Biochimica et Biophysica Acta 1804 (2010) 433–439

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

Dissecting the role of mTOR: Lessons from mTOR inhibitors

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ARTICLE INFO

Article history:
Received 29 October 2009
Received in revised form 27 November 2009
Accepted 1 December 2009
Available online 11 December 2009

Keywords:
Mammalian target of rapamycin (mTOR)
S6 kinases (S6Ks)
eIF4E-binding proteins (4E-BPs)
Eukaryotic translation initiation factor eIF4E (eIF4E)
Rapamycin
Active-site mTOR inhibitor
Translation

ABSTRACT

Recent years have observed significant advances in our understanding of how the serine/threonine kinase target of rapamycin (TOR) controls key cellular processes such as cell survival, growth and proliferation. Consistent with its role in cell proliferation, the mTOR pathway is frequently hyperactivated in a number of human malignancies and is thus considered to be an attractive target for anti-cancer therapy. Rapamycin and its analogs (rapalogs) function as allosteric inhibitors of mTORC1 and are currently used in the treatment of advanced renal cell carcinoma. Rapamycin and its derivatives bind to the small immunophilin FKBP12 to inhibit mTORC1 signalling through a poorly understood mechanism. Rapamycin/FKBP12 efficiently inhibit some, but not all, functions of mTOR and hence much interest has been placed in the development of drugs that target the kinase activity of mTOR directly. Several novel active-site inhibitors of mTOR, which inhibit both mTORC1 and mTORC2, were developed in the last year. In this manuscript, we provide a brief outline of our current understanding of the mTOR signalling pathway and review the molecular underpinnings of the action of rapamycin and novel active-site mTOR inhibitors as well as potential advantages and caveats associated with the use of these drugs in the treatment of cancer.

1. Introduction

1.1. mTOR signalling in health and disease

The serine/threonine kinase mammalian target of rapamycin (mTOR) plays a central role in regulating critical cellular processes such as growth, proliferation, cytoskeletal organization, transcription, protein synthesis and ribosomal biogenesis. mTOR is present in two distinct protein complexes commonly referred to as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2; Fig. 1). mTORC1 modulates cap-dependent translation in response to nutrients, hormones and growth factors and is composed of mTOR, the scaffolding protein raptor (regulatory associated protein of TOR), the GTPase β-subunit like protein GβL (also known as mLST8) and the recently identified deptor (disheveled, Egl-10, pleckstrin [DEP] domain containing mTOR interacting protein) [1,2]. Some of the mTORC1 components are also present in mTORC2: these include mTOR, mLST8 and deptor. Other proteins, like rictor (rapamycin insensitive companion of TOR), mSIN1 (mammalian stress-activated protein kinase (SAPK)-interacting protein) and PRR5 (Proline-rich protein 5, also known as protor) are found exclusively in mTORC2 [3–7].

mTORC1 and mTORC2 phosphorylate different substrates to regulate distinct cellular functions. For instance, mTORC2 phosphorylates AKT, SGK1 and PKC (members of the AGC kinase family) which control cell survival and cytoskeletal organization [1,8–10]. mTORC1, on the other hand, stimulates cell growth and proliferation by increasing cap-dependent translation initiation and this is mediated by its two major downstream targets: the eIF4E-binding proteins (4E-BPs) and S6 kinases (S6K1 and S6K2) [11]. S6 kinases are believed to control translation by modulating the activity of their downstream targets including ribosomal protein S6, eukaryotic initiation factor 4B (eIF4B) and programmed cell death 4 protein (PDCD4) [12]. 4E-BPs are a family of small molecular weight translational repressors that include 4E-BP1, 4E-BP2, and 4E-BP3. These proteins suppress translation of a subset of transcripts referred to as "eIF4E-sensitive mRNAs" by competing with eIF4G for binding to eIF4E, and thereby preventing formation of the eIF4F initiation complex [13]. eIF4E-sensitive mRNAs are characterized by long and complex 5′UTR regions and encode proliferation and survival promoting proteins such as cyclins, c-Myc and Bcl-xl [14,15]. Due to the complexity of their 5′UTRs, eIF4E-sensitive mRNAs are translated less efficiently than mRNAs bearing short, unstructured 5′UTRs, such as mRNAs encoding housekeeping proteins [16–18]. The most extensively studied and best-understood member of the 4E-BP family is 4E-BP1. Upon activation, mTORC1 phosphorylates Thr37 and Thr46 on 4E-BP1, which acts as a priming event essential for the phosphorylation of Ser65 and Thr70 leading to the release of eIF4E and subsequent assembly of the eIF4F complex [19,20]. mTORC1 is also thought to modulate protein synthesis indirectly through the activation of TIF-IA and consequent stimulation of transcription of ribosomal RNA and ribosomal biogenesis [21], as well as through phosphorylation of eIF4G [22].

In addition to its well-established role in translation, the mTORC1 pathway is suggested to play a role in a variety of important cellular

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doi:10.1016/j.bbapap.2009.12.001

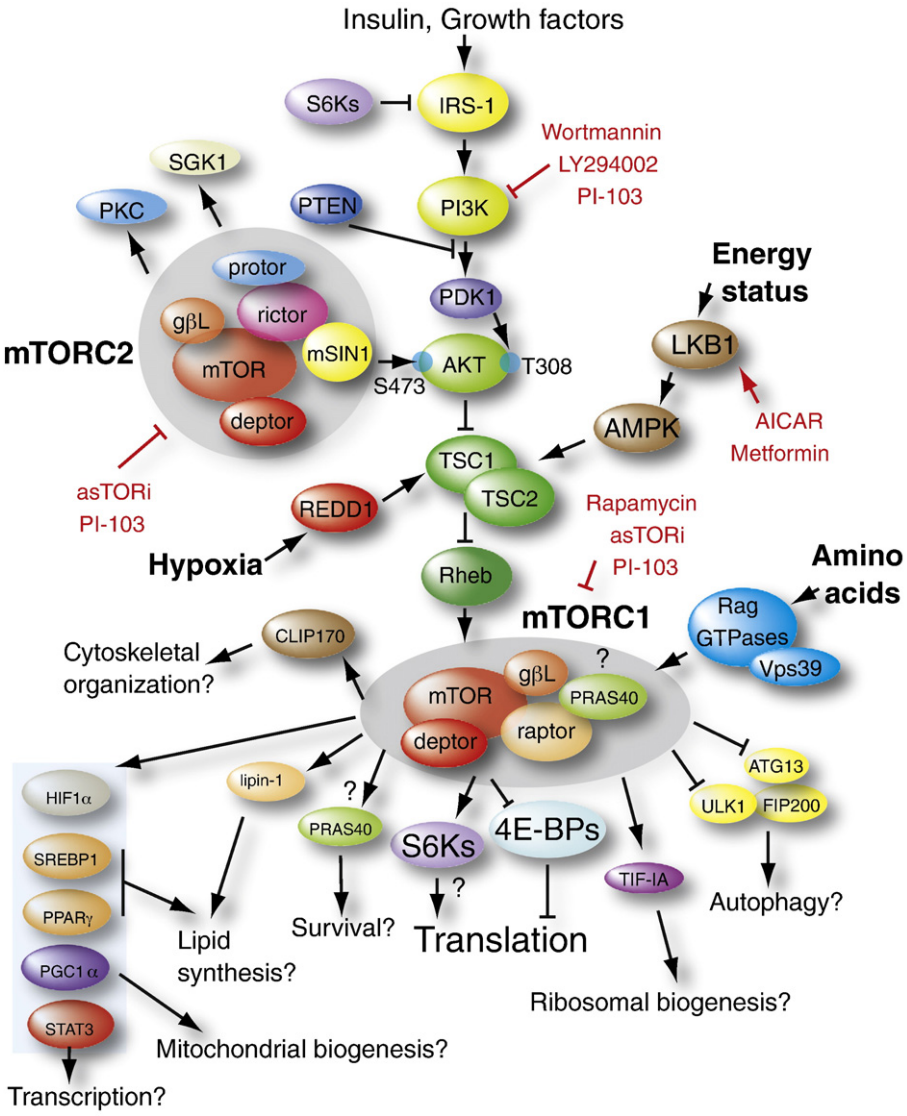

Fig. 1. Schematic representation of mTOR signalling pathway. Mammalian target of rapamycin (mTOR) exists in two distinct complexes: mTORC1 and mTORC2. mTORC1 is activated by various extra- and intracellular cues which regulate a multitude of signalling pathways including PI3K/AKT, TSC1/TSC2/Rheb, LKB1/AMPK and Vam6/Rag GTPases. In yeast, amino acids activate Vam6/VPS39 (the guanine nucleotide exchange factor) that loads GTP onto Gtr1/Gtr2 GTPases (the yeast homologs of Rag GTPases in mammals). In turn, in mammals, Rag GTPases are believed to activate mTORC1 by recruiting this complex into close proximity of Rheb, a small GTPase that switches mTORC1 signalling on through a poorly understood mechanism. GTP loading onto Rheb is controlled by the tuberous sclerosis complex that comprises both the scaffolding protein, TSC1 and the GTPase activating protein (GAP), TSC2. TSC2 is phosphorylated at multiple sites by AKT and AMPK. Phosphorylation of TSC2 by AKT and AMPK has been proposed to inhibit (block) or activate (arrow) the TSC1/TSC2 complex, respectively. mTORC1 is believed to modulate protein synthesis through activation of S6Ks and inhibition of 4E-BPs. Several additional downstream targets of mTORC1 have been described over the years and their proposed roles in mediating functional outputs of mTORC1 pathway are outlined. Kinase inhibitors that directly or indirectly affect mTORC1 signalling are shown in red. Abbreviations: mTOR: mammalian target of rapamycin; asTORi: active-site mTOR inhibitors; AICAR: aminoimidazole carboxamide ribonucleotide; TSC: tuberous sclerosis complex; SGK1: serum-and glucocorticoid-induced protein kinase 1; PKC: protein kinase C; LKB1: Serine/threonine kinase 11; AMPK: AMP-activated protein kinase; PI3K: Phosphoinositide 3-kinase; PRAS40 Proline-rich AKT substrate of 40 kDa; HIF1α: hypoxia-inducible factor 1α; IRS1 insulin receptor substrate 1; PDK1 phosphoinositide-dependent kinase 1; PTEN: phosphatase and tensin homologue; REDD1: protein regulated in development and DNA damage response 1; 4E-BP: 4E-binding protein; S6K: S6 kinase. ULK1: autophagy promoting factors unc-51-like kinase 1; ATG13: autophagy-related gene 13; SREBP1: sterol regulatory element binding protein 1; PPARγ: peroxisome proliferator-activated receptor-γ; PGC1-α: PPARγ coactivator 1; STAT3: signal transducer and activator of transcription 3; TIF-IA: transcription initiation factor IA; mSIN1: mammalian stress-activated protein kinase SAPK-interacting protein; gβL: GTPase β-subunit like protein; raptor: regulatory associated protein of TOR; rictor: rapamycin insensitive companion of TOR.

processes. For instance, mTORC1 inhibits autophagy, an evolutionarily conserved catabolic process triggered by nutrient deprivation in which cellular organelles and/or long-lived proteins are degraded by the lysosomal machinery [23]. Recent studies revealed that this is achieved through the phosphorylation and subsequent repression of the autophagy promoting factors unc-51-like kinase 1 (ULK1) and autophagy-related gene 13 (ATG13) [24–26]. An emerging body of data suggests that mTORC1 also plays an important role in the regulation of lipid synthesis and mitochondrial metabolism and biogenesis. At the molecular level, the effects of mTORC1 on lipid homeostasis are thought to be mediated by lipogenic transcription factors (e.g. sterol regulatory element binding protein 1 (SREBP1) [27] and peroxisome proliferator-activated receptor-γ (PPARγ) [28]), and a phosphatidic acid phosphatase, lipin-1 [29]. mTORC1 stimulates mitochondrial biogenesis and oxidative metabolism [30] possibly by modulating the interaction between PPARγ coactivator 1 (PGC1-α) and the transcription factor yin-yang 1 (YY1) [31]. mTOR was also shown to phosphorylate CLIP-170/Restin, which is a member of a family of conserved microtubule associated proteins [32]. The phosphorylation of CLIP-170/Restin was abrogated by rapamycin, indicating a possible role for mTORC1 in cytoskeletal organisation [32]. Additional substrates of mTORC1 include HIF1α [33] and STAT3

[34]. Albeit the precise role of these substrates in mTORC1 signalling needs to be established, these findings implicate the mTORC1 pathway in transcriptional regulation during hypoxia and inflammation.

mTORC1 signalling is regulated by a multitude of signalling cascades (depicted in Fig. 1). For instance, the tuberous sclerosis complex (TSC1/TSC2) functions upstream of mTORC1 to inhibit signalling through this complex (see for example [35] for review). Consistent with the inhibitory effect of TSC1/TSC2 on mTORC1, overexpression of TSC2 drastically reduces the phosphorylation of mTORC1 targets: 4E-BPs and S6Ks [36]. Phosphorylation of 4E-BPs and S6Ks is also dramatically reduced by overexpression of a recently identified component of mTORC1 termed PRAS40 (for Proline Rich AKT Substrate 40 kDa) ([37–41]). It is not entirely clear how overexpression of PRAS40 inhibits the phosphorylation of 4E-BPs and S6K: early reports suggested PRAS40 inhibited mTORC1 by acting upstream of this complex [37,38] but other mechanisms may also exist. PRAS40 binds to raptor via a TOR signalling (TOS) motif, commonly found in other mTORC1 targets: 4E-BPs, S6Ks and HIF1α and is subject to phosphorylation by mTORC1 at multiple sites, including rapamycin-sensitive (Ser 183 and Ser 221) and rapamycin-insensitive (Ser 212) residues [40,41]. The observation that PRAS40 contains a TOS motif and is itself a target for phosphorylation by mTORC1 [39,41,42] suggests that PRAS40 is not an upstream regulator, but rather, a downstream target of mTORC1 which inhibits mTORC1 signalling by competing with 4E-BPs and S6Ks for binding and phosphorylation by mTORC1. The exact mechanism whereby PRAS40 exerts its inhibitory effects on mTORC1 remains unclear to date and further work is required to elucidate the significance of its phosphorylation by mTORC1.

mTORC1 signalling is frequently dysregulated in cancer [1,43]. Loss or inactivation of tumor suppressors such as p53, LKB1, PTEN, and TSC1/2, which antagonize PI3K-dependent activation of mTORC1, can promote tumorigenesis via increased signalling through mTORC1 [44–47]. Moreover, increased levels and/or phosphorylation of downstream targets of mTORC1 have been reported in various human malignancies in which they correlate with tumor aggressiveness and poor prognosis [1,43,48]. S6K1 is reported to be overexpressed in breast cancer [49] and 4E-BP1 is downregulated and/or hyperphosphorylated (i.e. inactivated) in breast, ovarian, and other cancers [50–52]. Accordingly, in our recent study we show that 4E-BPs act as tumor suppressors in p53-null mice and that the loss of 4E-BPs results in premature senescence in fibroblasts derived from p53-wild type mice [53]. Alterations in components of the eIF4F complex have also been linked to a wide variety of human malignancies. For example, high levels and/or hyperactivity of eIF4E have been reported in head and neck squamous cell carcinoma, lung, colon, breast cancer, leukemias and lymphomas [54–57]. Taken together, these findings link aberrant mTORC1 signalling with dysregulated translational control in cancer. As a result, mTORC1 has emerged as an important target for anti-cancer therapy.

2. mTOR inhibitors

2.1. Rapamycin and rapalogs

Rapamycin is a naturally occurring macrolide triene antibiotic that acts as a specific, allosteric inhibitor of mTORC1 [11,58,59]. Although rapamycin has been widely used to study mTORC1 signalling for more than a decade, the molecular underpinnings of rapamycin's actions are still poorly understood. Rapamycin associates with its intracellular receptor, FKBP12 (FK 506-binding protein of 12 kDa) and the resulting complex interacts with the FRB (FKBP12-rapamycin binding) domain located in the C-terminus of mTOR [60]. Binding of rapamycin/FKBP12 to the FRB domain of mTOR precludes the binding of mTOR to raptor and this is believed to uncouple mTORC1 from its substrates (e.g. 4E-BPs and S6Ks) [61,62]. A recent study using high micromolar doses of the rapamycin analogue temsirolimus indicated that rapamycin and its derivatives can directly bind to the FRB domain of mTOR, thus disputing the absolute requirement of FKBP12 for rapamycin-mediated inhibition of mTORC1 signalling [63].

In contrast to mTORC1, mTORC2 does not bind rapamycin/FKBP12 and this is thought to confer mTORC2 its resistance to acute rapamycin treatment. mTORC2 is sensitive, however, to prolonged rapamycin treatment which (as proposed by [64]) interferes with *de novo* assembly of mTORC2. According to this model binding of rapamycin/FKBP12 to mTOR impedes the subsequent binding of the mTORC2-specific components mSin1 and rictor which are required for signalling downstream of mTORC2. This model is corroborated by the observation that rapamycin treatment leads to dephosphorylation and sub-cellular re-localization of mSin1 and rictor in non-immortalized human diploid fibroblasts and NIH 3T3 cells [65]. However, these effects are cell type specific and the factor(s) that render mTORC2 sensitive to rapamycin in some but not all cell types still need to be determined [64].

The ability of rapamycin to suppress both cellular proliferation and growth (through inhibition mTORC1) suggested that rapamycin and its analogs could serve as potent anti-cancer agents [59,66]. This observation prompted the development of rapamycin analogs (rapalogs), which share the mechanism of action of rapamycin but display improved pharmacokinetic properties. Presently, rapamycin and several rapalogs are in clinical development for the treatment of a wide variety of human malignancies. It has been reported that rapamycin and rapalogs effectively inhibit cell proliferation and angiogenesis in some human tumors [67,68]. At the molecular level, this could be partly explained by the mTORC1/4E-BP1 mediated suppression of translation of "eIF4E-sensitive" mRNAs such as cyclin D1, cyclin D3 and VEGF.

2.2. Molecular mechanisms underlying the relative inefficacy of rapamycin and rapalogs in cancer treatment

The US FDA (United States Food and Drug Administration) has recently approved the rapamycin analogs temsirolimus (CCI-779) and everolimus (RAD001) for the treatment of advanced stage renal cell carcinoma and sarcoma, respectively [58,68,69]. However, the efficacy of rapamycin and rapalogs as broad based monotherapies for cancer does not appear as promising as initially expected [54,58]. This has been attributed to the inability of rapamycin to inhibit the phosphorylation of 4E-BPs in an effective and sustained manner and thus suppress translation of eIF4E-sensitive mRNAs (Fig. 2). The relative inefficacy of rapamycin in cancer treatment has been attributed to rapamycin-mediated upregulation of PI(3)K/AKT phosphorylation resulting from the loss of the negative-feedback loop from S6K to IRS-1. [54,58,70,71]. Upon activation, mTORC1 activates S6K1, which in turn phosphorylates inhibitory sites (i.e. Ser 636/639) on the insulin receptor substrate-1 (IRS-1), thereby suppressing IRS-1 mediated activation of the PI3K/AKT pathway. Inhibition of mTORC1 by rapamycin results in the attenuation of negative feedback to IRS-1, leading to increased AKT activity. The full activation of AKT requires two phosphorylation events, on Ser473 which lies in its hydrophobic motif, and on Thr308 within its activation loop. PDK1 has long been known to be the major kinase for Thr308 [72]. Recently, it was revealed that mTORC2 is also implicated in the activation of AKT, through phosphorylation of Ser473 [9,73]. As mentioned above, mTORC2 is rapamycin-insensitive in the majority of cancer cell lines [64,74], suggesting that mTORC2-dependent phosphorylation of AKT on Ser473 still persists during rapamycin treatment.

Taken together these findings indicate that at the molecular level, rapamycin treatment leads to hyperactivation of AKT through loss of the mTORC1/S6K1/IRS-1/PI3K negative feedback loop, which in some types of cancer is further reinforced by the inability of rapamycin to efficiently suppress mTORC2 signalling towards AKT. Accordingly,

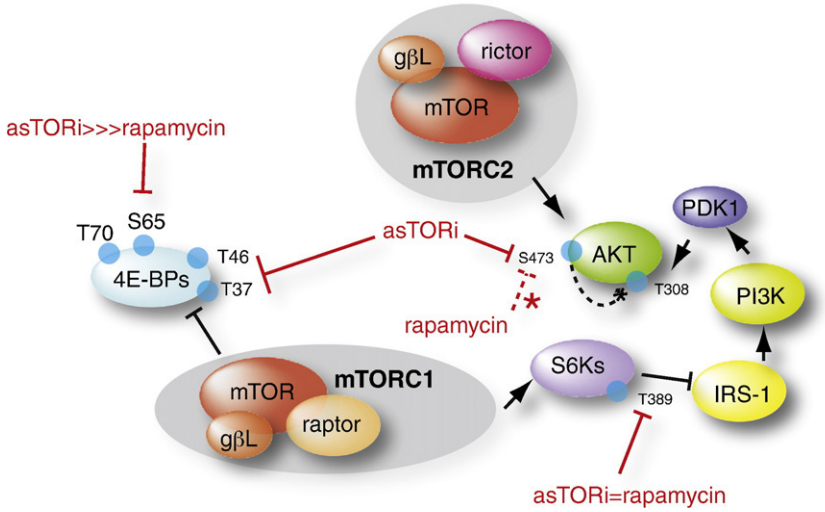

Fig. 2. Diagram depicting differences in the effects of the active-site mTOR inhibitors and rapamycin on mTOR signalling. Active-site mTOR inhibitors suppress the phosphorylation of 4E-BPs on Ser65 to a higher extent than rapamycin. Furthermore, these compounds completely abolish the phosphorylation of rapamycin-insensitive Thr37/46 sites on 4E-BPs. In turn, the effects of the active-site mTOR inhibitors on S6 kinase (S6K) activation through inhibition of Thr389 phosphorylation are comparable to those of rapamycin. Prolonged rapamycin treatment leads to a loss of the negative feedback between S6K1 and IRS-1, resulting in increased phosphorylation of AKT on Thr308 and its subsequent activation. It is suggested that the active-site mTOR inhibitors counteract the effect of the loss of the negative feedback between S6K1 and IRS-1, by inhibiting mTORC2 dependent phosphorylation of AKT on Ser473. Intriguingly, it has been reported that in a limited number of cell lines, mTORC2 signalling towards AKT is inhibited by prolonged rapamycin treatment (*). Relatively limited success of rapamycin as an anti-cancer agent has been attributed to activation of the AKT pathway as well as the inability of rapamycin to inhibit phosphorylation of 4E-BPs.

phosphorylation of AKT is reportedly increased in tumors derived from patients treated with everolimus [66]. The activation of AKT-dependent pro-survival mechanisms may not only hinder the anti-neoplastic activity of rapamycin, but also promote tumorigenesis and lead to resistance of tumor cells to other anti-cancer agents.

2.3. New generation of mTOR inhibitors: targeting the active site

In addition to the emerging role of mTORC2 in the activation of AKT, it was recently shown that mTORC2 activity is necessary for PTEN-dependent tumorigenesis. A study by Guertin at al., [75] revealed that mTORC2 signalling is necessary for the development of prostate cancer caused by *Pten* deletion. Intriguingly, mTORC2 activity was dispensable for the function of normal prostate epithelial cells. These findings highlight the importance of developing specific inhibitors of mTORC2 in order to efficiently target mTOR signalling in cancer. A number of active-site mTOR inhibitors have now been developed, which specifically suppress mTOR signalling by competing with ATP for binding to the kinase domain of mTOR [76–79]. These compounds, PP242, Torin1, WYE-354 and Ku-0063794 suppress both mTORC1 and mTORC2 activity with significant selectivity over phosphatidylinositol 3-kinase (PI3K) isoforms and display more dramatic effects on cell growth, proliferation, cell cycle and cap-dependent translation than rapamycin [76–79]. Furthermore, it was shown that Torin1, unlike rapamycin, potently induces autophagy in mouse embryonic fibroblasts [78]. As expected, active-site mTOR inhibitors, but not rapamycin, suppressed mTORC2-mediated phosphorylation of AKT on Ser473 [76,78]. Surprisingly, the active-site mTOR inhibitors inhibited cell proliferation, growth and cell cycle progression to the same extent in wild type and mouse embryonic fibroblasts lacking rictor or mSin1 which are deficient in mTORC2 activity [76,78]. These findings indicate that the effects of the active-site mTOR inhibitors are not mediated by mTORC2. Furthermore, the active-site mTOR inhibitors and rapamycin reduced the phosphorylation of S6Ks and its substrate, ribosomal protein rpS6, to the same extent [76–78]. In stark contrast, the effects of active site mTOR inhibitors on 4E-BP1 phosphorylation were much stronger than rapamycin. Rapamycin has little effect on the phosphorylation of Thr37 and Thr46 on 4E-BP1, whilst Ku-0063794, PP242 and Torin1 completely inhibited the phosphorylation of these residues *in vivo* [76–78]. In addition, Ku-0063794, PP242 and Torin1 caused a greater reduction in Ser65 phosphorylation compared to rapamycin [76–78]. These findings are consistent with recent studies showing that 4E-BP1 phosphorylation is resistant to rapamycin in several cancer cell lines, especially after prolonged treatment [58,70].

Thus, the ability of active-site mTOR inhibitors to exert more prominent effects on mTOR functional outputs than rapamycin is largely due to their ability to suppress rapamycin-resistant mTORC1 signalling towards 4E-BPs [76–78]. A question that remains unanswered is how does rapamycin cause complete deactivation of S6K, while having only a moderate effect on 4E-BP1 phosphorylation? One possible explanation to this conundrum is that mTORC1 binds to 4E-BPs and S6Ks with a different affinity and/or conformation, wherein the rapamycin-mediated structural changes in mTORC1 are sufficient to disrupt its association with S6Ks, but not with 4E-BPs [80,81]. This would allow mTOR to still signal to 4E-BP1 while simultaneously loosing its ability to phosphorylate S6K. Whilst assessing the binding affinity of the mTORC1 complex to its substrates is experimentally challenging, the observation that raptor binds more efficiently to 4E-BP1 than S6K supports this model of rapamycin-resistant signalling to 4E-BP1 [80,82]. Alternatively, it has been proposed that kinases other than mTOR regulate the phosphorylation of 4E-BP1 on Thr 37/46 [83]. Thus, the discrepancy in the effects of rapamycin and the active-site mTOR inhibitors on the phosphorylation of 4E-BPs and S6Ks, could also be explained by the inability of rapamycin to inhibit the kinase responsible for the phosphorylation of 4E-BPs on Thr 37/46. Finally, it is plausible that the differential effects of the active-site mTOR inhibitors and rapamycin on the phosphorylation of S6Ks and 4E-BPs are the consequence of the activation of a hitherto unidentified phosphatase. Undoubtedly, future studies will be necessary to answer the conundrum of differential sensitivity of S6Ks and 4E-BPs to the active-site mTOR inhibitors and rapamycin.

2.4. New active-site mTOR inhibitors in cancer treatment

While new active-site mTOR inhibitors have been important in elucidating the molecular mechanisms of mTORC1 and 2 signalling, their most critical role may be in the treatment of cancer. Due to the

existence of the rapamycin-resistant mTORC2 complex and its role in AKT activation, active-site mTOR inhibitors have the potential to be potent anti-cancer agents. These new inhibitors may function as more effective anti-cancer therapies because they counteract the activation of AKT, which can occur as a result of rapamycin-mediated disruption of the mTOR/S6K/IRS-1 negative feed back loop. To date, Ku-0063794, PP242 and Torin1 have not been tested in animal models for the inhibition of tumor growth. However, new active-site inhibitors (WAY-600, WYE-687, and WYE-354) have been developed by Wyeth (Pearl River, NY) and tested on a variety of cancer cells *in vitro* and *in vivo* [79]. In a xenograft model, the mTOR active-site inhibitor WYE-354 significantly inhibited the growth of tumors established with PC3-MM2 prostate cancer cells, indicating that these new inhibitors represent future potential anti-cancer agents [79].

When considering compounds as anti-cancer agents, it is important to identify factors that may be responsible for governing sensitivity or resistance to treatment. For example, increased activation of AKT and over-expression of S6K have correlated with rapamycin sensitivity [84,85]. In addition, loss of the tumor suppressor PTEN is thought to increase the sensitivity of cells and tumors to treatment with rapamycin [86]. However using PTEN status as a marker of sensitivity to rapamycin treatment has been somewhat unreliable in human patients [54]. The level of eIF4E activation may correlate with resistance to rapamycin as over-expression of eIF4E, or decreased expression of its inhibitor, 4E-BP1, have been associated with poor responses to rapamycin based therapies [87,88].

In addition to identifying markers of rapamycin sensitivity, it is of paramount importance to identify biomarkers to assess the efficacy of rapamycin and other mTOR inhibitors in human patients. In general, inhibition of mTOR activity can be monitored by assessing the phosphorylation status of its downstream targets, S6K and 4E-BP1. Indeed, several clinical studies have demonstrated a decrease in the phosphorylation of S6K and 4E-BP1 in skin, blood, and tumor samples from cancer patients undergoing therapy with rapamycin derivatives [66,89]. However, the existence of rapamycin-resistant phosphorylation sites on 4E-BP1 renders the use of 4E-BP1 as a biomarker for rapamycin based anti-cancer therapies problematic.

3. Conclusions and future directions

The dysregulation of mTOR signalling is implicated in a number of human diseases including cancer. Consequently, a great deal of research has focused on elucidating the mechanisms linking mTOR signalling to the control of cell growth, proliferation, differentiation and transformation. Despite recent advances in the understanding of mTOR structure and function, much work still needs to be done. The development of the novel active-site mTOR kinase inhibitors has already yielded interesting findings on mTORC1 and mTORC2 signalling. The use of these mTOR inhibitors will likely reveal new targets for phosphorylation by mTOR and further our current understanding of mTOR signalling and its role in health and disease. Furthermore, the optimization of new active-site mTOR inhibitors for use in patients and the identification of biomarkers of efficacy of mTOR inhibition in patients will be key for the effective treatment of human cancers with mTOR-targeted therapies.

Acknowledgements

The authors thank Colin Lister and Pam Kirk for excellent technical assistance. The work in author's laboratory is supported in part by the Canadian Cancer Society. Nahum Sonenberg is a Howard Hughes Medical Institute International Research Scholar and a James McGill Professor. Ryan Dowling is supported by a Research Studentship provided by the Terry Fox Foundation through the National Cancer Institute of Canada. Ivan Topisirovic is a Special Fellow of the Leukemia and Lymphoma Society, USA. Bruno D. Fonseca gratefully acknowledges financial support from a Canadian Institute for Health Research Consortium Training Fellowship Award. The authors have no conflicts of interest that relate to the content of this review article.

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