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Cutting through the Complexities of mTOR for the Treatment of Stroke

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Abstract

On a global basis, at least 15 million individuals suffer some form of a stroke every year. Of these individuals, approximately 800,000 of these cerebrovascular events occur in the United States (US) alone. The incidence of stroke in the US has declined from the third leading cause of death to the fourth, a result that can be attributed to multiple factors that include improved vascular disease management, reduced tobacco use, and more rapid time to treatment in patients that are clinically appropriate to receive recombinant tissue plasminogen activator. However, treatment strategies for the majority of stroke patients are extremely limited and represent a critical void for care. A number of new therapeutic considerations for stroke are under consideration, but it is the mammalian target of rapamycin (mTOR) that is receiving intense focus as a potential new target for cerebrovascular disease. As part of the phosphoinositide 3-kinase (PI 3-K) and protein kinase B (Akt) cascade, mTOR is an essential component of mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) to govern cell death involving apoptosis, autophagy, and necroptosis, cellular metabolism, and gene transcription. Vital for the consideration of new therapeutic strategies for stroke is the ability to understand how the intricate and complex pathways of mTOR signaling sometimes lead to disparate clinical outcomes.

Keywords

Akt; apoptosis; autophagy; Deptor; mammalian target of rapamycin (mTOR); mLST8; mSIN1; mTORC1; mTORC2; necroptosis; oxidative stress; PI 3-K; PRAS40; Protor-1; p70S6K; Raptor; Rictor; SGK1; stroke

Incidence and Current Therapeutic Strategies for Cerebrovascular Disease

For 2011, an approximate 1% decrease in the age-adjusted death rate was reported for the United States population derived from information on mortality data for the years of 2000 through 2011 (1). Life expectancy is now believed to be approaching almost 80 years for all individuals. The five leading causes of death are cardiac disease, cancer, chronic lower respiratory disease, stroke, and traumatic accidents (2). Interestingly in this analysis, stroke is no longer ranked as the third leading cause of death. A number of factors may have contributed to this lower ranking for stroke that include improved long-term care with

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disorders tied to hypertension and low-density lipoprotein cholesterol management, reduction in tobacco consumption, improved public education and awareness for the need for rapid treatment of cerebrovascular disorders, and improved management of metabolic disorders such as diabetes (3, 4). Furthermore, treatment with recombinant tissue plasminogen activator in an applicable sub-group of patients that requires a narrow therapeutic window also has led to a reduction in mortality and morbidity in patients presenting with stroke (5, 6). However, overall therapeutic strategies for patients presenting with stroke remain limited for the majority of patients. A number of new therapeutic considerations for stroke, ischemic vascular disease, and central nervous system inflammation under investigation focus upon cytokines (7–19), growth factors (20), progenitor cells (21), normobaric hyperoxia (22), metallic ions (23), cellular metabolism (24), small molecular regulators of hypoxia inducible factor (25), tissue kallikrein (26), and retinoblastoma protein (27). Yet, gaining exceptional and more recent interest as a novel strategy for stroke and cerebrovascular disease is the role of the mammalian target of rapamycin (mTOR) (28–30).

Mechanistic Avenues of Consideration for mTOR

mTOR (also known as the mechanistic target of rapamycin and FK506-binding protein 12-rapamycin complex-associated protein 1) is a 289-kDa serine/threonine protein kinase. It was initially isolated in yeast in *Saccharomyces cerevisiae* with the identification of the genes *TOR1* and *TOR2* that encode two isoforms in yeast Tor1 and Tor2 (31). A single gene *FRAP1* encodes mTOR in mammals, is ubiquitously expressed throughout the body, and modulates metabolism, cellular survival, gene transcription, and cytoskeletal components (29, 32–36).

The protein complexes mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) each contain the protein mTOR and have been identified based on their components and their sensitivity to rapamycin (28, 35–39) (Figure 1). mTORC1 has the regulatory-associated protein of mTOR (Raptor) protein. Phosphorylation of Raptor that controls mTORC1 activity can proceed through a number of pathways that involve the protein Ras homologue enriched in brain (Rheb) (40). Rheb phosphorylates Raptor residue serine^{863} as well as other residues that include serine^{859}, serine^{855}, serine^{877}, serine^{696}, and threonine^{706}. mTORC1 activity can be limited if serine^{863} remains unphosphorylated, (41). mTOR itself can phosphorylate Raptor following stimulation by insulin. In contrast, rapamycin, a macrolide antibiotic from Streptomyces hygroscopicus, inhibits mTOR activity (41). mTORC1 is more sensitive to the inhibitory effects of rapamycin than mTORC2 (42). Rapamycin inhibits mTORC1 by binding to immunophilin FK-506-binding protein 12 (FKBP12) that attaches to FKBP12 - rapamycin-binding domain (FRB) at the C-terminal of mTOR to prevent the phosphorylation of mTOR (43). Chronic exposure of rapamycin also can inhibit mTORC2 that may involve a mechanism that disrupts the assembly and the integrity of mTORC2 (42).

The N-terminal portion of mTOR has at least a 20 HEAT (Huntingtin, Elongation factor 3, A subunit of Protein phosphatase-2A, and TOR1) repeat (34). This region promotes binding with two important, and mutually exclusive, regulatory proteins, Raptor (regulatory-associated protein of mTOR) and Rictor (rapamycin-insensitive companion of mTOR) (32,

44). It is the association with either Raptor or Rictor that determines whether mTOR is a component of mTORC1 or mTORC2.

mTORC1 consists of a number of other components in addition to Raptor that include the proline rich Akt substrate 40 kDa (PRAS40), Deptor (DEP domain-containing mTOR interacting protein), and mLST8/GβL (mammalian lethal with Sec13 protein 8, termed mLST8). PRAS40 competitively inhibits the binding of mTORC1 to Raptor (45). The maintenance of mTORC1 activity occurs through the inhibitory phosphorylation of PRAS40 by protein kinase B (Akt). PRAS40 is phosphorylated on several residues that include serine^{183}, serine^{212}, serine^{221}, and threonine^{246} (46, 47). The serine sites are targets of mTOR and the residue of threonine^{246} is the phosphorylation target of Akt. The phosphorylation of PRAS40 leads to its dissociation with Raptor (48) and promotes the binding of PRAS40 to the cytoplasmic docking protein 14-3-3 (49–51). This removes PRAS40 from interacting with Raptor and facilitates the activation of mTORC1 (52). Deptor also is an inhibitory subunit of mTORC1. Deptor binds to the FAT domain of mTOR (for FKBP associated protein, Ataxia-telengiectasia, and Transactivation/transformation domain-associated protein) to inhibit the activity of mTORC1. In the absence of Deptor, the activity of Akt, mTORC1, and mTORC2 increase (53). mLST8 is a 36 kDa peripheral membrane protein that is a component of both mTORC1 and mTORC2. mLST8 promotes mTOR kinase activity with p70S6K and 4EBP1 (54), controls insulin signaling through the transcription factor FoxO3 (55), is necessary for the phosphorylation of Akt and protein kinase C-α (PKCα) (55), and is required for the association between Rictor and mTOR (55).

Two important targets of mTORC1 are p70S6K and the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) (33, 35). mTORC1 fosters mRNA biogenesis, translation of ribosomal proteins, and cell growth through p70S6K phosphorylation (56). Amino acids, such as glutamate and leucine, also have been shown to phosphorylate p70S6K. Through amino acid activation of the mTOR-p70S6K pathway, glutamate may control neuronal synaptic signaling (57) and leucine can decrease food intake (58). In contrast, phosphorylation of 4EBP1 results in its inactivation. When 4EBP1 is hypophosphorylated, it can block protein translation by binding to eukaryotic translation initiation factor 4 epsilon (eIF4E) through eIF4 gamma (eIF4G), a protein that helps transport mRNA to the ribosome. mTORC1 phosphorylation of 4EBP1 leads to the dissociation of 4EBP1 from eIF4E, allowing eIF4G to begin mRNA translation (59). Binding of 4EBP1 and p70S6K to Raptor can be prevented during activation of PRAS40.

Different from mTORC1, mTORC2 contains the rapamycin-insensitive companion of mTOR termed Rictor. Similar to mTORC1, mTORC2 has the components of mTOR, mLST8, and Deptor. mTORC2 contains additional components that are the mammalian stress-activated protein kinase interacting protein (mSIN1) and the protein observed with Rictor-1 (Protor-1). Rictor and mSIN1 can form the structural basis of mTORC2. mTORC2 utilizes Rictor to activate and phosphorylate Akt at Ser^{473}, facilitating threonine^{308} phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) (60). mSIN1 is necessary for mTORC2 to activate Akt. mTOR has also been shown to phosphorylate mSIN1, preventing the lysosomal degradation of mSIN1 (61). Protor-1 is a Rictor-binding subunit of

mTORC2 that does not appear to alter other mTORC2 components in a way that would lead to the phosphorylation of Akt or PKCα. However, Protor-1 may function to activate serum and glucocorticoid induced protein kinase 1 (SGK1). Loss of Protor-1 in animal models reduces the hydrophobic motif phosphorylation of SGK1 and its substrate NRDG1 (N-Myc downregulated gene 1 in the kidney) (62).

Targets of mTORC2 are Akt, protein kinase C alpha (PKCα), P-Rex1, P-Rex2, Rho GTPases, and SGK1. mTORC2 promotes cell survival through the activation of Akt and uses PKCα for cytoskeleton remodeling. mTORC2 phosphorylates and activates SGK1, is a member of the protein kinase A/protein kinase G/protein kinase C (AGC) family of protein kinases, and is activated by growth factors to control ion transport and growth (63). mTORC2 modulates cell migration through activating Rac guanine nucleotide exchange factors P-Rex1 and P-Rex2 and uses Rho signaling during cell-to-cell contact (64). P-Rex1 and P-Rex2 are phosphorylated by Akt through mTORC2 acting as a catalytic complex and are linked to Rac activation and cell migration (64).

Akt activates mTORC1 in response to growth factors and several other Akt mediated pathways (33). Tuberous sclerosis complex (TSC) 1 (hamartin)/TSC2 (tuberin) complex is an inhibitor of mTORC1 and one of the targets of Akt for the regulation of mTORC1 activity. Although several regulatory phosphorylation sites are known to exist for TSC1, phosphorylation of TSC2 by Akt, extracellular signal-regulated kinases (ERKs), activating protein p90 ribosomal S6 kinase 1 (RSK1), AMP activated protein kinase (AMPK), or glycogen synthase kinase -3β (GSK-3β) appear to be more significant for controlling the TSC1/TSC2 complex. TSC2 functions as a GTPase-activating protein (GAP) converting a small G protein Ras homologue enriched in brain (Rheb-GTP) to the inactive GDP-bound form (Rheb-GDP). Once active, Rheb-GTP can directly interact with Raptor to activate mTORC1 and also regulate the binding of 4EBP1 to mTORC1 (65). Akt phosphorylates TSC2 on multiple sites that leads to the destabilization of TSC2 and disruption of its interaction with TSC1. The phosphorylation of TSC2 (serine^{939}, serine^{981}, and threonine^{1462}) can increase its binding to protein 14-3-3 and lead to cellular sequestration, disruption of the TSC1/TSC2 complex, and subsequent activation of Rheb and mTORC1 (66). In contrast to mTORC1, TSC1/TSC2 fosters the activity of mTORC2 (67). Loss of a functional TSC1/TSC2 complex can lead to the loss of mTORC2 kinase activity *in vitro* (67). The TSC1/TSC2 complex can associate with mTORC2 to promote mTORC2 activity that involves the N-terminal region of TSC2 and the C-terminal region of Rictor.

On an additional note, it is important to recognize that mTOR signaling is part of cascade of pathways that include phosphoinositide 3 –kinase (PI 3-K), Akt, and AMPK (68). AMPK phosphorylates TSC2 to lead to increased GAP activity to turn Rheb-GTP into Rheb-GDP and thus inhibits the activity of mTORC1 (69). AMPK also can control TSC1/2 activity through RTP801 (REDD1/ product of the *Ddit4* gene) (70). During hypoxia, AMPK activity can increase REDD1 expression to suppress mTORC1 activity by releasing TSC2 from its inhibitory binding to protein 14-3-3 (70). Increased AMPK activation has been shown to reduce myocardial infarct size in experimental models of diabetes (71). However, down-regulation of the AMPK pathway may be detrimental. For example, loss of AMPK activity can increase insulin resistance in skeletal muscle (72). In addition, the liver kinase B1

(LKB1) can regulate the activation of AMPK through phosphorylation (73). Loss of LKB1 impairs cardiac function during ischemic conditions (74), illustrating the importance of AMPK signaling in the mTOR pathway for the vascular system.

Cell Injury Through Apoptosis, Autophagy, and Necroptosis

Ischemic injury as aresult of oxidative stress in the brain (75, 76) can ultimately initiate programmed cell death pathways that oversee apoptosis, autophagy, and necroptosis (77–79) (Figure 1). In acute and chronic degenerative disorders, apoptosis, autophagy, and necroptosis have been associated with cell injury. Apoptotic DNA degradation and the presence of caspase 3 in neurons has been reported in the postmortem nigra of Parkinson's disease patients, suggesting that apoptosis results in neuronal cell death (80). Apoptotic DNA fragmentation (81) and caspase activation (82) also have been reported in the brains of patients with Alzheimer's disease as well as in cell models of Alzheimer's disease and cognitive loss (83–88). Apoptotic cell loss has also been associated with acute traumatic injury (84, 88).

mTOR has been shown through Akt to protect endothelial cells against apoptosis (89) and to block “pro-apoptotic” forkhead transcription factors, such as FoxO3a (89, 90). Inflammatory cells also can undergo apoptotic injury during oxidative stress if deprived of Akt and mTOR activation (86, 91). Apoptotic cell death in dopaminergic neurons can be blocked during application of agents that increase Akt and mTOR activity (92). Akt also functions to modulate apoptosis with mTOR through the inhibition of PRAS40. Phosphorylation of PRAS40 by Akt can block the activity of this substrate, lead to its dissociation from mTORC1 to promote mTOR activation and prevent apoptosis (49, 87, 93).

mTOR also can regulate apoptotic cell death through downstream signaling pathways such as p70S6K and BAD. Phosphorylation of BAD leads to the dissociation of this protein from the “anti-apoptotic” protein Bcl-2/Bcl-x_L and increases BAD binding to protein 14-3-3. Activation of p70S6K promotes the phosphorylation of BAD in astrocytes to limit apoptotic cell injury (94). The activation of mTOR and p70S6K may also decrease apoptosis through pathways that can increase “anti-apoptotic” Bcl-2/Bcl-x_L expression (94). In addition, insulin prevents apoptosis in rat retinal neuronal cells against serum deprivation through the activation of mTOR and p70S6K (95). Activation of p70S6K in the PI3-K/Akt pathway that may not involve BAD also can foster neuronal (96) and cardiac protection (97). Over-expression of wild type p70S6K or a rapamycin resistant form of the p70S6K kinase enhances the cytoprotective effect of insulin (95). Other growth factors similar to insulin, such as erythropoietin (EPO) (98), also have been reported to be dependent upon mTOR activation for cytoprotection against apoptosis (86, 99, 100).

Yet, activation of mTOR does not consistently block apoptosis. During Alzheimer's disease, post-mitotic neurons that attempt to enter the cell cycle do not replicate, but can result in apoptotic cell death (27, 101). In studies with amyloid oligomer exposure, neurons can be prevented from entering the cell cycle during the inhibition of mTOR and thus be protected from apoptosis (102).

In contrast to apoptosis that can lead to the ultimate destruction of a cell, autophagy allows cells to recycle cytoplasmic components and remove defective organelles for tissue remodeling. Of the three categories of autophagy that include microautophagy, macroautophagy, and chaperone-mediated autophagy, macroautophagy is the most prominent and involves the degradation of cytoplasmic material and the sequestration of the cytoplasmic protein and organelles into autophagosomes (78, 79, 103, 104). mTOR controls autophagy through the regulation of autophagic genes. mTOR phosphorylates the mammalian homologue of autophagy related gene 13 (Atg13) and the mammalian Atg1 homologue ULK1 and ULK2 to prevent the progression of autophagy (105). The focal adhesion kinase family interacting protein of 200 kDa (FIP200) has been identified as a ULK binding protein. FIP200 and Atg13 are vital for the stability and activation of ULK1. Mammalian Atg13 binds to ULK1/2 and FIP200 to activate ULKs and facilitates the phosphorylation of FIP200 by ULKs (105). It is believed that mTOR activation prevents autophagy in mammalian cells through the inhibition of the ULK-Atg13-FIP200 complex by phosphorylating Atg13 and ULKs.

In the nervous system, it remains unclear under what specific circumstances pathways such as autophagy may be beneficial. Autophagy can lead to cell death in cerebral astrocytes (106), in purkinje neurons (107), in sympathetic neurons (108), in cortical neurons (109), and in spinal cord motor neurons (110). Activation of the mTOR pathway that blocks autophagy may be necessary to protect against spinal cord injury (111) and maintain synaptic plasticity (112). Yet, in other scenerios, autophagy also may invoke a protective component (113). Trophic factor neuronal protection may be mediated through the induction of autophagy (114). Induction of autophagy also may be necessary with combined inhibition of mTOR signaling to improve cognitive function, limit amyloid (Aβ) cell injury (115), and clear mutant huntingtin in Huntington's disease (116). Activation of autophagy also may be required to protect against neuronal cell loss and α-synuclein toxicity in Parkinson's disease (117).

Given the varied outcomes that can occur with programmed cell death pathways, it may come as no surprise to learn that apoptosis and autophagy also share a complex relationship. For example, inhibition of mTOR activity in squamous carcinoma cell lines can lead to the combined activation of apoptosis and autophagy (118). Methamphatamine leads to cell death not only through apoptosis, but also through autophagy by inhibiting the disassociation of the Bcl-2/Beclin 1 complex (119). Bcl-2/Bcl-x_L is an “anti-apoptotic” protein that blocks autophagy through its inhibitory interaction with Beclin 1 (120). Autophagy and apoptosis also may have opposing roles. Induction of apoptosis may conversely require the inhibition of autophagy (78, 121, 122).

Necroptosis is a regulated necrotic cell death pathway that is controlled by receptor-interacting protein (RIP-1 and RIP-3) kinases and cylindromatosis (turban tumor syndrome) (CYLD). Necroptosis can be closely tied to autophagy and has been shown that inhibition of mTOR in acute lymphoblastic leukemia leads to autophagy dependent cell loss with features that are consistent with necroptosis (77). In human carcinoma cell lines, agents that can slow cell cycle progression have been shown to be dependent upon necroptosis (123), suggesting a potential new pathway of treatment for neurodegenerative disorders such as Alzheimer's

disease. Furthermore, the ability to proliferate by glioblastoma cells has been linked to a number of mechanisms that include inhibition of Akt, mTORC1 and mTORC2, cell-cycle block at G2-M, and the initiation of necroptosis and autophagy (124).

Targeting mTOR in Cerebrovascular Disease

Given the potential role of mTOR in a number of neurodegenerative disorders (29, 35, 36, 39, 125–130), new enthusiasm is now focusing upon the mTOR pathway for the treatment of cerebrovascular disease. In an experimental model of ischemic-reperfusion injury, the agent salvianolate has been shown to decrease stroke volume that was associated with the up-regulation of Golgi phosphoprotein-3 and mTOR phosphorylation (131). The neuroprotective agent ferulic acid reduces middle cerebral artery infarction in a rat experimental model with phosphorylation and activation of mTOR signaling to include mTOR and p70S6K (132). In animal models of ischemic preconditioning, activation of mTOR pathways are also believed to be necessary for neuroprotection. Phosphorylation and activation of mTOR has been observed during remote ischemic preconditioning of the hippocampus that was neuroprotective and improved memory function during global brain ischemia (133). In models of ischemic post-conditioning, long-term cerebral focal ischemic damage and neurological disability were reduced and mediated by enhanced Akt and mTOR activity (134).

Cerebrovascular protection in these models may be mediated in part through modulation of glutamate uptake and a reduction in excitotoxicity modulated through mTOR signaling in glia. During oxygen-glucose deprivation, the Akt-mTOR axis through mTORC1 and mTORC2 has been demonstrated to be necessary for glutamate transporter subtype 2 (GLT-1) expression that would promote glutamate uptake during brain ischemia and limit ischemic injury (135). Prior work supports such a premise with the illustration that mTOR signaling is vital to protect other non-neuronal cells such as those that involve microglia (86, 91, 100). An additional mechanism of protection through mTOR to consider involves small non-coding micro RNAs (miRNAs). In adult animal models of stroke, a small cohort of circulating miRNAs that were related to PI3-K, Akt, and mTOR were associated with a greater degree of neuroprotection in adult females, suggesting that miRNAs with the presence of a sex factor could offer clinical protection through mTOR signaling (136).

Despite the number of investigations that support a role for mTOR activation in neuroprotection during cerebral ischemia, other experimental studies offer a counter perspective. Some studies suggest that inhibition of mTOR through PRAS40 activation may reduce cerebral infarction through work that over-expresses PRAS40 as well as eliminates the presence of PRAS40 in murine models of stroke (137). However, other experimental models employing neuronal cell lines and microglia have shown that PRAS40 either in conjunction with the inhibition of mTOR signaling or independently can lead to detrimental cell injury and that cell protection requires the reduction of PRAS40 activity (49, 87). Studies also show that antagonism of the histamine H3 receptor leads to protection following cerebral ischemia and reperfusion through inhibition of mTOR phosphorylation and induction of autophagy (138). In hippocampal neurons, damage from excitotoxicity can be reduced with promotion of autophagy and mTOR inhibition (139). During oxygen-

glucose deprivation in human umbilical vein endothelial cells, rapamycin with subsequent inhibition of mTOR protectes vascular cells from injury in conjunction with autophagy activation (140). Rapamycin also was found to be protective during oxygen-glucose deprivation in cortical neuronal cells. Application of rapamycin prevented the activation of mTORC1 and mTORC2 and led to increased neuronal survival (141). Using sub-lethal ischemic precondition to result in ischemic brain tolerance, rapamycin also was found to promote autophagy, reduce brain damage, and improve neurological scores that was suggested to be mediated through TSC1 (142).

The current work with examining the role of mTOR during cerebrovascular injury clearly suggests that a number of parameters may determine whether promotion of the mTOR signaling pathway or blockade of the mTOR axis is necessary for protection in the brain. Different experimental models as well multiple factors in clinical trials may impart variables that lead to a variety of outcomes. However, a case also can be made for the degree of mTOR activation that may be necessary to prevent or at least limit an injury process and subsequently lead to cellular and tissue protection. For example, activation of mTOR can prevent oxidative stress mediated autophagy in dopamine neurons (92). Yet, prolonged activation of mTOR can lead to dyskinesia in patients with Parkinson's disease (143). Furthermore, in chronic disorders such as Alzheimer's disease, it is the inhibition of mTOR with the activation of autophagy that may be necessary to impart clinical benefit (115).

Conclusions and Future Perspectives

Cerebrovascular disease is one of the five leading causes of disability and death in the US and affects over 800,000 people year at a cost of greater than 75 billion US dollars annually. Although the incidence of stroke has declined placing it in rank from the third leading cause of death to the fourth, multiple factors rather than any single entity are most likely contributors to this result. These factors would include improved management of vascular disease in patients, reduction of tobacco use, and more rapid time to treatment in patients during the initial onset of stroke. Yet, stroke continues to remain a significant cause of death and disability worldwide and the available treatments for stroke are markedly restricted. In addition, therapies such as recombinant tissue plasminogen activator are only applicable for a small subset of patients. New therapeutic strategies continue to be investigated for the treatment of stroke but none may be as groundbreaking as well as complex as those that focus upon mTOR signaling.

Targeting mTOR can offer a wide variety of outcomes that appear beneficial with either the activation or the inhibition of mTOR pathways, suggesting that the degree of mTOR activity may play a significant role in attempts to achieve neuroprotection during the treatment of stroke. However, current studies also indicate that mTOR most likely does not function in isolation and the axis that involves PI 3-K, Akt, and mTOR should be considered more broadly in relation to potential mechanisms that affect cellular survival. For example, regulation of the combined PI 3-K, Akt, and mTOR cascade has been shown to be important to promote increased radiosensitivity against tumor cell growth and the vascular supply of tumors (144). Furthermore, combined loss of 70S6K activity with the loss of the mTORC2 substrate Akt2 is necessary for defective insulin activity and β-cell function (145),

suggesting that both of these pathways may require targeting when considering therapeutic strategies to maintain glycemic control. New studies also indicate that metformin may limit prostate cancer growth and disrupt membrane initiated androgen signaling through combined mTOR, 70S6K, and AMPK signaling (146). Additional studies also have highlighted the critical role of other linked pathways to the PI3-K, Akt, and mTOR axis for cellular survival and injury that involve wingless (Wnt) signaling (86, 100, 147–150), the CCN family (87, 151, 152), cytokines such as EPO (49, 86, 99, 100), sirtuins (153–155), forkhead transcription factors (55, 156–159), neurotransmitter modulation (160), and lipid metabolism (28).

One also must be cognizant of the potential for tumorigenesis with the activation of the mTOR pathway. Inhibition of tumor growth and development of metastases usually requires blockade of the proliferative mTOR pathway (39, 161–163). Experimental studies show that inhibition of mTOR can block lung cancer growth (39, 163), prostate cancer (164), breast cancer (165), and colorectal cancer (166). At the clinical level, rapamycin (sirolimus) and rapamycin derivative compounds (“rapalogs”) are currently approved by the Food and Drug Administration for the treatment of subependymal giant cell astrocytoma associated with tuberous sclerosis (everolimus) and neuroendocrine pancreatic tumors (everolimus) (35, 167, 168). As result, activation of mTOR signaling may foster protection against neurodegenerative disorders but such focus must also consider the potential for unintended and unchecked cellular growth. Ultimately, knowledge of how the intricate cellular signaling pathways of mTOR can lead to sometimes very different clinical outcomes will be essential for the development of clinical strategies for stroke that rely upon mTOR.

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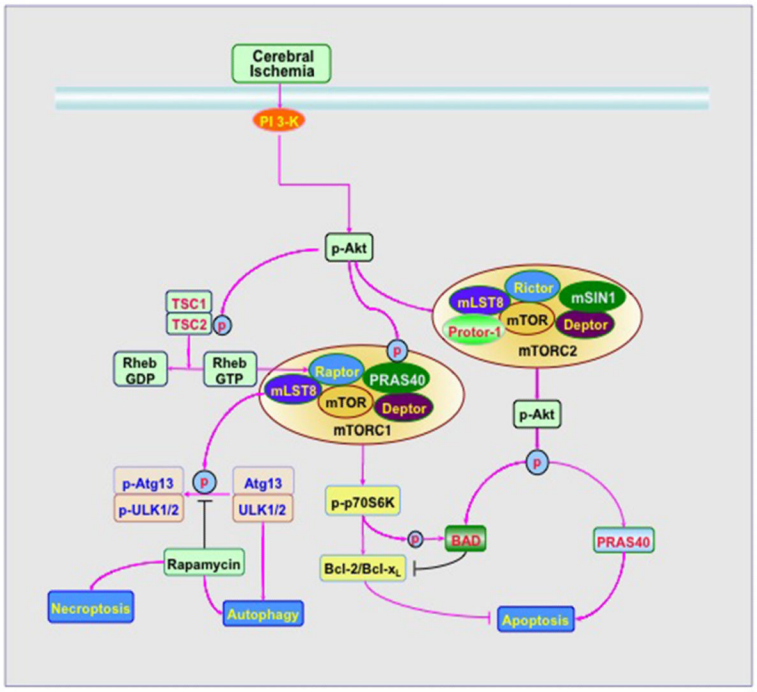

Figure 1. mTOR signaling in Stroke

Activation of phosphoinositide 3 kinase (PI 3-K) following cerebral ischemia onset leads to phosphorylation and activation of Akt (protein kinase B). Subsequently, Akt activates mTORC1 through phosphorylating TSC2 and disrupting the interaction between TSC2 and TSC1. Akt also can directly phosphorylate proline rich Akt substrate 40 kDa (PRAS40) to reduce its binding to regulatory associated protein of mTOR (Raptor), lead to activation of mTORC1, and prevent apoptosis. mTORC1 phosphorylates the downstream target p70 ribosome S6 kinase (p70S6K) to phosphorylate pro-apoptotic protein BAD and increase the expression of Bcl-2/Bcl-x_L which functions as an anti-apoptotic protein. mTORC1 activation also inhibits autophagic proteins autophagy related gene 13 (Atg13) and UNC-51 like kinase 1/2(ULK1/2) through phosphorylation to prevent autophagy. During the inhibition of mTOR with agents such as rapamycin, autophagy and necroptosis can be initiated.