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The Protective Effect of mammalian Target of Rapamycin (mTOR) in Cisplatin Induced Nephropathy

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Abstract

Cisplatin, a simple inorganic compound, has been one of the leading antitumor drugs especially for solid tumors for near 30 years. The mechanisms of cisplatin include denaturation of DNA and cell mitochondria, arresting cell cycle in the G2 phase and eventually causing apoptosis, inflammation, necrosis and death in cells. Apoptosis is a process of programmed cell death through cystein proteases named 'caspases'. Pathways of caspase-mediated apoptosis can classified as 'mitochondrial' pathway and 'death receptor' pathway Especially caspase-3 plays a crucial role in cisplatin-induced nephrotoxicity through the pathways of apoptosis. The mammalian target of rapamycin (mTOR), is a serine/threonine kinase that regulates both cell growth and cell cycle progression through the phosphotidyl 3 kinase (PI3K)/protein kinase B (Akt) signaling pathway. mTOR regulates both cell growth, cell cycle progression and angiogenesis. By targeting mTOR, the immunsuppressant and antiproliferative agent Rapamycin inhibits signals required for cell cycle progression, cell growth, cell proliferation and angiogenesis. Angiogenesis is extremely important in tumor progression and metastasis. Although rapamycin is proapoptotic agent especially in cancers, there is an evidence that rapamycin can also have antiapoptotic properties through pleiotropic function in the regulation of cell death depending on the cell type and activation state as well as downstream targets of antiapoptotic molecules such as p53 and Bcl-2 proteins. Activation of caspase signaling pathways and dysregulation of pro- and antiapoptotic Bcl-2 proteins have been described previously. So there are links between the mTOR and caspase signaling pathways. Despite its effectiveness, the dose of cisplatin that can be administered is limited by its nephrotoxicity such as acute tubuler necrosis (ATN) causing acute renal failure (ARF). Several agents have been tested to see whether they could ameliorate or augment the nephrotoxicity of cisplatin. Therefore, we hypothesize that mTOR inhibitor rapamycin can inhibit cisplatin induced ATN and ARF through the mechanisms including PI3K/Akt signaling and mitochondrial cell death pathway by affecting apoptosis. If this hypothesis will be proved by experimental and clinical studies, the patients with solid tumors receiving cisplatin may also be treated with mTOR inhibitors to reduce cisplatin induced nephrotoxicity.

Keywords
Cisplatin, Nephrotoxicity, Mammalian Target of Rapamycin,Apoptosis, mTOR inhibitors

Introduction

Apoptosis is a process of programmed cell death characterized by volume reduction, cell surface blebbing, chromatin condensation, internucleosomal cleavage of DNA, and formation of apoptotic bodies. A family of cysteine proteases, the caspases, are the major mediators of apoptosis. Pathways of caspase-mediated apoptosis can be classified as 'mitochondrial' pathway and 'death receptor' pathway (1). Caspase-3 plays a crucial and extensively studied role in the promotion of apoptotic cell death (2). Calpains are another important family of cysteine proteases which perform an important role in various cellular processes in mammals, such as signal transduction, cell proliferation and differentiation, apoptosis and necrosis (3).

Hypoxia is a common environmental stress which can also induces apoptosis through intracellular signaling pathways (4).

Cisplatin (cis-diamminedichloroplatinum (II)) is an effective agent against various solid tumors, including ovarian, head and neck, and testicular germ cell tumors (5). Cisplatin denaturates DNA chain and also damages cell mitochondria, arrests cell cycle in the G2 phase, inhibits ATPase activity, alters the cellular transport system, and eventually causing apoptosis, inflammation, necrosis and death in cells (5). The toxic effects of the drug in humans and animals include nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression, but its main dose-limiting side effect is nephrotoxicity such as acute tubuler necrosis (ATN) causing acute renal failure (ARF) (6) (Figure 1).

The mammalian target of Rapamycin (mTOR), which is a member of the phosphatidylinositol-3 kinase related kinase (PIKK) family, plays a critical role in transducing proliferative signals mediated through the PI3K/Akt signaling pathway, principally by activating downstream protein kinases that are required for both ribosomal biosynthesis and translation of key mRNAs of proteins required for progression of G1 to S phase (Figure 2). PI3K/AKt/mTOR-pathway plays a role in the regulation of cell proliferation, cell survival, angiogenesis and resistance to anti-tumor treatments. In many tumor types the PI3K/AKt/mTOR-pathway is found activated through several different underlying mechanisms. There are four mTOR-inhibitors, 'rapamycin, everolimus, temsorimus, and deforolimus' are evaluated for clinical use. (7) By targeting mTOR, these immunsuppressant and antiproliferative agents inhibit signals required for cell cycle progression, cell growth, cell proliferation and angiogenesis (8). Therefore mTOR is a prime strategic target for anti-cancer therapeutic development. Although Rapamycin is proapoptotic especially in cancers, there is an evidence that Rapamycin can also have antiapoptotic properties through pleiotropic function in the regulation of cell death depending on the cell type and activation state as well as downstream targets of antiapoptotic molecules such as p53 and Bcl-2 proteins (2) (Figure 1).

Hypothesis

We hypothesize that mTOR inhibitor, Rapamycin, can inhibit cisplatin-induced ATN and ARF through the mechanisms including PI3K/Akt signaling and mitochondrial cell-death pathway by affecting apoptosis (Figure1,2). If this hypothesis will be proved by experimental and clinical studies, the patients that have solid cancers receiving cisplatin may also be treated with mTOR inhibitors to reduce cisplatin induced nephrotoxicity.

Evaluation of the hypothesis

In the mitochondrial pathway of apoptosis, the balance of pro- and antiapoptotic Bcl-2 proteins control cytochrome c release from mitochondria. When proapoptotic Bax or Bad is in excess, cells execute a death command, but when antiapoptotic Bcl-2 or Bcl-XL dominates, apoptosis is inhibited and cells survive (1). Cytochrome c binds to the cytosolic protein, apoptosis protease-activating factor-1 (APAF-1), which activates caspase-9. In the death receptor pathway, the binding of a ligand to its death receptor recruits an adaptor protein that in turn recruits and activates procaspase-8. For example, Fas ligand (FasL) binds to its death receptor Fas that recruits an adaptor protein called Fas-associated death domain (FADD). FADD in turn recruits and activates procaspase-8. The initiator caspases-8 and 9 in turn activate caspase-3 which plays a crucial and extensively studied role in the promotion of apoptotic cell death (2) (Figure 1). Calpains are a family of calcium-dependent cysteine proteases found in all eukaryotes. It has been implicated that calpains perform an important role in various cellular processes in mammals, such as signal transduction, cell proliferation and differentiation, apoptosis and necrosis. The potential role of calpains in apoptosis is indicated by a growing list of calpains substrates, including p53, Bax, Bid, apoptosis-inducing factor (AIF) and several cytoskeletal proteins (3).

Hypoxia is also another important inducer of apoptosis. The central mediator of hypoxia is a DNA binding complex named HIF-1, plays a key role in the regulation of angiogenesis especially in cancer cells by induction of vascular endothelial growth factor (VEGF) (4). Numerous past studies have suggested a critical role of the paracrine effect between tumor vascular endothelial growth factor (VEGF)-C and lymphatic FLT-4 in solid tumor-

associated lymphangiogenesis (24). Hypoxia also triggers adaptive mechanisms to insure cell survival. The prosurvival effects of HIF-1 in endothelial cells are mediated by NOR-1. The over-expression of NOR-1 decreased the rate of endothelial cells undergoing apoptosis in cultures exposed to hypoxia, while the inhibition of NOR-1 increased cell apoptosis (9).

Cisplatin, an effective anticancer agent, can induce tumor cell apoptosis via caspase-dependent and-independent pathways. However, the precise mechanism that regulates the pathways remains unclear.

The mammalian target of Rapamycin (mTOR), is a serine/threonine kinase that regulates both cell growth and cell cycle progression through the phosphotidyl 3 kinase (PI3K)/protein kinase B (Akt) signaling pathway. Phosphatidylinositol-3 kinase (PI3K) converts the lipid phosphatidylinositol biphosphate (PIP2) into phosphatidylinositol triphosphate (PIP3), which localizes protein kinase B (Akt) to the membrane. mTOR phosphorylates both ribosomal protein S6 kinases (p70S6K1) and eukaryotic initiation factor 4E-binding proteins (4E-BP1) via independent pathways, resulting in activation of S6K1 and inactivation of 4E-BP1. Increased p70S6K1 and eIF4E act independently to promote cell growth and cell-cycle progression. Therefore mTOR plays a critical role in transducing proliferative signals mediated through the PI3K/Akt signaling pathway, principally by activating downstream protein kinases that are required for both ribosomal biosynthesis and translation of key mRNAs of proteins required for progression of G1 to S phase (8) (figure 2). mTOR can also be activated by numerous oncogenic signals, such as endothelial growth factor (EGF), insulin like growth factor (IGF) and vascular endothelial growth factor (VEGF), mutation and silencing of the PTEN tumor suppressor gene, activating mutations in the PI3K catalytic subunit, and the Ras-Raf-MEK pathway. These deregulations permit the survival, growth, proliferation and migration of cancer cells and promote tumor angiogenesis. Therefore targeting mTOR pathway has been a successful anticancer strategy (10).

Experimental data

There are links between the mTOR, cisplatin-induced apoptosis and calpain-caspase signaling pathways. Edelstein et al. showed that Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease (PKD) through apoptosis (11). Houghton et al. also discussed the effects of mTOR inhibitors in cancer therapy (12). Mita et al. evaluate the effects of targeting the mTOR pathway using deferolimus in cancer therapy (10). Recently Scotte et al showed the beneficial effects of everolimus in the treatment of metastatic renal cell carcinoma (13) These effects can be partly attributed to inhibition of angiogenesis through VEGF signaling pathway (4).

There is much evidence that Rapamycin is proapoptotic especially in cancers, resulting in apoptotic death of the cancer cells (14); however, mTOR may have a pleiotropic function in the regulation of cell death depending on the cell type and activation state as well as downstream targets such as p53 and Bcl-2 proteins (15). And also there is evidence that Rapamycin can be antiapoptotic (16,17). For example, Rapamycin inhibits death of syncytia via inhibition of proapoptotic Bax and inhibition of the mitochondrial cell death pathway (18,19). Chen et al. showed that mu-calpain mediated both caspase-dependent and-independent pathways during cisplatin-induced apoptosis in human lung adenocarcinoma cells. In this study after cisplatin treatment, calpain activation was an early event, taking place well before AIF release and caspase-9/-3 activation. Inhibition of mu-calpain not only significantly reduced caspase-9/-3 activities but also completely blocked AIF redistribution. The study demonstrated that activation of mu-calpain played an essential role in regulating both caspase-dependent and AIF-mediated caspase-independent apoptotic pathways in cisplatin-induced apoptosis (3). Faubal et al. showed that caspase-1 contributes to cisplatin-induced ARF and ATN. Furthermore, caspase-1 affects caspase-3 activation and apoptosis in cisplatin induced ARF (20). Calpain activation may also inhibit the anabolic signaling of Akt, since a molecular chaperone previously shown to mediate Akt activity, heat shock protein 90 (HSP 90), is a calpain substrate. Thus, an additional objective was to determine whether calpain activation affects the Akt signaling pathway (23).

There are associations between the mTOR and caspase signaling pathways (Figure 1). Activation of caspase signaling pathways and dysregulation of pro-and antiapoptotic Bcl-2 proteins have been described previously. Although S6 protein is the best characterized substrate of p70S6K, p70S6K is also known to inactivate the proapoptotic protein BAD by preventing phosphorylation of Ser136 on BAD and blocking cell survival induced by insulin like growth factor –I (IGF-I) (14) (Figure 2). Several agents have been tested to see whether they could ameliorate or augment the nephrotoxicity of platinum drugs. The agents that have been shown to ameliorate experimental cisplatin nephrotoxicity include antioxidants (e.g. melatonin, vitamin E, selenium, and many others), modulators of nitric oxide (e.g. zinc histidine complex), agents interfering with metabolic pathways of cisplatin (e.g. procaine HCL), diuretics (e.g. furosemide and mannitol), and cytoprotective and antiapoptotic agents (e.g. amifostine and erythropoietin) (21). Chan et. al also showed benefecial effects of

Rapamycin in a rat model of adriamycin-induced nephropathy (22).

Discussion

Cisplatin is a widely used antineoplastic agent especially for solid tumors such as ovarian, head, neck, and testicular germ cell tumors for several years. Despite its beneficial effects, dose-limiting side effect of cisplatin is nephrotoxicity such as acute tubuler necrosis (ATN) causing acute renal failure (ARF). The side effects of cisplatin can be attributed to both apoptosis and necrosis. The interaction of caspase and calpain pathways of apoptosis is extremely important in cisplatin-induced ARF. mTOR regulates both cell growth, cell cycle progression and angiogenesis. By targeting mTOR, the immunsuppressant and antiproliferative agent Rapamycin inhibits signals required for cell cycle progression, cell growth, cell proliferation and angiogenesis. Angiogenesis is extremely important in tumor progression and metastasis. Recent studies demonstrate these effects by inhibition of PI3K/AKt/mTOR-pathway in solid cancer therapy (10,13). So we hypothesize that mTOR inhibitor Rapamycin can inhibit cisplatin induced ATN and ARF through the mechanisms including PI3K/Akt signaling and mitochondrial cell death pathway by affecting apoptosis. If this hypothesis will be proved by experimental and clinical studies, the patients that have solid cancers receiving cisplatin may also be treated with mTOR inhibitors to reduce cisplatin-induced nephrotoxicity.

OVERVIEW BOX

First Question: What do we already know about the subject?
Answer: Acute renal failure can be seen in cisplatin-treated patients through the apoptosis pathways. mTOR inhibitors may induce antiapoptotic properties along the caspase and calpain interactions. Several agents, except Rapamycin, used to prevent cisplatin-induced nephropathy. Recent studies showed beneficial effects of mTOR inhs in solid cancer therapy.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?
Answer: Several studies were performed to evaluate the mechanisms of apoptosis. However the interactions between calpain and caspase mediated apoptosis remain unclear. If our hypotheses will be proved, both the pathogenesis of apoptosis and its reflection may provide benefial clinical effect in patients with cisplatin-induced ARF.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?
Answer: An experimental study could be performed searching the effects of Rapamycin in rats with cisplatin-induced ARF. Further clinical trials will be needed if the results of this experimental study are significant.

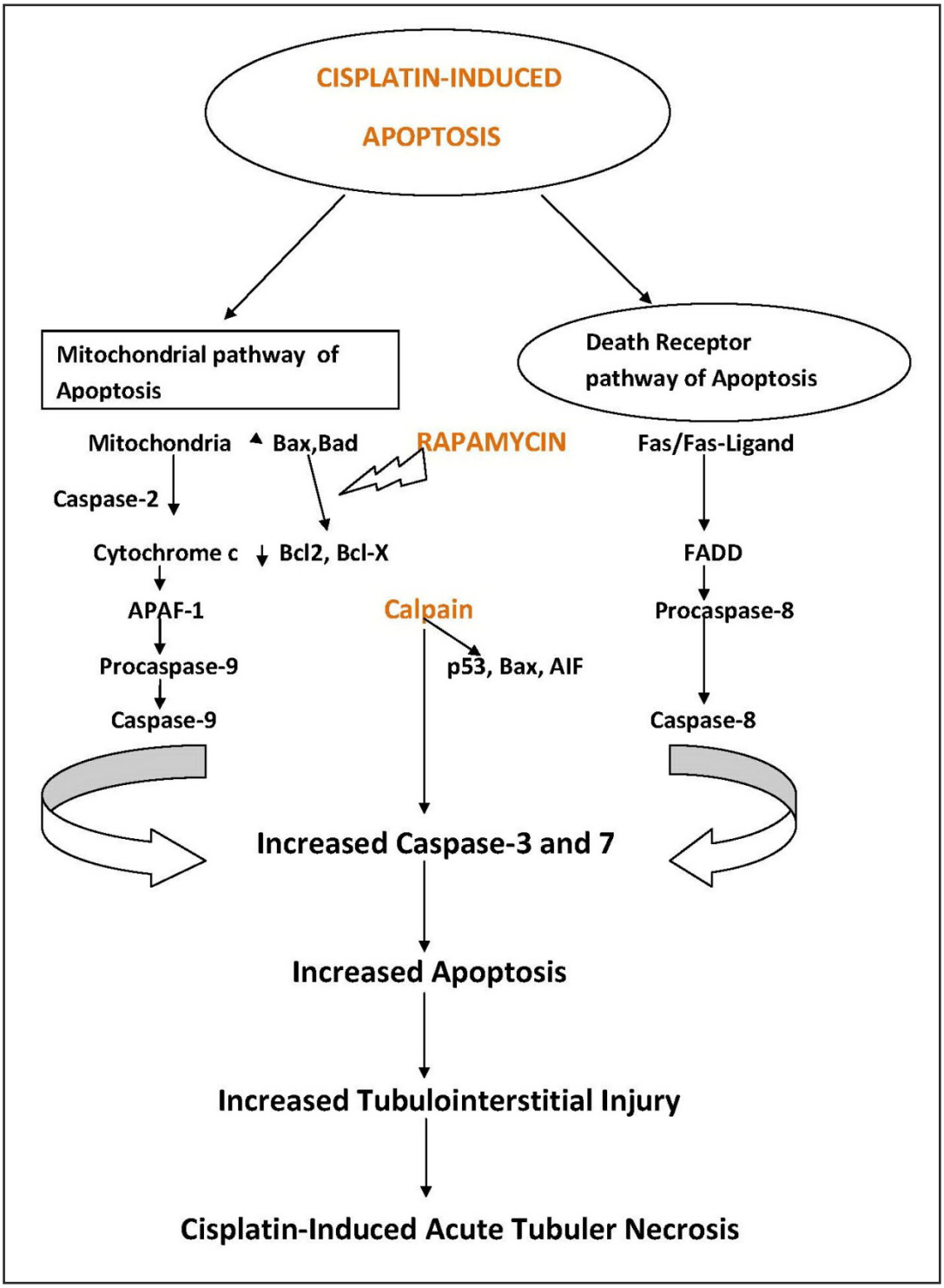

Figure 1: Mechanisms of Cisplatin-Induced Acute Tubuler Necrosis due to Apoptosis and Protective effect of Rapamycin (APAF-1: apoptosis protease-activating factor 1, FADD: Fas-associated death domain, AIF: Apoptosis Inducing Factor

: inhibition

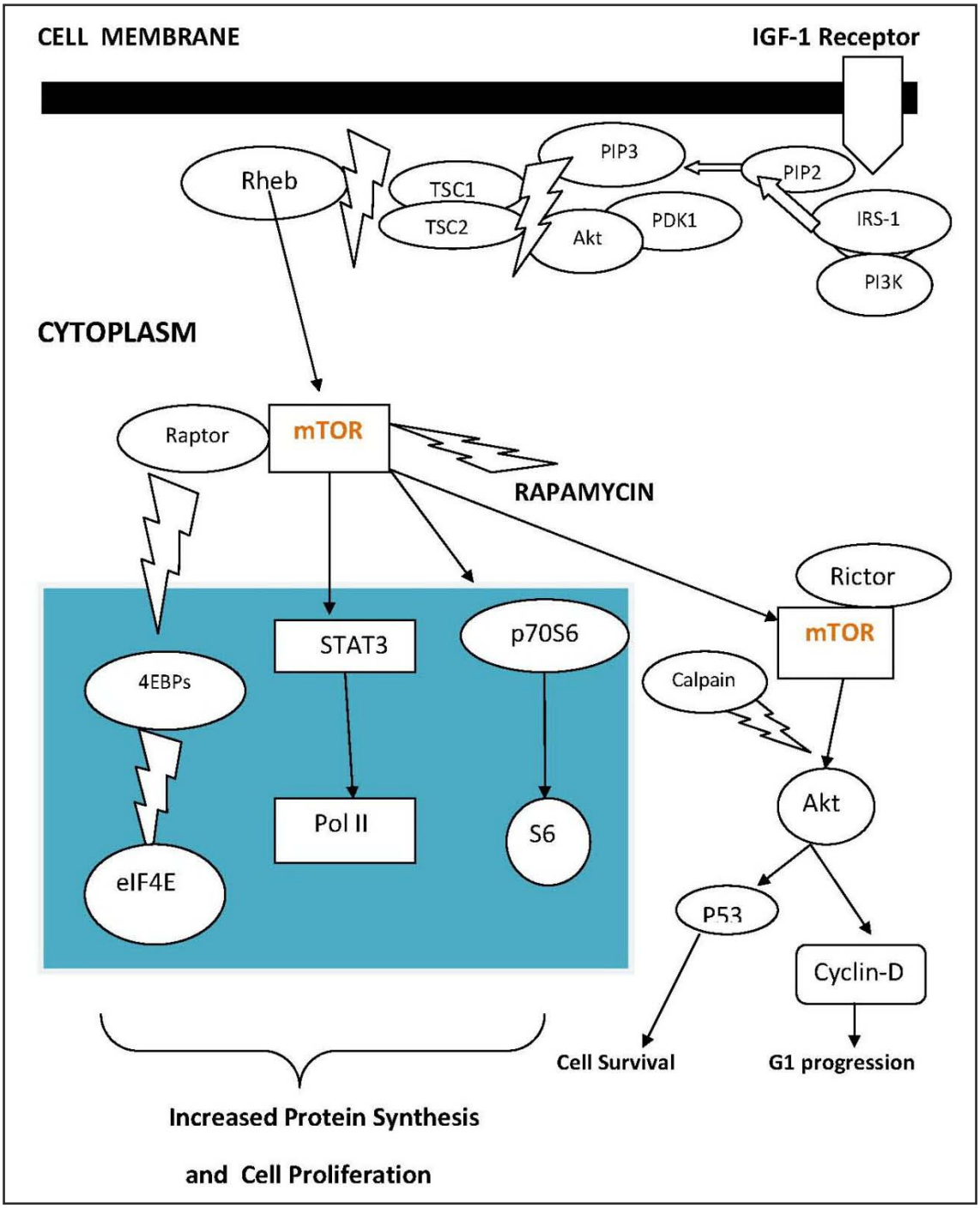

Figure 2: Intracellular effects of Rapamycin through mammalian target of rapamycin pathway (mTOR). mTOR-Raptor complex is sensitive to Rapamycin however mTOR-Rictor complex is resistant to effects of Rapamycin.
eukaryotic initiation factor 4EIGF-1; Insulin-like growth factor 1, PI3K; Phosphotidylinositol 3 kinase,
PDK1; Phosphotidylinositol-dependent-kinase-1, Akt; protein kinase B, TSC1,2; Tuberosclerosis complex 1,2 Rheb; Ras-homolog-enriched in brain act as Ras-related small GTPase
Raptor; Rapamycin-sensitive adaptor protein, eIF4E; eukaryotic initiation factor 4E, 4EBP; eukaryotic initiation factor 4E binding protein, Pol II; polymerase II.

: **inhibition**

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