct complexes: rapamycin-sensitive mTOR complex (C) 1 and rapamycin-insensitive mTORC2. While the role of mTORC1 in shaping immune responses, including transplant rejection, and the influence of its antagonism in promoting allograft tolerance have been studied extensively using rapamycin, lack of selective small molecule inhibitors has limited understanding of mTORC2 biology. Within the past few years, however, intracellular localization of mTORC2, its contribution to mitochondrial fitness, cell metabolism, cytoskeletal modeling and cell migration, and its role in differentiation and function of immune cells have been described. Studies in mTORC2 knockdown/knockout mouse models and a new class of dual mTORC1/2 inhibitors, have shed light on the immune regulatory functions of mTORC2. These include regulation of antigen-presenting cell, NK cell, T cell subset and B cell differentiation and function. mTORC2 has been implicated in regulation of ischemia/reperfusion injury and graft rejection. Potential therapeutic benefits of antagonizing mTORC2 to inhibit chronic rejection have also been described, while selective in vivo targeting strategies using nanotechnology have been developed. We briefly review and discuss these developments and their implications.

1. Introduction

The mechanistic target of rapamycin (mTOR) is a conserved, nutrient-sensing, serine/threonine kinase that coordinates cell growth and metabolism with environmental input. It functions in two distinct complexes: rapamycin-sensitive mTOR complex (C) 1 and

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rapamycin-insensitive mTORC2. Extensive research has established a central role for mTOR signaling in regulating numerous fundamental cell processes including metabolism, protein synthesis and autophagy[1]. The role of mTORC1 in shaping immune responses, including allograft rejection, and the influence of its antagonism by rapamycin in promoting experimental transplant tolerance have been studied extensively. However, there is a relative paucity of information regarding the functional immunobiology of mTORC2. This is due, in part, to the lack of an mTORC2-specific pharmacological inhibitor and to the absence of a constitutively active mTORC2 model. Within the past three years, new fundamental observations at the cellular level, accompanied by studies in which mTORC2 has been deleted specifically in either antigen-presenting cells (APCs) or lymphocytes, together with the use of knockdown or knockout (KO) mouse models and the advent of a new class of adenosine triphosphate (ATP) competitive, dual mTORC1/2 inhibitors, have begun to shed light on the roles of mTORC2 in regulation of immune cell differentiation and function. mTORC2 has also been implicated in regulation of ischemia/reperfusion (I/R) injury, allograft rejection, tumor growth and aging.

2. Biology of mTORC2

The seminal discovery in 1991[2] of two related genes,- *TOR1* and *TOR2*, in the yeast *Saccharomyces cerevisiae* led to demonstration of their kinase activity and their requirement for cell proliferation[3]. This was soon followed by identification of their mammalian counterparts and of upstream and downstream regulators of mTOR (reviewed in ref [1]), that defined a signaling pathway[4] fundamental to control of cell growth and metabolic homeostasis. The biology of the two distinct mTOR complexes is depicted in Figure 1. Structurally, mTORC2 consists of several components,- i.e. mTOR, the essential mTORC2 component rapamycin-insensitive companion of mTOR (Rictor), DEP domain-containing mTOR interacting protein (Deptor), protein observed with Rictor (Protor), mammalian lethal with SEC13 protein 8 (mLST8), and mammalian stress-activated MAP kinase-interacting protein 1 (mSIN1).

Unlike with mTORC1, the guanosine triphosphate-binding protein Rheb (Ras homolog enriched in brain) is not an upstream activator of mTORC2 and indeed, upstream regulators of mTORC2 have not been defined. Furthermore, how or even if mTORC2 is regulated by extracellular cues has remained unclear. Insulin can activate mTORC2, but only if the complex contains two specific SIN isoforms[5]. There is also evidence that, in human embryonic kidney 293 T cells, mTORC2 can be activated directly by phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5) P_3)[6]. In yeast, TORC2 is regulated in part by plasma membrane tension[7] and this may also be the case in mammalian cells, e.g. epithelial and vascular smooth muscle cells, as cell stretching induces mTORC2-dependent phosphorylation of Akt at the Ser473 site. In neutrophils, increasing plasma membrane tension acts through a pathway containing the phospholipase D2 and mTORC2 to limit actin network assembly. Without this negative feedback loop, neutrophils display larger leading edges, greater membrane tension and markedly defective chemotaxis[8]. However, in addition to cell membrane localization, mTORC2 has also been localized to intracellular compartments[9]. By examining localization of the obligate mTORC2 component mSIN1 using imaging and biochemical approaches, Ebner et al[10] have demonstrated that within

mammalian cells, mTORC2 activity localizes to the plasma membrane, mitochondria and a subpopulation of endosomal vesicles. mTORC2 located to the plasma membrane and mitochondria can be activated toward Akt through the mSIN1 pleckstrin homology domain that is independent of PI3K and growth factor, suggesting that mTORC2 may exist in subpopulations that are not sensitive to PI3K inside cells[10].

Active mTORC2 phosphorylates multiple protein kinase (PK) A, PKC, and PKG family kinases, including Akt, PKCα (a regulator of the actin cytoskeleton) and serum/glucocorticoid regulated kinase 1 (SGK1). Activation of Akt by mTORC2 couples extracellular growth and survival cues with pathways controlling cell growth and proliferation, but how growth factors target mTORC activity towards Akt is not known. Elucidation of the downstream effector pathways of mTORC2 remains difficult, since these phosphorylation events may not impact kinase activity as much as substrate specificity.

Canonically, mTORC2 has been described as insensitive to rapamycin[11]. However, prolonged exposure of many cell types to rapamycin reduces levels of mTORC2 needed to maintain Akt/PKB signaling[12]. It has also been shown[13] that rapamycin-mediated mTORC2 inhibition is determined by the relative expression of FK506-binding proteins (FKBP),-primarily FKBP12 and FKBP51. Significantly, mTORC2 signaling is also regulated by mTORC1 due to a negative feedback loop between mTORC1 and insulin/PI3K signaling[14]. This has multiple implications for pharmacological targeting of mTOR and provides a rationale for developing mTORC2-specific inhibitors that do not perturb this feedback loop.

3. Regulation of mitochondrial fitness and cell metabolism

Conditional deletion of mTOR, Raptor or Rictor in primary hematopoietic cells has revealed that mTOR plays a developmental stage-specific role in regulation of mitochondrial fitness, that is independent of conventional mTOR complexes and kinase activity[15]. By comparing the effects of mTOR deletion with that of Raptor or Rictor on mitochondrial fitness of progenitor stage (lineage [Lin])$^-$ and lineage-committed (Lin$^+$) hematopoietic cells, it has been shown[15] that mTOR regulates glycolysis, mitochondrial mass and mitochondrial membrane potential in Lin$^-$ cells and mitochondrial mass and oxidative phosphorylation (OXPHOS) in Lin$^+$ cells depending on mTORC1. By contrast, mTOR regulates OXPHOS in Lin$^-$ cells and glycolysis in Lin$^+$ cells depending on both mTORC1 and mTORC2. Whether this phenomenon occurs in other cells or tissues, as well as its underlying mechanism, are uncertain.

The role of mTOR in cell metabolism in immunity has been reviewed extensively[1, 16]. Separately, both mTORC1 and mTORC2 are involved in the control of glucose metabolism. In contrast to mTORC1, the most important role of mTORC2 is likely as an effector of insulin/PI3K signaling[17]. mTORC2 inhibition disrupts the physiological response to insulin and liver-specific KO mice exhibit severe insulin resistance and glucose intolerance[18].

4. Role of mTORC2 in innate immune cells (Table 1)

4.1 Macrophage activation and function

Phosphorylation of the cytoplasmic protein NDRG1 (N-Myc Downstream Regulated 1), which is a downstream target of mTORC2, is lost in Rictor$^{-/-}$ macrophages, but increased in Raptor$^{-/-}$ macrophages, suggesting that mTORC1 may restrict mTORC2 activation in these cells[19]. Macrophage colony-stimulating factor synergizes with IL-4 to facilitate elevated glycolysis during activation of M2 macrophages (that are associated with wound healing/tissue repair) by enhancing mTORC2 signaling involved in the PI3K/mTORC2/Akt pathways[19]. Furthermore, the mTORC2 pathway enhances interferon regulatory factor (IRF)-4 expression that is essential for metabolic reprogramming to support M2 macrophage activation[19].

In response to lipopolysaccharide stimulation, Rictor$^{-/-}$ macrophages exhibit enhanced levels of proinflammatory gene expression and lower levels of IL-10 expression than control cells[20]. Other evidence[21] shows that mTORC2 negatively regulates the generation of tissue-resident peritoneal macrophages, but not monocyte-derived peritoneal macrophages, through inhibiting GATA6 transcription factor expression by negatively controlling forkhead box protein O1 (FoxO1) activation. Rictor deficiency in peritoneal tissue-resident macrophages results in enhanced phagocytosis and proliferation in the resolution phase of zymosan-induced peritonitis[21]. Additionally, Rictor-deleted peritoneal tissue-resident macrophages have increased mitochondrial mass compared with wild-type (wt) counterparts, suggesting a critical role for mTORC2 in metabolic reprogramming[21]. Therapeutically, mTORC2 deletion in macrophages can suppress B16 melanoma tumor growth and loss of resistance to helminth parasites[19].

4.2. Dendritic cell (DC) function

In mice, epidermal Langerhans cell homeostasis appears to depend strictly on mTORC1 and not mTORC2 function[22]. On the other hand, several studies have documented enhanced pro-inflammatory and T cell stimulatory activity of DC lacking mTORC2 activity. Thus, Rictor$^{-/-}$ conventional DC that lack mTORC2 display decreased programed death-ligand 1 (PD-L1) expression, suggesting that mTORC2 positively regulates this T cell co-regulatory molecule[23]. Enhanced PD-L1 expression induced by the dual mTORC1/2 inhibitor Torin1 occurs through a signal transducer and activator of transcription 3 (STAT3)-dependent but IRF-1-independent pathway[23]. Torin1-treated DC can promote regulatory T cell (Treg) induction through PD-L1 and IL-1β cooperative activation[23]. Conditional or CD11c-specific Rictor deletion in DC results in stronger alloreactive T helper (Th)1 and Th17 cell stimulatory ability after Toll-like receptor 4 ligation compared to control DC[24]. This enhanced pro-inflammatory profile of Rictor-deficient DC is partially dependent on glycogen synthase kinase 3 (GSK-3) regulation[24]. Therapeutically, intra-tumoral Rictor$^{-/-}$ DC injection inhibits B16 melanoma growth in wt B6 mice by promoting interferon (IFN)-γ and granzyme-B^{+} cytotoxic CD8^{+} T cell infiltration into the tumor[25]. In agreement with these findings, CD8^{+} T effector cell responses are augmented in skin grafts transplanted from major histocompatibility complex (MHC)- or minor histocompatibility antigen (Ag) (HY)-mismatched CD11c Rictor$^{-/-}$ donors[26], with the latter undergoing accelerated

rejection. In the absence of Rictor specifically in cutaneous DC, $CD8^+$ T cell responses are also enhanced in a mouse cutaneous delayed-type hypersensitivity model[26].

4.3 NK cells

mTORC1 and 2 play distinct roles and are essential for NK cell development and function. Conditional deletion of mTORC2 in mice blocks the transition of double positive ($CD27^+CD11b^+$) cells to terminally mature CD11b single positive NK cells[27]. This defect is associated with impaired induction of the T-box transcription factor T-bet through a mechanism involving the mTORC2-Akt S473-FoxO1 signaling axis. Other recent data[28] provide evidence of positive and negative cross-talk between mTORC1 and mTORC2 via cytokine signaling that variegates the extent of NK cell maturation and effector function.

5. Role of mTORC2 in T cell subsets (Table 2)

Immune cells, including T cells, are auxotrophs for various essential and non-essential amino acids (aa)[29]. Little is known about how proliferating Th cells integrate signals from limiting aa[29]. Recent studies demonstrate that mTORC2 rather than mTORC1 plays a critical role in aa sensing that arrests Th cells in G1 when environmental aa are limiting[30]. Rictor-deficient $CD4^+$ T cells proliferate normally in MLR cultures with limiting arginine or leucine, suggesting that mTORC2 determines the aa-sensitive checkpoint for Th cell proliferation[30].

T follicular helper (Tfh) cells are a unique helper $CD4^+$ subset that highly express B cell lymphoma 6 (Bcl6), the chemokine receptor CXCR5, T cell inhibitory receptor programed death 1 (PD1) and T cell inducible co-stimulator (ICOS). They are essential for B cell-mediated humoral immunity. Deletion of Rictor in $CD4^+$ T cells results in less T cell accumulation, germinal center (GC) B cells and Tfh cells in Peyer's patches[31]. Mechanistically, mTORC2 is activated by ICOS to further drive glycolysis and lipogenesis and enhance Tfh differentiation after foreign Ag immunization[31]. In the absence of mTORC2, FoxO1 activity is upregulated. However, ablation or reduction of FoxO1 can largely or partially restore defective Tfh cells in mTORC2 KO T cells, suggesting that mTORC2 promotes Tfh cell responses via FoxO1 inhibition[31]. Consistently, there is evidence that mTORC2 enhances Tfh differentiation through Akt activation and subsequently T cell factor 1 (TCF1) upregulation[32]. Other studies also show that mTORC2 kinase activity integrates T cell receptor (TCR) with ICOS to promote late maturation, but not early induction of virus-specific Tfh cells. Moreover, mTORC2 can ensure Tfh cells maintain their phenotype, migratory ability towards B cell follicles and effector function to promote B cell differentiation[33].

Th9 cells secrete high levels of IL-9 upon stimulation. There is recent evidence that mTORC2 controls Th9 polarization and allergic airway inflammation. Thus, in an allergic airway inflammation model, Th9 differentiation is diminished in the absence of Rictor[34]. Interestingly, negative regulation of Th9 polarization is FoxO1-independent, but relies on IRF4 reduction due to decreased Akt or STAT6 activation[34]. Of note, IL-9 acting on Treg has been implicated in allograft tolerance.[35]

mTORC2 has been reported as dispensible for $CD8^+$ effector T cell differentiation[36]. On the other hand, Rictor deficiency in $CD8^+$ T cells results in enhanced memory precursor cell generation and stronger recall responses to viral infection, but reduced short-lived effector cells in a FoxO1-dependent manner[37]. FoxO1 is increased in Rictor-deleted $CD8^+$ T cell nuclei to enhance expression of the transcription factors Eomes and TCF1 that further enhance $CD8^+$ memory T cell differentiation, highlighting the roles of the mTORC2-Akt-FoxO1 signaling axis in memory T cell development[37].

Several studies have demonstrated that over-activation of either TORC1 or TORC2 impairs Treg stability, accompanied by defects in their ability to suppress specific effector T cells[38, 39]. As with TORC1, an optimal level of mTORC2 activity is required for Treg differentiation, trafficking and function[40].

mTORC2 is required for NKT-17 cell development, optimal NKT cell cytotoxicity, expansion and survival[41]. In the absence of Rictor, the overall frequency and absolute number of invariant NKT (iNKT) cells are decreased dramatically in the thymus[41], as well as in the spleen and liver[42]. Rictor regulates the generation of GATA-3-expressing iNKT cells that affects IL-4-producing cells, however this effect is independent of the promyelocytic leukemia zinc-finger which is a critical transcriptional factor for iNKT cell development[42].

6. Role of mTORC2 in B cells (Table 2)

Early Rictor deletion in $CD19^+$ cells leads to reduced B cell receptor (BCR) signaling through decreasing phosphorylated Brutons tyrosine kinase and increasing phosphorylated SH2-containing inositol phosphatase, that are the key positive and negative molecules of upstream BCR signaling[43]. Mechanistically, absence of Rictor enhances actin polymerization to restrict BCR movement that eventually reduces the humoral immune response[43]. Inducible deletion of Rictor in Cre-ERT2 transgenic mice impairs Ab production, marginal zone and B1a B lymphocyte generation through regulation of nuclear factor (NF)-κB and NFκB2/p52 generation[44], highlighting the vital role of Rictor in mature B cell function[44]. Genetic ablation of SIN1, a key component of mTORC2, in mouse B cells, impairs their metabolism and proliferation and humoral immunity[45].

Rapamycin and the ATP-competitive dual mTORC inhibitor PP242 can attenuate BAFF (B cell-activating factor from the tumor necrosis family)-mediated normal and neoplastic B cell proliferation and survival by inhibiting mTORC1/2 signaling[46].

7. Regulation of ischemia/reperfusion (I/R) injury

Observations of the regulatory functions of mTORC2 in I/R injury are summarized in Table 3. During hepatic ischemia/reperfusion (I/R) injury, Rictor expression is up-regulated, whereas Rictor deficiency aggravates I/R injury by increasing macrophage/neutrophil accumulation, proinflammatory cytokine production and apoptosis induction, with enhanced activation of MAPK signaling[47]. In an acute kidney I/R injury model in mice, Rictor deficiency specifically in $CD11c^+$ DC worsens renal tissue damage, with enhanced neutrophil infiltration and increased proinflammatory cytokine production compared to

littermate controls[48]. Ablation of Rictor in DC results in enhanced T cell costimulatory, but reduced coinhibitory molecule expression, with enhanced migration of the DC to the injured kidney[48]. Tubule-specific ablation of Rictor exacerbates cisplatin-induced acute kidney injury with reduced tubular cell autophagy, but increased apoptosis compared to littermate counterparts[49]. In a model of chronic kidney injury after I/R or unilateral ureteric obstruction, Rictor is upregulated in macrophages from the fibrotic kidneys. Conversely, deletion of Rictor in macrophages attenuates kidney fibrosis, inflammatory cell infiltration, macrophage proliferation and M2 polarization[50]. Collectively, these findings highlight the potential therapeutic value of Rictor in both acute and chronic tissue injury.

8. Pharmacologic targeting of mTORC2 and transplant outcome

Novel insights into the roles of mTORC1 and mTORC2 in regulation of immune cell homeostasis and function are improving our understanding of the complex effects of mTOR targeting on immune responses, including those that impact transplant outcomes[51]. Since there is no specific pharmacologic inhibitor that selectively targets mTORC2, most investigators have used dual mTORC1 and mTORC2 inhibitors to examine the potential roles of mTORC2 in transplantation (Table 3). AZD8055 is an ATP competitive dual mTORC1/2 inhibitor that suppresses both $CD4^+$ and $CD8^+$ T cell proliferation and decreases inflammatory cytokine production[52]. A short course of AZD8055 prolongs heart allograft survival, increases Treg within the grafts and decreases IFN-γ production, with no impairment of wound healing[52]. Another dual mTORC1/2 inhibitor (AZD2014), with a more favorable pharmacokinetic profile, decreases DC generation and T cell proliferation in vitro, as well as T cell and B cell responses in vivo[53]. In addition, a nine-day course of AZD2014 prolongs heart allograft mean survival time by preventing mononuclear cell infiltration into the grafts and elevating the ratio of Treg to T effector memory cells in the spleen[53]. However, the immunoregulatory effects AZD2014 are not maintained after drug withdrawal and thus differ from the longer-lasting effects of Rapamycin. This may possibly be due to mTORC2 inhibition resulting in a pro-inflammatory effect that would be consistent with mTORC2 deletion in DC promoting an inflammatory phenotype[24] or/and to limited bioavailability. Recently, Chen et al[54] reported that coinhibition of the mTORC1/2 (with everolimus) and RhoA/ROCK pathways in rat heart allograft recipients prevented chronic rejection. There is also evidence that the rapalogue everolimus is more effective than sirolimus at antagonizing both mTORC1 and mTORC2, the latter of which is critical in endothelial cell functional changes leading to transplant vasculopathy in human organ transplantation after HLA I crosslinking[55].

The dual mTORC1/2 inhibitor CC214–1 decreases Th1/Th2 cytokine production and T cell activation in vitro[56], while in allogeneic hematopoietic stem cell transplantation, injection of CC214–2 prolongs mouse survival from graft-versus-host disease[56].

9. Conclusions and future prospects

The past few years have seen important advances in our understanding of mTORC2 biology, including its intracellular localization and role in aa sensing and mitochondrial fitness. mTORC2 also regulates cytoskeletal modeling and cell migration. Various strategies, that

include genetic deletion of Rictor, transient or stable knockdown/targeting of Rictor (using siRNA or shRNA respectively), or/and use of new generation dual mTORC inhibitors, have helped define multiple diverse roles of mTORC2 in innate and adaptive immune cells. These include roles of mTORC2 signaling in the generation, differentiation, metabolism, survival, activation and function of APC, T cell subsets, B lymphocytes and NKT cells. mTORC2 also affects endothelial cell proliferation/angiogenesis and tumor growth[57, 58]. Of relevance to transplantation, studies in mice suggest a potential therapeutic value of the Rictor/mTORC2 axis in alleviation of hepatic or renal I/R injury. There may also be potential therapeutic benefits of targeting/antagonizing both mTORC1 and 2, rather than mTORC1 alone, to inhibit chronic allograft rejection. More work is needed to ascertain how these inhibitors compare with rapamycin and its analogues in suppressing acute and chronic allograft rejection, including their use in combination with other anti-rejection agents. Although a lack of selective small molecule inhibitors of mTORC2 has limited testing of the role of this complex in vivo, -selective in vivo targeting is feasible in pre-clinical models using nanobiologic approaches. Much remains to be understood regarding the role of mTORC2 and the impact of its antagonism in transplantation.

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Abbreviations:

4EBP1	eukaryotic initiation factor 4E binding protein 1
Deptor	Dep-domain-containing partner of mTOR
FKBP12	FK506 binding protein of 12kDa
mLST8	mammalian lethal with SEC13 protein 8
mSIN1	mammalian stress-activated MAP kinase-interacting protein 1
PI3K	phosphatidylinositol 3-kinase
PRAS40	proline-rich Akt sustrate 40kDa
Protor	protein observed with Rictor
Rheb	Ras homologue-enriched in brain
Rictor	rapamycin-insensitive comparison of mTOR
S6K1	ribosomal protein S6 kinase
SGK1	serum and glucocorticoid-regulated kinase 1

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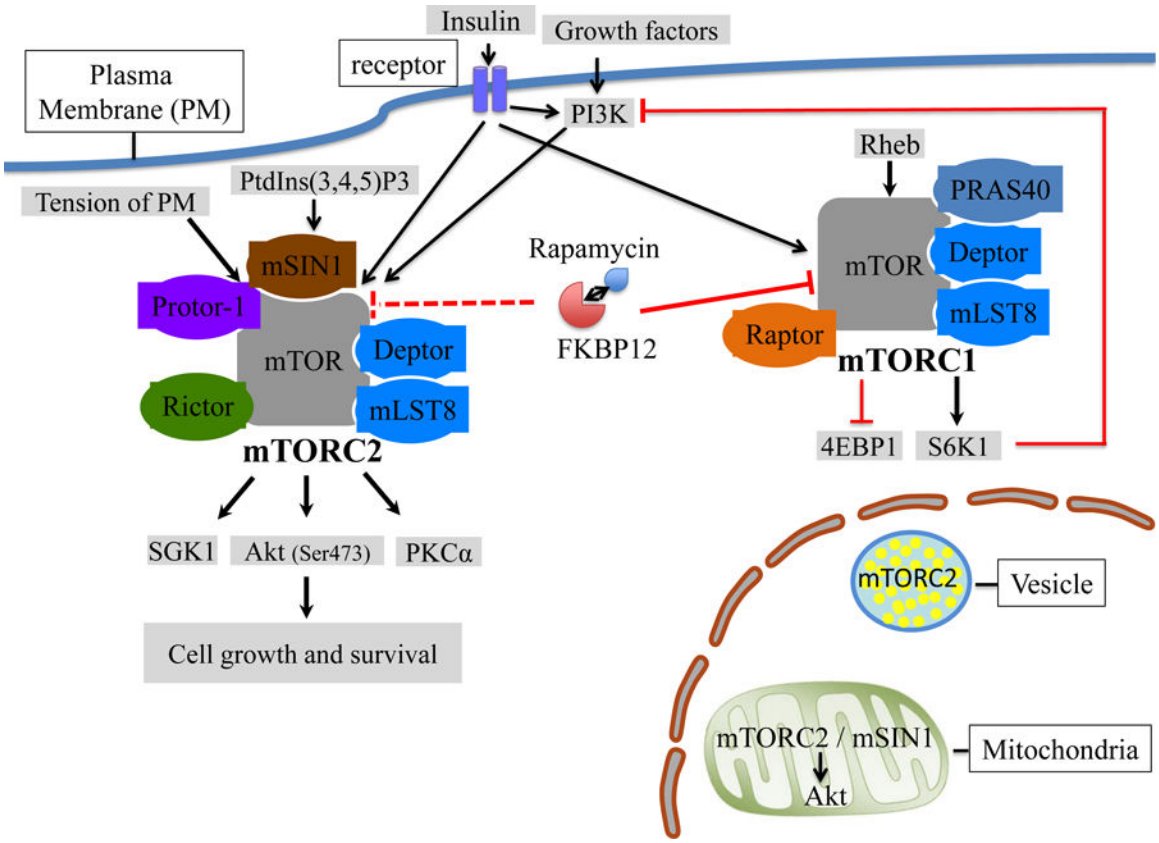

Figure 1.

Biology of mTORC2. mTORC2 consists of several components, including mTOR, Rictor, Protor, Deptor, mSIN1 and mLST8. In contrast, mTORC1 consists of mTOR, Raptor, PRAS40, Deptor and mLST8. mTORC2 has been localized in both cell membrane and intracellular compartments, including mitochondria and endosomal vesicles. PtdIns(3,4,5) P_3, plasma membrane tension and growth factors, including insulin, can activate mTORC2. Active mTORC2 phosphorylates multiple protein kinase (PK) PKA, PKC, and PKG family kinases, including Akt, PKCα and SGK1 to support cell growth and survival. Rapamycin inhibits mTORC1 through binding to the immunophilin FKBP12. mTORC2 has been described as insensitive to rapamycin (sirolimus). mTORC2 signaling is also regulated by mTORC1 through a negative feedback loop between mTORC1-S6K1 and insulin/PI3K signaling.

TABLE 1

Functions of Rictor/mTORC2 in innate immune cells

Cell type	Observation	Reference
Macrophages	Rictor/TORC2 signaling promotes macrophage activation, migration, M2 polarization	50
	mTORC2 pathway activation is required for enhanced glucose metabolism during M2 activation	19
	mTORC2 signaling regulates generation/function of tissue-resident macrophages	21
	Rictor/mTORC2 regulates macrophage proliferation and survival	20
	Chronic mTORC2 activity impairs lysosome degradation in lupus-prone mice	59
Dendritic cells	Absence of Rictor does not affect Langerhans cell homeostasis	22
	Rictor deficiency enhances conventional DC maturation and T cell (allo) stimulatory function in vitro and in vivo	24
	Intratumoral delivery of Rictor$^{-/-}$ DC enhances CD8^{+} T cell-mediated anti-tumor immunity	25
	Rictor deficiency in skin CD11c^{+} DC enhances cutaneous CD8^{+} T cell inflammatory responses and minor histocompatibility Ag-mismatched skin graft rejection	26
NK cells	mTORC2 represses TORC1-modulated NK cell effector functions	28
	mTORC2 deficiency impairs terminal NK cell maturation, proliferation and migration	27

TABLE 2

Functions of Rictor/mTORC2 in adaptive immune cells

Cell type	Observation	Reference(s)
T cell subsets		
Tfh cells	mTORC2 is essential for Tfh cell differentiation and germinal center formation under steady-state, immunization and viral infection conditions	31, 32
Th9 cells	mTORC2 controls Th9 cell polarization	34
Th1/Th2 cells	mTORC2 regulates Th1/Th2 cell differentiation via distinct signaling pathways	60
$CD8^+$ effector T cells	mTORC2 is dispensible for $CD8^+$ T cell differentiation	36
$CD8^+$ memory T cells	mTORC2 controls $CD8^+$ Tmem differentiation in a FoxO1-dependent manner; Rictor deficiency enhances $CD8^+$ Tmem formation	36, 37
Treg cells	Loss of mTORC2 increases thymic-derived Treg generation and enhances induced Treg differentiation; excessive mTORC2 activation disrupts Treg stability and function	38, 40
B cells	Rictor regulates B cell receptor signaling via actin reorganization	43
	Rictor is required for early B cell development in bone marrow and for homeostasis and function of mature B cells	44, 61
NKT cells	mTORC2 regulates multiple aspects of invariant NKT cell subset development and function	41, 42

Abbreviation: Tfh cell, T follicular helper cell

TABLE 3

Regulatory functions of Rictor/mTORC2 in I/R injury and transplant models

Disease model	Observation	Reference(s)
I/R injury (kidney)	Rictor deficiency specifically in DCs augments inflammatory response and tissue injury	48
	Ablation of Rictor in macrophages reduces kidney fibrosis and inflammatory cell accumulation after I/R injury	50
I/R injury (liver)	Rictor deficiency enhances liver injury by increasing macrophage and neutrophil infiltration, cytokine and chemokine release	47
Skin graft rejection	DC-specific Rictor deletion in donor skin augments $CD8^+$ T effector cell responses and accelerates minor HA-mismatched graft rejection	26
Heart allograft rejection	Dual mTORC1/2 targeting using ATP-competitive inhibitors AZD8055 or AZD2014 suppresses rejection (mouse)	52, 53
	Co-inhibition of mTORC1/2 and RhoA/ROCK pathways prevent chronic rejection (rat)	54

Abbreviations: ATP, adenosine triphosphate; DC, dendritic cell; HA, histocompatibility Ag; I/R, ischemia-reperfusion; RhoA, Ras homolog gene family, member A; ROCK, rho-associated, coiled-coil-containing protein kinase