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Invited Mini Review

The functions of mTOR in ischemic diseases

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Mammalian Target of Rapamycin (mTOR) is a serine/threonine kinase and that forms two multiprotein complexes known as the mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTOR regulates cell growth, proliferation and survival. mTORC1 is composed of the mTOR catalytic subunit and three associated proteins: raptor, mLST8/GβL and PRAS40. mTORC2 contains mTOR, rictor, mLST8/GβL, mSin1, and protor. Here, we discuss mTOR as a promising anti-ischemic agent. It is believed that mTORC2 lies down-stream of Akt and acts as a direct activator of Akt. The different functions of mTOR can be explained by the existence of two distinct mTOR complexes containing unique interacting proteins. The loss of TSC2, which is upstream of mTOR, activates S6K1, promotes cell growth and survival, activates mTOR kinase activities, inhibits mTORC1 and mTORC2 via mTOR inhibitors, and suppresses S6K1 and Akt. Although mTOR signaling pathways are often activated in human diseases, such as cancer, mTOR signaling pathways are deactivated in ischemic diseases. From *Drosophila* to humans, mTOR is necessary for Ser473 phosphorylation of Akt, and the regulation of Akt-mTOR signaling pathways may have a potential role in ischemic disease. This review evaluates the potential functions of mTOR in ischemic diseases. A novel mTOR-interacting protein deregulates over-expression in ischemic disease, representing a new mechanism for controlling mTOR signaling pathways and potential therapeutic strategies for ischemic diseases. [BMB reports 2011; 44(8): 506-511]

INTRODUCTION

The serine/threonine kinase Mammalian Target of Rapamycin (mTOR) is a key regulator of protein synthesis and plays a role in other biological functions such as cell growth and cell survival. mTOR forms two complexes, mTOR complex 1 (mTORC1) and mTOR complex2 (mTORC2). mTORC1 is composed of mTOR, Raptor, mLST8, DEPTOR and PRAS40. mTORC1 activates S6K1 and inactivates 4E-BP1. mTORC2 is composed of mTOR, Rictor, mSin1, DEPTOR and mLST8. It is known that mTORC2 phosphorylates Akt at Ser473 but the function of mTORC2 is not fully understood, mTOR is a down-stream substrate of Akt and can phosphorylate and activate Akt, which is a member of the AGC kinase family. Thus, mTOR functions both up-stream and down-stream of Akt. In general, mTORC1 is rapamycin-sensitive, and mTORC2 is rapamycin-insensitive. Within the mTORC1 and mTORC2 complexes, mTOR is resistant to acute rapamycin treatment, but prolonged exposure to rapamycin can block the assembly of mTORC2 components (1) and Akt activation (2). In recent studies focused on ischemic disease, the role of signaling pathways has not been identified clearly (2, 3). To address the functions of the mTOR pathway in ischemic diseases, we first review the basic elements of the PI3K/Akt/mTOR pathways and how these pathways are involved in ischemic disease. Then, we discuss the pathway specificity of each mTOR complex and the value of mTOR signaling pathways in the treatment of ischemic diseases. Further study into the nuances mTOR pathway regulation is necessary to understand the potential for mTOR as a promising target in ischemic diseases.

COMPONENTS OF THE mTOR COMPLEXES

The serine/threonine kinase mTOR is a key regulator of growth factors, nutrients, stresses, cell cycle progression, autophagy, and cell survival (3, 4). mTOR serves as the catalytic subunit of two distinct multiprotein complexes, mTORC1 and mTORC2. mTORC1 has four associated proteins: Raptor (Regulatory associated protein of mTOR), mLST8/GβL (mammalian lethal with Sec13 protein 8), PRAS40 (proline-rich AKT substrate 40 kDa), and DEPTOR (DEP-domain-containing mTOR interacting protein) and is highly sensitive to rapamycin (5). Raptor is a regulatory-associated protein, whereas PRAS40 is a negative regulator. DEPTOR has been identified recently as both a positive and negative regulator in mTORC1 and mTORC2 (3, 6). mTORC2 contains several components: Rictor (Rapamycin-insensitive companion of mTOR), mLST8/GβL, mSin1, DEPTOR and PROTOR (4) and is relatively rapamycin insensitive (1, 2, 6, 7) (Fig. 1). Thus, both mTORC1 and mTORC2 function biochemically and structurally as dimers, these mTOR complexes modulate many fundamental biological processes, including

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transcription, translation, and autophagy, by integrating intracellular and extracellular signals, such as growth factors, nutrients, energy levels, and cellular stress (8). Many mTOR studies have focused on human diseases such as cancer, diabetes mellitus, obesity, cardiovascular diseases and neurological disorders.

mTOR SIGNALING PATHWAYS

Over the past few decades, the mTOR-mediated pathway has received considerable attention due to its critical roles in cell growth control and various human diseases. mTORC1 is controlled by diverse energy-sensing pathways and nutrient signals. The most well-characterized mTORC1 substrates are the AGC family S6K1 and 4E-BP1/2 proteins (9). mTOR signaling occurs in concert with the upstream PI3K/Akt and the negative regulator of Tuberous Sclerosis Complex (TSC) 1/2 (10). Furthermore, TSC is one of the most important sensors involved in the regulation of mTORC1 activity. TSC, a syndrome characterized by mTORC1 hyperactivation, is thought to have limited growth potential due to PI3K inactivation caused by a feedback loop (11,12). There is close functional interaction between the two TSC proteins, TSC1 and TSC2. These two proteins suppress mTORC1 and inactivate TSC2, a GTPase-activating protein (GAP) that negatively regulates mTORC1 activity. mTORC1 is stimulated by growth factors, and the stimulation of this pathway increases phosphorylation of TSC2 by protein kinase B (PKB, also known as Akt) and inhibits Akt (13). In addition, mTORC1 positively regulates growth factors via the PI3K/Akt pathway, and Akt activation can activate mTORC1 in a TSC1/2-dependent manner. Therefore, mTORC1 activity is dependent on modifications that functionally inhibit or activate the TSC1-TSC2 complex (10).

The PI3K/Akt/mTOR signaling pathway is implicated in numerous cellular processes, including cell survival, proliferation, differentiation, apoptosis, motility, metabolism and autophagy (11). Although mTOR is generally considered to be downstream of Akt, Akt can be phosphorylated when bound to Rictor (a positive regulator of mTORC2) in the mTORC2 complex (14-17). For full activation, Akt requires phosphorylation at two sites: Thr308 of the activation loop by PDK1 and Ser473 in the hydrophobic motif of the C-terminal tail (4, 11, 18). As previously mentioned, the mTORC2 complex regulates cell survival metabolism and cell proliferation in part by phosphor-

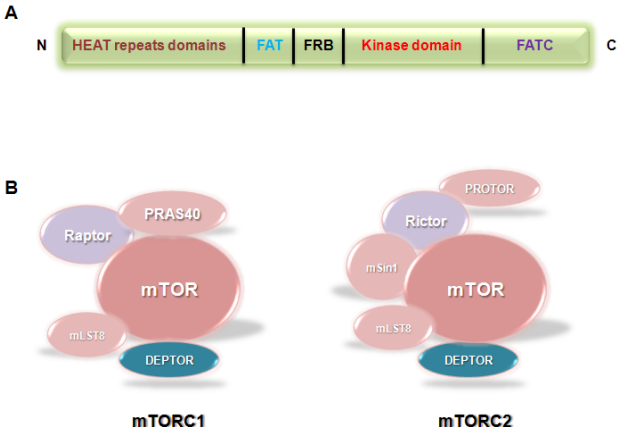

Fig. 1. The structures of mTOR and mTORC components. (A) mTOR contains six functional domains: HEAT repeats domain at the N-terminus, a FAT domain, a FRB (FKBP12-rapamycin-binding) domain, a kinase domain, and a FATC domain at the C-terminus. (B) The components of the mTOR complexes, mTORC1 and mTORC2. mTORC1 contains mTOR, Raptor, PRAS40, mLST8 and DEPTOR, while mTORC2 is comprised of mTOR, Rictor, PROTOR, mSIN1, mLST8 and DEPTOR.

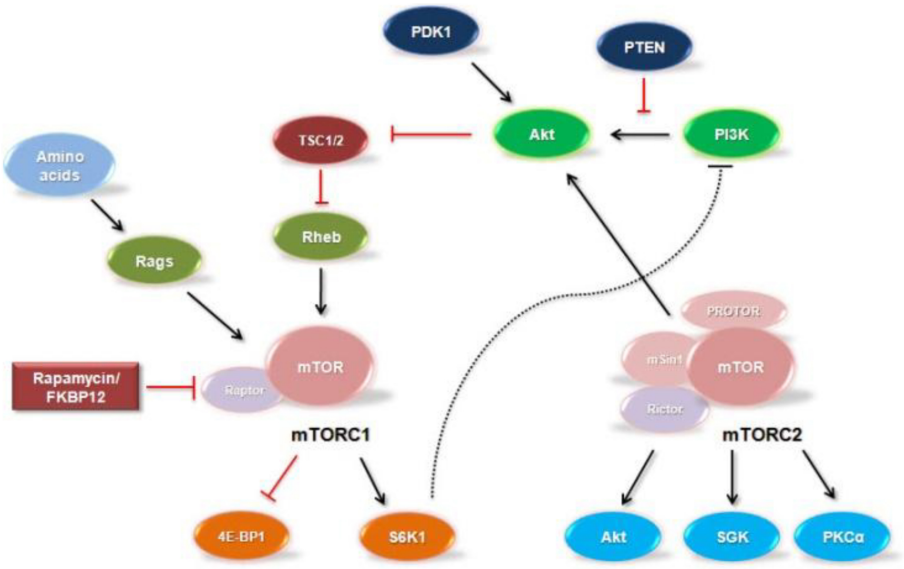

Fig. 2. Schematic representation of mTOR signaling pathways. mTOR is a component of two complexes, mTORC1 and mTORC2. The activation of mTORC1 is regulated by PI3K, Akt, and TSC. Amino acids activate mTORC1 via the Rag GTPases. Activation of mTORC1 by growth factors or amino acids promotes protein synthesis through phosphorylation of S6K1 and 4E-BP1. The mechanism of activation of mTORC2 has not been well characterized functionally, but it is known that mTORC2 directly activates Akt by phosphorylation. Akt controls cell survival and cell cycle progression. *Black arrows indicate activating connections; red lines indicate inhibitory connections. Dotted lines indicate interactions between proteins that are not known to be direct.*

ylating both Akt and SGK1. Many papers have shown that deletion of the gene or knock-down of the mRNAs of mTORC2 components involve the interaction of Akt and SGK1 (7). Other reports suggest that deletion of mTORC2 components specifically inhibits Akt phosphorylation of some, but not all, targets (19) (Fig. 2). Recently, studies have shown that mTORC2 activation can be promoted by the TSC1/2 complex (20, 21). Although the functions and regulation of mTORC2 are unclear, we know that mTOR is involved both upstream (mTORC2) and downstream (mTORC1) of Akt and that the regulation of mTORC2 plays an important role in identifying other cellular signals. Finally, Akt and the mTORCs may compete with each other specific protein components, and further knowledge of the relationship between Akt and the mTORCs may help in the interpretation of mTORC2-mediated cellular processes.

mTORS PATHWAYS IN ISCHEMIC DISEASES

A major characteristic of mTOR is its specific inhibition by rapamycin and its role in cellular growth and proliferation through the regulation of protein translation (22, 23). mTOR may play a role in ischemic injury, as some reports have suggested that rapamycin treatment provides a protective effect in Ischemia/Reperfusion (I/R). Other studies have indicated that mTOR is involved in preconditioning and that activation of the PI3K/Akt pathway plays a significant role in I/R preconditioning (24). Rapamycin stimulates a metabolic state that protects cardiomyocytes from I/R injury (25-27), and inhibition of mTOR promotes the myocardial protection effect of insulin at reperfusion (28). In contrast, other studies reported that the administration of rapamycin before the onset of ischemia reduces the cardioprotective effect of ischemic preconditioning, and prior activation of Akt or S6K1 are important for cell survival after I/R (28, 29). mTOR has been identified as a negative regulator of autophagy in mammalian cells, and rapamycin conveys a neuroprotective effect by blocking the prosurvival pathway (PI3K/Akt) (30-34). In myocardial ischemic disease, autophagy is stimulated by myocardial ischemia and associated with mTOR inhibition in myocardial I/R. Autophagy is an intracellular bulk degradation process for proteins and organelles. In the heart, autophagy is usually observed during acute and chronic ischemia, heart failure, and aging (35). Autophagy is accompanied by activation of mTOR signaling, Beclin and LC3-II (36), and the myocardial protective effect mediated by the regulation of autophagy through AMPK- and Akt-mTOR signaling during ischemia and reperfusion (37). It has been shown that long-term activation of Akt/mTOR signaling pathways links cardiovascular disease (2) and ischemic cardiomyocyte apoptosis, and that this process is mediated by activation of the Akt-mTOR pathway (38).

The expression of Rictor, a component of mTOR complex 2, activates its downstream survival kinase Akt phosphorylation of Ser473 and induces autophagy involving the mTOR-Rictor survival pathway (39). In brain ischemia disease, mTOR is a downstream kinase in the PI3K/Akt signaling pathway, and Ser473 phosphorylation of Akt is significantly decreased in ischemic stress, which is related to decreased mTOR phosphorylation at Ser2448 (40, 41). Ischemic brain injury induces decrease of mTOR and p70S6 kinase phosphorylation (42, 43); thus, the mTOR signaling pathway is critical for controlling neuronal cell death after ischemic brain injury, and the neuroprotective effects of mTOR are mediated by increasing injury-induced mTOR phosphorylation (44). It is known that mTOR is a downstream target of Akt and a central regulator of protein synthesis, cell growth, and cell cycle progression. In *Drosophila* as well as in human cells mTOR is necessary for Ser473 phosphorylation of Akt, and the Akt-mTOR pathways are interrupted when cells undergo necrotic cell death (1, 34). Phosphorylation of Akt in epithelial cells is associated with elevated phosphorylation of mTOR (45) and the regulation of autophagy mediated by Akt-mTOR signaling cascades during ischemia and reperfusion (46). Activation of the Akt and mTOR pathways contributes to the promotion of neovascularization (46, 47) and the I/R induced phosphorylation of mTOR, p70S6K, and 4E-BP1 that correlate with a significant reduction in infarct size after ischemia (48). It is still controversial whether inhibition or activation of mTOR mediates important effects following ischemic diseases. However, mTOR has been associated with the inhibition of apoptosis, and many neurodegenerative diseases are caused by neuronal death through apoptosis. It is possible that the signaling pathways of mTOR may provide protection against apoptosis. In particular, a number of neurological diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and other diseases, such as tuberous sclerosis, neurofibromatosis, fragile X syndrome, epilepsy, autophagy, traumatic brain injury, and ischemic stroke, may gain substantial benefits by either activating or inhibiting mTOR activity (49). *Glial* cell line-derived neurotrophic factor and hepatocyte growth factor are strongly associated with not only the anti-apoptotic but also the anti-autophagic effects of mTOR signaling pathways (50). Although mTOR inhibition has been characterized in various cell types, the activation of mTOR may play a much more significant role than inhibition of mTOR.

PROSPECTIVE ROLES FOR mTOR SIGNALING PATHWAY

Rapamycin, also known as sirolimus, is a macrocyclic antibiotic produced by the bacterium *Streptomyces hygroscopicusfound* in the soil of Easter Island (51). It possesses potent antifungal properties and immunosuppressant activities (52). Rapamycin is an oral drug, and the bioavailability of rapamycin is low. Rapamycin does not target the catalytic site of mTORC1, but it binds FKBP12 (FK506 binding protein 12) in a complex that inhibits downstream signaling events. Thus, mTORC1 is relatively sensitive to rapamycin. In addition, many rapamycin analogs, such as Temisirolimus (CC1-779),

Table 1. PI3K/Akt/mTOR inhibitors

mTOR inhibitors	Akt inhibitors	PDK-1 inhibitors	PI3K inhibitors
Rapamycin	Perifosine	UCN-01	PI-103
Temsirolimus (CC1-779)	GSK-690693		BGT-226
Everolimus (RAD001)			BEZ-235
Deforlimus (AP-23573)			XL-765
AZD-8055			XL-147
O'Donnell *et al*., 2003 (58); Ma and Jimeno 2007 (57); Chresta *et al*., 2010 (53)	Kondapaka *et al*., 2003 (56)	Granville *et al*., 2006 (55)	Sausville *et al*., 2001 (59); Dees *et al*., 2005 (54)

Everlimus (RAD001), Deforolimus (AP-23573), and AZD8055, have been developed in clinical trials (Table 1). Several lines of evidence have demonstrated that mTOR is a key player in the PI3K/Akt/mTOR signaling pathways and that inhibition of mTOR is a strong candidate for therapeutic strategies. Until now, clinical studies have indicated that mTOR inhibitors are effective in cancer treatment, as the mTOR pathway is deregulated in a number of cancers. Thus, mTOR is linked to diverse human diseases, including cancer, obesity, diabetes mellitus, cardiovascular disease, neurodegenerative disease, brain injury, and ischemia. We predict that regulation of mTOR will be utilized as an important mechanism for controlling cell growth and survival.

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