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Inhibiting 4EBP1 in glioblastoma

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Abstract

Glioblastoma is the most common and aggressive adult brain cancer. Tumors show frequent dysregulation of the phosphatidylinositol-3 kinase-mechanistic target of rapamycin pathway. While a number of small molecules target the PI3K-AKT-mTOR axis, their preclinical and clinical efficacy has been limited. Reasons for treatment failure include poor penetration of agents into the brain, and observations that blockade of PI3K or AKT minimally affects downstream mTOR activity in glioma. Clinical trials using allosteric mTOR inhibitors (rapamycin and rapalogs) to treat glioblastoma patients have also been unsuccessful or uncertain, in-part because rapamycin inefficiently blocks the mTORC1 target 4EBP1, and also feeds back to activate PI3K-AKT signaling. Inhibitors of the mTOR kinase (TORKi) such as TAK-228/MLN0128 interact orthosterically with the ATP and substrate-binding pocket of mTOR kinase, efficiently block 4EBP1 in-vitro, and are currently being investigated in the clinical trials. Preclinical studies suggest that TORKi have poor residence times of mTOR kinase, and our data suggests that this poor pharmacology translates into disappointing efficacy in glioblastoma xenografts. RapaLink-1, a TORKi linked to rapamycin, represents a drug with improved pharmacology against 4EBP1. In this review, we clarify the importance of 4EBP1 as a biomarker for the efficacy of PI3K-AKT-mTOR inhibitors in glioblastoma. We also review mechanistic data by which RapaLink-1 blocks p-4EBP1, and discuss future clinical strategies for 4EBP1 inhibition in glioblastoma.

Introduction

Glioblastoma remains one of the major challenges in pediatric and adult cancer. Despite surgery, radiation and chemotherapy, patients survive a median of 18 months or less from diagnosis (1). Glioblastomas frequently activate signaling through phosphatidylinositol-3 kinase (PI3K), AKT, and mTOR (mechanistic target of rapamycin) (2). A number of inhibitors that target key components of this pathway are being tested clinically, and to date have shown limited efficacy (3, 4).

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mTOR integrates the abundance of nutrients and growth factor to cell growth and metabolism (5). Signaling functions of mTOR are distributed between at least two distinct protein complexes: mTORC1 and mTORC2. In mTORC1, mTOR is associated with proteins including PRAS40 and the rapamycin-sensitive adapter protein of mTOR (Raptor) whereas in mTORC2, mTOR is associated with a separate protein complex including the rapamycin-insensitive companion of mTOR (Rictor). The mTORC1 complex signals primarily through two effectors. One is p70 ribosomal protein S6 kinase (S6K). Phosphorylation and activation of S6K result in phosphorylation of S6K targets such as eIF4B and ribosomal protein S6 (RPS6). The second major output of mTOR signaling is via regulation of the eukaryotic initiation complex eIF4F, which recruits mRNA to the ribosome and consists of three proteins: 1). eIF4A, an RNA helicase, 2). eIF4E, a protein that binds and recruits the m^7GTP cap of mRNA to the eIF4F complex, and 3). eIF4G, which serves a scaffolding function by directly binding to eIF4E, eIF4A, and ribosome-associated eIF3 (6). Interaction of eIF4E with both the m^7GTP cap and eIF4G is rate limiting in translation. Regulation of this step occurs through 4E-binding protein (4EBP), which binds to eIF4E at the eIF4E-eIF4G interaction interface, to prohibit its participation in the initiation complex. Hypophosphorylated 4EBP binds eIF4E with high affinity, whereas direct phosphorylation by mTOR causes 4EBP to dissociate from eIF4E. Free eIF4E can then participate in the translation initiation complex, leading to an increase in cap-dependent translation, and driving proliferation.

How mTORC2 contributes to translation regulation and growth control generally, and in glioblastoma specifically, remains less clear (Fig. 1, ref. 7). This is partly because there are no specific inhibitors of TORC2. The mTORC2 complex is stimulated by growth factors that promote PI3K-dependent activation of mTORC2. PI3K-independent mechanisms of mTORC2 activation have also been described, and include WNT-LRP5 and Notch signaling (8). Activated mTORC2 can phosphorylate several members of the AGC subfamily of kinases, including AKT (Ser 473), SGK1 (Ser 422), PKCα (Ser 657), as well as the actin-crosslinking protein filamin A (FLNA) on Ser 2152, to regulate tumor growth, metabolism, chemotherapy resistance, and cytoskeletal organization in glioblastoma (9, 10). Therefore, mTORC2 may also represent a therapeutic target in glioblastoma.

A number of mTOR inhibitors are currently in pre-clinical or clinical trials for cancer (Table 1; Ref. 4, 11–40). Allosteric mTOR inhibitors (rapamycin and rapalogs, Fig. 2) bind to FK506 Binding Protein 12 (FKBP12). The rapamycin-FKBP12 complex subsequently binds to a region of mTOR kinase called FK506-Rapamycin Binding (FRB), outside of the ATP/substrate binding pocket. Binding of FKBP12 and rapamycin to FRB changes the conformation of mTOR allosterically (41) limiting substrate access, and resulting in blockade of S6K but not 4EBP1. The FRB is not accessible in mTORC2, so rapalogs are mTORC1 selective, and only inhibit one output of mTORC1 (42, 43). In addition, rapalogs activate AKT due to a well-described negative feedback loop (Fig. 1), potentially reducing their benefit as anticancer agents (44). Indeed, while some rapalogs have gained FDA approval for the treatment of specific cancers (Table 1), the survival benefit with rapalogs is on the order of months and not years, likely due in-part, to these drugs having only a cytostatic effect. In lesions driven primarily by mTORC1 activation, however, rapalogs have shown significant efficacy. Patients with Tuberous Sclerosis have germline inactivation of

either TSC1 or TSC2 which link AKT to mTOR (Fig. 1), and develop benign growths in multiple organs, including their heart, kidneys, and brain (45). These lesions are extremely sensitive to rapalogs, with everolimus associated with a 75% durable objective response rate in patients with subependymal giant cell astrocytomas (46), a benign brain tumor found in 15% of patients with TS.

The identification of PI3K/mTOR inhibitors (47, 48) led to further chemical efforts to dial out PI3K inhibition, resulting in mTOR kinase inhibitors, TORKi (24, 28, 30, Fig. 2). In contrast to rapalogs, TORKi act through orthosteric interactions with the ATP binding pocket of mTOR kinase. As a result, TORKi block both mTORC1-dependent phosphorylation of 4EBP and S6K, and mTORC2-dependent phosphorylation of AKT. While these agents are consequently more active than rapalogs, this increased activity is due to better inhibition of mTORC1, rather than to inhibition of mTORC2 (24, 28, 30). The activity of these agents against p-AKT produces a broad acting agent that may limit the therapeutic index of active site inhibitors of mTOR, as mTORC2 but not mTORC1 is essential for normal cells (49). Also, mTORC2 promotes lipogenesis, glucose uptake, glycolysis, and cell survival through the downstream targets, such as AKT, serum/glucocorticoid-regulated kinase (SGK), and protein kinase C (50–52). Due to its role in mediating lipid and glucose homeostasis, blockade of mTORC2 signaling can lead to dose-limiting toxicities related to insulin resistance and diabetes. Likely due to mTORC2 inhibition, the TORKi Torin 1 is actually more toxic to pancreatic islet cells than rapamycin (53, 54).

TORKi, such as OSI-027, TAK-228/MLN0128, AZD8055, and CC-223 are being tested clinically (Table 1). While many studies are still ongoing, early reports do not show clinical efficacy using TORKi's as monotherapy in unselected populations. TORKi's have shown significant, durable antitumor efficacy in preclinical models and in patients that harbor RICTOR amplification, found in ~14% of small cell lung cancers and 4% of gastric carcinomas (55–57). Cell based screens using AZD8055 have identified activating mutations driving resistance (39). The third generation mTOR inhibitor RapaLink-1 (Fig. 2), developed to block mTOR activity in the setting of mutational activation of mTOR kinase, linked an active site inhibitor of mTOR to the mTORC1 specific targeting domain of rapamycin. RapaLink-1 was able to overcome resistance to either rapamycin or TAK-228 and to a combination of the two in kidney and breast cancer lines (39).

The canonical pathway linking PI3K and AKT to mTOR is inactive in glioblastoma

PI3Ks are lipid kinases activated by a wide range of upstream receptor tyrosine kinases to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3). PIP_3 couples PI3K to downstream effectors such as AKT, a serine-threonine kinase that suppresses apoptosis, promotes growth, and drives proliferation. PIP_3 also indirectly activates the mTOR. Activation of PI3K signaling may result from amplification and mutation of receptor tyrosine kinases such as EGFR, or from mutation of components downstream of PI3K. Examples include inactivation of the *PTEN* (phosphatase and tensin homolog), a lipid

phosphatatase and negative regulator of PI3K, and mutational activation of a class I PI3K enzymatic subunit *PIK3CA*. Stimulation of PI3K in response to growth factors leads to phosphorylation and activation of AKT. Activated AKT is recruited to the plasma membrane by PIP_3 through a direct interaction with the PH domain of AKT. AKT is phosphorylated by PDK1 on T308, and by mTORC2 on S473 (Fig. 1). Activated Akt phosphorylates well over 100 validated and candidate downstream substrates (58) that are mostly inhibitory, including PRAS40 and the TSC1/2 (Hamartin-Tuberin) complex. PRAS40 inhibits mTORC1, while Tuberin inhibits the GTPase RHEB, which in turn inhibits mTORC1 (59).

A numbers of small molecule inhibitors of PI3K and AKT have been developed, agents that block all Class I isoforms, isoform-specific Class I PI3K inhibitors, and inhibitors of AKT (60). These clinical agents were derived from chemotypes now available preclinically, including the p110α-selective PIK90, INK1437, the p110β-selective TGX221, the p110δ-selective IC87114, and the p110γ-selective AS252424; Pan-PI3K inhibitor, such GDC-0941; AKT inhibitor, such as the AKT inhibitor VIII, MK-2206. Comparing the anti-proliferative and biochemical activities of these inhibitors in multiple human glioblastoma lines, we demonstrated that inhibitors of class I PI3Ks and AKT minimally impacted proliferation, despite observing that p110α-selective inhibitors of PI3K, and AKT inhibitors potently blocked phosphorylation of AKT (40). These results were consistent with earlier studies, in that neither blockade of PI3K/AKT, nor knockdown of AKT1–3 affected the abundance of the mTORC1 target p-RPS6 (61, 62).

Also aligned with these preclinical observations, preliminary clinical studies using the blood brain barrier penetrant PI3K inhibitor buparlisib (BKM120) demonstrated intratumoral concentrations sufficient to inhibit p-AKT (S473), with minimal single agent efficacy (63). These preclinical and clinical data suggest that activation of mTOR in glioblastoma is not linked canonically to upstream PI3K and AKT. Our preclinical observations also suggest that PI3K and AKT inhibitors in clinical use will not block mTOR signaling in glioblastoma. The best way to block mTOR is to use agents that inhibit mTOR directly, rather than agents that block upstream signaling.

p-4EBP1 represents a robust biomarker for the antiproliferative effect of mTOR inhibitors in glioblastoma

Having demonstrated that neither PI3K nor AKT represented biomarkers for proliferation in glioblastoma, we next sought to identify a biomarker that did correlate with therapeutic response. Glioma cells were treated with the pan-class I PI3K inhibitor GDC-0941, the allosteric mTOR inhibitor rapamycin, the TORKi KU-0063794, and a dual PI3K/mTOR inhibitor NVP-BEZ235. GDC-0941, at doses selective for class I PI3K, minimally affected proliferation, although levels of p-AKT were clearly reduced. GDC-0941 did display potent anti-proliferative effects at doses high enough to inhibit mTOR. Rapamycin led to reduced abundance of p-RPS6 but not of p-4EBP1, with increased levels of PIP3 and p-AKT, in accordance with a well-established mTORC1 negative feedback loop leading to reactivation of PI3K signaling (44). This agent also minimally affected proliferation. In contrast, the TORKi KU-0063794 and the PI3K/mTOR inhibitor NVP-BEZ235 showed dose-dependent

responses against p-RPS6, p-4EBP1, and p-AKT with corresponding blockade of proliferation (40). These data suggested that blockade of p-4EBP1 was critical, whereas blockade of mTORC1 target RPS6 or the mTORC2 target AKT were dispensable for the anti-proliferative activity of mTOR inhibitors in glioblastoma.

RapaLink-1 is more potent than first- and second-generation mTOR inhibitors in glioblastoma

We next compared rapamycin, TAK-228, and RapaLink-1 for effects on proliferation and mTOR signaling in human glioblastoma cell lines and in short-term cultures isolated from patient derived xenografts. Both growth inhibition and arrest at G0/G1 were more potent in response to RapaLink-1, as compared to rapamycin or TAK-228. As expected, rapamycin only inhibited the mTORC1 target p-RPS6. TAK-228, in contrast, inhibited the mTORC1 targets p-RPS6 and p-4EBP1, as well as mTORC2 target p-AKT in a dose-dependent manner. The antiproliferative effects of TAK-228 correlated with inhibition of p-4EBP1. To address a role for mTORC2 in this activity, we also combined TAK-228 with the AKT inhibitor MK-2206 (64). This combination did not enhance the efficacy of TAK-228. Additionally, RapaLink-1 selectively inhibited p-RPS6 and p-4EBP1 at doses as low as 1.56 nM, while the mTORC2 target p-AKT was inhibited only at five-ten fold higher doses, without further affecting proliferation. These results suggest that additional blockade of mTORC2 did not improve the efficacy of mTORC1 inhibitors.

To evaluate penetration across the blood-brain barrier, we treated normal BALB/C$^{nu/nu}$ mice with RapaLink-1 and examined insulin signaling in brain tissues. RapaLink-1 inhibited p-RPS6 and p-4EBP1 in a dose-dependent manner in brain, but did not inhibit the mTORC2 substrate p-AKT in vivo. These data suggest that RapaLink-1 is able to cross the blood-brain barrier. The absolute mTORC1 selectivity in vivo contrasted with only partial mTORC1 selectivity in-vitro. These differences either reflect a limited ability to cross the blood brain barrier, or a fundamental difference between in-vitro and in vivo pharmacology.

We next compared RapaLink-1, TAK-228 and rapamycin in both cell line and patient-derived orthotopic glioblastoma xenografts. Inhibition of tumor growth was more potent in response to RapaLink-1, as compared to rapamycin or TAK-228. Western blotting of treated tumors demonstrated that RapaLink-1 efficiently blocked p-4EBP1, whereas TAK-228 only modestly blocked p-4EBP1. Despite observations in multiple cell lines, that rapamycin failed to block p-4EBP1 even at high doses, rapamycin and TAK-228 were equivalent in their ability to block p-4EBP1 in vivo. These results are consistent with observations that rapamycin is partially able to inhibit the p-4EBP in some settings (65, 66). All treatments blocked p-RPS6, while TAK-228 uniquely inhibited p-AKT. Surprisingly, rapamycin slowed tumor growth and prolonged survival more effectively than TAK-228. While both agents blocked p-4EBP to similar degrees in tumors from animals sacrificed 30 minutes after treatment, it is likely rapamycin did so more durably than TAK-228.

RapaLink-1 dramatically improved survival compared with rapamycin and TAK-228. RapaLink-1 led to initial regression of tumors, with subsequent recovery of growth, although subsequent growth was much slower when compared to rapamycin- or TAK-228-treated

mice. In cell-line based xenografts, all mice treated with rapamycin or TAK-228 had succumbed to glioblastoma by 55, days, a time point at which all animals treated with RapaLink-1 were still alive (40). RapaLink-1 was also tested in a genetically engineered "GTML" MYCN-driven model for medulloblastoma (67) in which tumors arise spontaneously without mechanical disruption of the blood brain barrier. Rapalink-1 again led to regression of established tumors in these barrier-intact animals, blocking both p-RPS6 and p-4EBP1.

RapaLink-1 durably blocks mTORC1 through a RapaLink-1-FKBP12-mTOR complex

To compare the durability of mTORC1 inhibition, glioblastoma cells were treated with RapaLink-1, rapamycin, or TAK-228 for one day, followed by washout. Recovery of proliferation in cells treated with RapaLink-1 was observed four days after washout, whereas biochemical recovery of p-RPS6 and p-4EBP1 was detectable at two days. Rapamycin showed much more modest blockade of proliferation, with no recovery of proliferation or of p-RPS6 blockade over four days, but inefficient inhibition of p-4EBP1. Surprisingly, recovery of proliferation in cells treated with TAK-228 started after one day, and nearly full recovery of signaling was observed at one hour after washout (earliest time point evaluated). TAK-228 thus shows poor residence time, the time that TAK-228 resides on mTOR kinase (68). To evaluate whether allosteric binding of RapaLink-1 to mTORC1 might augment orthosteric inhibition, rapamycin was tested in combination with TAK-228. Washout recovery of TAK-228 cells was identical in the presence or absence of rapamycin (QWF and WW, unpublished). To date, it is not clear whether allosteric blockade of mTORC1 contributes to the orthosteric activity of RapaLink-1.

Washout data suggested that even though TAK-228 was a more complete inhibitor of mTORC1 in-vitro as compared to rapamycin, TAK-228's poor in vivo pharmacology contributed to it underperforming compared to rapamycin in vivo. RapaLink-1, through binding to FKBP12 and FRB, improved this in vivo pharmacology, resulting in a more durable mTORC1 inhibitor. FKBP12 is an abundant cellular protein (69), with high-level expression across all of over a dozen primary human glioblastoma samples tested (40). Rapamycin binds to FKBP12 to form a drug-receptor complex that binds to the FRB domain of mTOR. The immunosuppressive FK-506 itself does not inhibit mTORC1, but competes with rapamycin for FKBP12 binding. RapaLink-1 also requires binding to FKBP12 for activity, verified by using FK-506 to treat human glioblastoma cells in combination with either RapaLink-1 or TAK-228 (40). As controls, rapamycin was tested in combination with either RapaLink-1 or TAK-228. Both FK-506 and rapamycin antagonized the inhibitory effects of RapaLink-1 on proliferation and on blockade of p-RPS6 and p-4EBP1. Neither FK-506 nor rapamycin blocked the cellular or biochemical effects of TAK-228. These results suggest that FKBP12 is required for the activity of RapaLink-1.

Lastly, the binding of rapamycin-FKBP12 to mTORC1 was compared with that of RapaLink-1-FKBP12. Immunoprecipitates of mTOR kinase were prepared from RapaLink-1- or rapamycin-treated glioblastoma cells, and analyzed by western blotting for

bound FKBP12. Levels of RapaLink-1-FKBP12 complex bound to mTOR were higher than those of the rapamycin-FKBP12 complex. The increased affinity of RapaLink-1 for FKBP12 and of the RapaLink-1-FKBP12 complex for mTOR could, in-part, underlie earlier observations that RapaLink-1 was more effective than rapamycin at suppressing mTORC1 activity and proliferation. However, these biochemical ideas are at some levels non-aligned with washout data, which demonstrated that rapamycin more durably blocked signaling, compared with RapaLink-1.

Concluding remarks and future directions

In the last decade, PI3K-AKT-mTOR pathway inhibitors have been developed as drugs for cancer, with testing of these agents now underway in patients with glioblastoma. PI3K and AKT inhibitors fail to block proliferation preclinically, with early evidence suggesting similar failure in patients. The canonical pathway linking PI3K and AKT to mTOR appears to be inactive in glioblastoma. In glioblastoma, activated AKT phosphorylates TSC2 without blocking mTOR, suggesting that the miswiring may occurs at the level of TSC1 or RHEB (62). Blockade of mTOR does show efficacy preclinically, with the downstream effector p-4EBP1 rather than S6K representing a robust biomarker of therapeutic response to mTOR inhibition. First generation mTOR inhibitor rapamycin and rapalogs have shown limited clinical impact in brain tumors (70), likely due to selectively inhibiting only S6K and not 4EBP1, and to feedback activation of PI3K-AKT. Inhibition of mTORC1 in some setting also feeds back to activate MAPK signaling, leading to therapy resistance (71). The cell-type specificity for this feedback up-regulation of AKT and MAPK remains unclear.

While prolonged rapamycin treatment inhibits mTORC2 and its downstream target AKT in certain cancer cell types (66), second generation TORKi more effectively block mTORC2-dependent phosphorylation of AKT, while also inhibiting mTORC1-dependent phosphorylation of 4EBP and S6K. The TORKi TAK-228 was more effective than rapamycin in glioblastoma cell lines, however this improved efficacy was not evident in vivo in preclinical experiments. This surprising result was traced to poor in vivo pharmacokinetics for TAK-228, resulting in short-lived in vivo activity of TAK-228 against the mTORC1 target p-4EBP1. RapaLink-1 in contrast, binds to FKBP12 and to the FRB in a manner analogous to the binding of rapamycin. The dual binding of RapaLink-1 to both FRB and the ATP/substrate binding pocket may serve to increase affinity and stability, leading to potent blockade of both mTORC1 downstream effectors 4EBP1 and S6K and accumulation of RapaLink-1 in brain tumor cells. RapaLink-1 was a more potent anti-cancer agent, and a better 4EBP1 inhibitor than rapamycin or TAK-228 in vivo.

The first-generation mTOR inhibitor rapamycin demonstrated some anti-tumor activity in a phase I trial for patient with recurrent PTEN-deficient glioblastoma (Table 1, Ref. 13). The results from a Phase II trial of rapamycin as monotherapy or in combination with erlotinib, however, were discouraging for patients with recurrent glioblastoma (Table 1 Ref. 4). Clinical trials in other cancers have also been disappointing, due to unfavorable pharmacokinetic properties, leading pharmaceutical companies to develop rapalogs including everolimus, temsirolimus, and ridaforolimus. A phase II trial of everolimus in combination with temozolomide (TMZ) and radiation failed to achieve promising results for

patients with newly diagnosed glioblastoma (Table 1, Ref. 15). A phase II trial of temsirolimus (CCI-779) showed limited activity against recurrent glioblastoma patients and in children with high-grade glioma (Table 1, Ref. 19, 20). In a phase I/pharmacodynamics trial ridaforolimus reduced the p-S6 levels in glioblastoma but this was not associated with any radiographic response (Table 1, Ref. 23). Rapalogs are currently undergoing clinical evaluation in various tumor types. The rapalogs temsirolimus and everolimus have been approved for cancer therapy, however rapalogs do not appear to be effective for the majority of solid tumors including glioblastoma.

Given that rapamycin and rapalogs exert an incomplete inhibition of mTORC1 and are inactive against mTORC2, in the last decade, second-generation selective mTORC1/2 inhibitors have been developed (Table 1). Among them, PP242, WYE-354, WAY-600, WYE-687, Torin 1, and KU-0063794 are still in preclinical development while early phase clinical trials have been initiated for OSI-027, TAK-228, AZD8055, AZD2014, and CC-223 in treatment of cancers including glioblastoma. Despite their superior potency in vitro and in vivo, thus far clinical efficacy has been limited to patients with RICTOR amplifications (55).

Although, third generation mTOR inhibitor shows improved potency compared with rapalogs and TORKi for treatment of glioblastoma in vitro and in vivo, it remains to be determined whether RapaLink-1 has immunosuppressive properties similar to rapamycin and whether RapaLink-1 induces autophagy to promote survival. Like other PI3K pathway inhibitors, RapaLink-1 as monotherapy was mainly cytostatic rather than cytotoxic, likely due to feedback activation of mitogenic pathways, rewiring, and other modes of intrinsic and acquired resistance. The effective combination of RapaLink-1 with agents that promote apoptosis and that block emergent resistance will help to position RapaLink-1 for clinical development.

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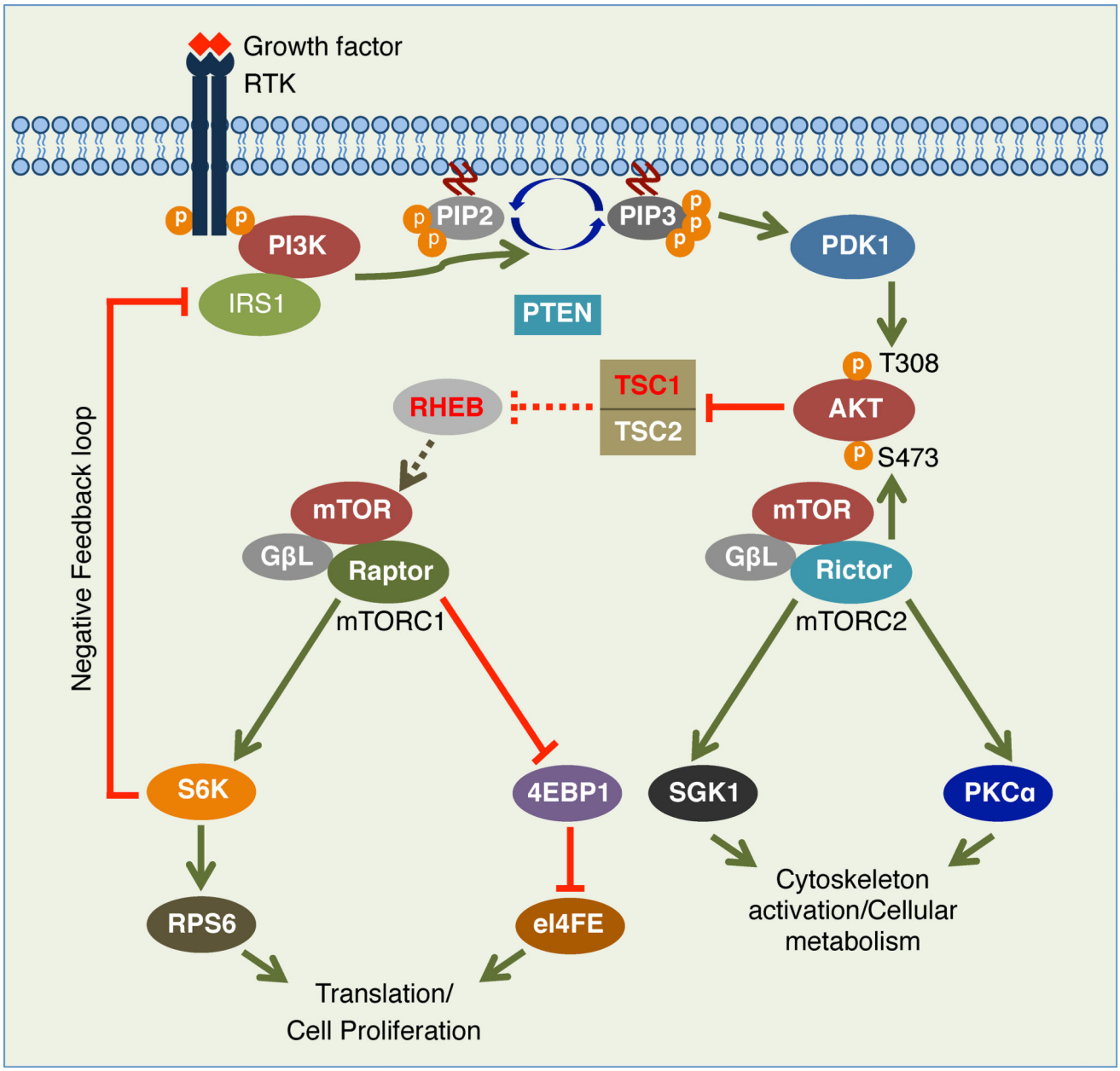

Figure 1.

PI3K-AKT-mTOR signaling pathways in glioblastoma. S6K negatively affects the insulin-PI3K-AKT pathway as displayed. This axis is activated in response to mTOR blockade (not shown). Note that our earlier work demonstrates that canonical upstream signaling from AKT to mTOR is not operative in glioblastoma. Activated AKT is able to phosphorylate TSC2 without blocking mTOR, suggesting that the miswiring may occur at the level of TSC1 or RHEB as displayed. RTK: Receptor tyrosine kinase; PI3K: phosphoinositide 3-kinase; IRS1: insulin receptor substrate 1; PTEN: phosphatase and tensin homolog; PDK1: phosphoinositide-dependent kinase 1; TSC1 and TSC2: tuberous sclerosis protein 1 and 2; RHEB: ras homolog enriched in brain; mTORC1: mTOR complex 1; mTORC2: mTOR complex2; S6K: S6 kinase; RPS6: ribosomal protein S6; eIF4E: eukaryotic initiation factor

4E; 4EBP1: elF4E-binding protein; SGK1: glucocorticoid-induced protein kinase 1; PKCα: protein kinase Cα.

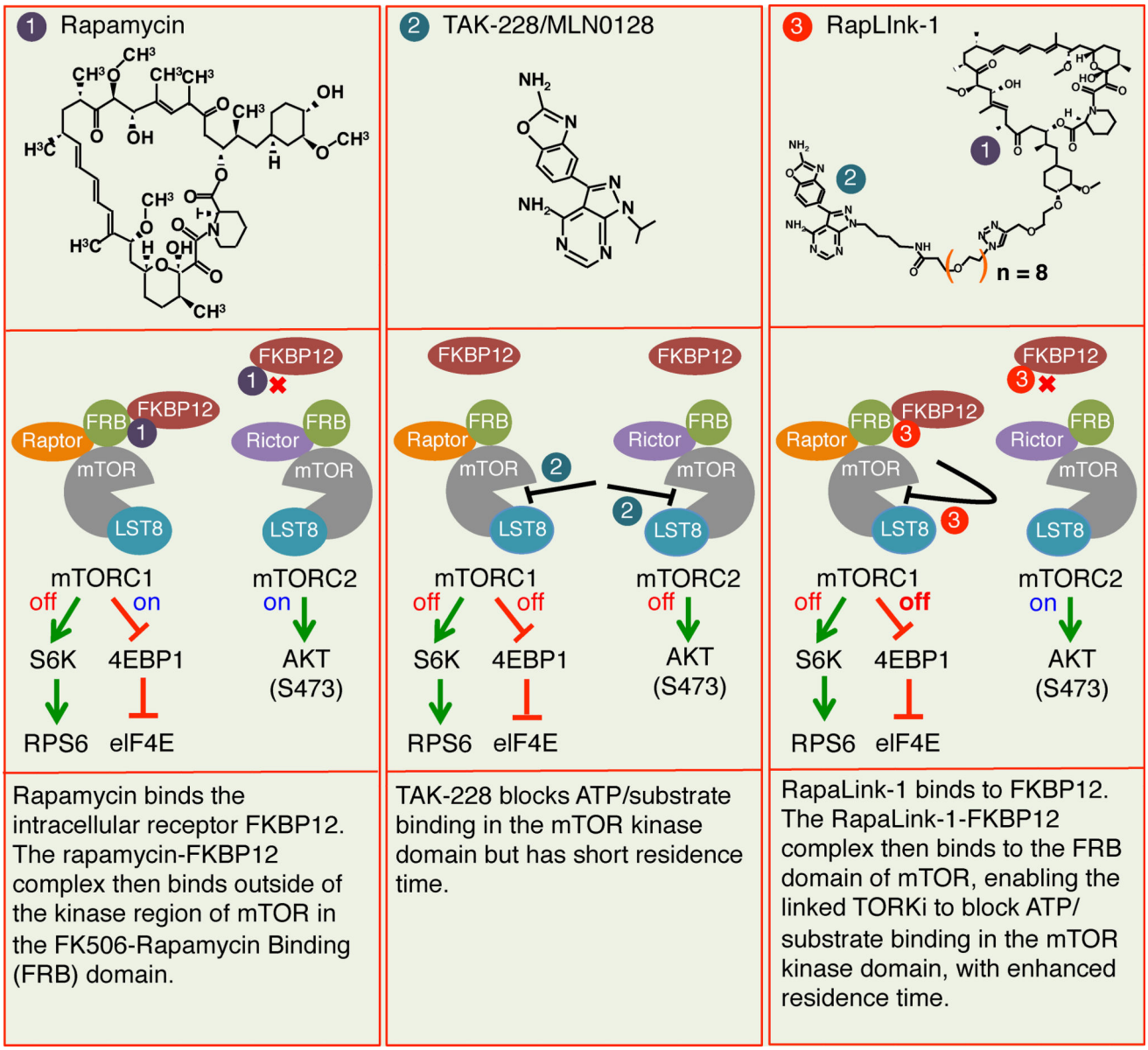

Figure 2.

FKBP12 is required for activities of rapamycin and RapaLink-1 but not for TAK-228. **Top panels:** Chemical structures of rapamycin, TAK-228, and Rapalink-1. **Middle panels:** Rapamycin or RapaLink-1 binds with FKBP12 to form a complex. The rapamycin-FKBP12 or RapaLink-1-FKBP12 complex binds to the FRB, which is distinct from the kinase region of mTOR. FKBP12 and FRB binding is not required for the activity of TAK-228. **Bottom panels:** Mechanisms of action for first-, second-, and third-generation mTOR inhibitors.

Table 1

mTOR inhibitors currently in preclinical and clinical development in cancer

Agents	Targets	GBM/ Phase	Other tumor types/Phase	References/ Ongoing GBM clinical trials
Rapamycin	mTORC1	Phase I/II	HCC Phase II	4, 11, 12, 13
Everolimus	mTORC1	Phase II	FDA approved for RCC, PNET, NET, SEGA associated with TS, and breast cancer in combination with exemestane	14, 15, 16, 17
Temsirolimus	mTORC1	Phase II	FDA approved for RCC, combination with sorafenib in HCC/Phase II, combination with irinotecan/temozolomide in neuroblastoma/Phase II	18, 19, 20
Ridaforolimus	mTORC1	Phase I	Breast, endometrial, prostate cancers and NSCLC/ Phase II Soft tissue and bone sarcomas/Phase III	21, 22, 23
PP242	mTORC1/2	preclinical	MM, leukemia, colon, breast cancers/preclinical	24, 25
WYE-354	mTORC1/2	preclinical	Breast, prostate, colon cancers, and RCC/ preclinical	26, 27
WAY-600	mTORC1/2	preclinical	Breast, prostate, colon cancers, and RCC/ preclinical	26, 27
WYE-687	mTORC1/2	preclinical	Breast, prostate, colon cancers, and RCC/ preclinical	26, 27
Torin 1	mTORC1/2	preclinical	Colon and prostate cancers/preclinical	28, 29
KU-0063794	mTORC1/2	preclinical	RCC T-cell ALL, pancreatic cancer, NSCLC, and HCC/preclinical	30, 31
OSI-027	mTORC1/2	Phase I/II	HCC, AML, bladder, head and neck, breast cancer/Phase I	32
TAK-228/MLN0128/Sapanisertib	mTORC1/2	Phase I/II	Breast, pancreatic, ovarian, colorectal cancers, and osterosarcoma/Phase I	33, NCT02133183
AZD8055	mTORC1/2	Phase I/II	HCC, RCC, breast cancer, and neuroblastoma/ Phase I	34, 35, NCT01316809
AZD2014	mTORC1/2	Phase I/II	NSCLC, ovarian, breast, renal, and prostate cancers/Phase I	36, 37, NCT02619864
CC-223	mTORC1/2	Phase I	NHL and MM/Phase I	38, NCT01177397
RapaLink-1	mTORC1	preclinical	Kidney and breast cancer/ preclinical	39, 40

HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; PNET, pancreatic neuroendocrine tumors; NET, Progressive, nonfunctional gastrointestinal and lung neuroendocrine tumors; SEGA, subependymal giant cell astrocytoma; TS, tuberous sclerosis; NSCLC, non-small cell lung cancer; MM, multiple myeloma; GBM, glioblastoma; AML, acute myeloid leukemia; NHL, Non-Hodgkin's lymphoma.