Current status of mTOR inhibition in cancer therapy

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Summary

The mTOR pathway is key in transducing several inputs like growth factors, nutrients, oxygen, stress and energy levels determining initiation of cell growth and proliferation. Discovered in 1964, it already showed promising immunosuppressive, antitumour and antifungal effects. mTOR is a PI3K-related kinase and the core-subunit of two complexes, mTOR complex 1 and 2. These two complexes differ in key components, regulators and downstream substrates, but are linked together via a feedback loop. Because mTOR signalling initiates cell growth and proliferation and is hyperactivated in many cancers, mTOR inhibitors are a possible treatment for cancer. First generation mTOR inhibitors like rapamycin and rapamycin analogues (rapalogs) are already FDA approved and used in clinic. There are cases that show great responses to these drugs, but that is not always the case. There are other limitations, like side effects, resistance and the fact that these drugs are cytostatic, not cytotoxic. Second- and third-generation mTOR inhibitors are currently in development, for example, ATP competitive mTOR kinase inhibitors, dual PI3K/mTOR inhibitors, and rapalink-1. These new drugs do show promising effects, but most are still in the preclinical stage of development.

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Introduction

The mechanistic target of rapamycin (mTOR) pathway is a key transductor of all types of environmental signals, including growth factors, nutrients, oxygen and stress levels, determining initiation of cell growth and proliferation. The pathway was discovered in 1964, when rapamycin was isolated from Streptomyces hygroscopicus found in a soil sample from the island of Rapa Nui. The macrolide immediately appeared promising, showing anti-fungal, immunosuppressive and antitumour activity[1,2,3]. However, the mechanism of action of this compound was only elucidated decades later, when it was found that rapamycin binds to FKBP12 to form a complex which inhibits cell growth and proliferation[4]. The next step was to find the target of this complex, which was a kinase that was subsequently called the mechanistic target of rapamycin, abbreviated as mTOR. Already shown by the effects of rapamycin treatment when it was discovered, the mTOR complexes are crucial transductors of many signals controlling cell growth and proliferation, including growth factors, nutrient levels and stress levels. mTOR is a kinase in the PI3K-related protein kinases family. In mammals, mTOR is the catalytic subunit of two complexes, namely mTORC1 and mTORC2. These complexes differ in their regulators, effectors, accessory proteins and their response to rapamycin. As mentioned earlier, the mTOR pathways are important regulators of cell growth and proliferation. mTORC1 accomplishes this by activating protein synthesis, lipid and nucleotide synthesis, and repressing autophagy and catabolism. mTORC2 activates protein kinase C (PKC), Akt, and glucocorticoid-induced protein kinase (SGK). These induce cytoskeleton rearrangement and mobility, anabolic metabolism, glucose homeostasis, ion transport and inhibition of apoptosis. Activity of mTORC1 and mTORC2 is regulated by the presence of nutrients, growth factors, energy, oxygen, and stress levels. Because the pathway is an important regulator of cell growth and proliferation, a link between this pathway and cancer is easily made. The mTOR signalling pathways are frequently hyperactivated in human cancers, although the kinases itself are rarely mutated. Hyperactivation of mTORC1/2 in cancers occurs through constitutive activation of PI3K-Akt and/or ERK signalling pathways, which are the same pathways which growth factors use to signal mTORC1. Common mutations that activate these two pathways are often found in malignant cancers, contributing to increased mTORC1 activation even without presence of growth factors[5]. Because hyperactivation of the mTOR pathways is common in cancers, and the fact that rapamycin already showed antitumour activity in early studies, mTOR inhibitors are a hot topic in cancer research. There are already FDA approved mTOR inhibitors available, with second- and third generation being developed. The purpose of this thesis is to look at the current progress in the research of mTOR inhibitors in cancer treatment, and to see if this approach is a potential and realistic new therapy in cancer treatment.

mTOR signalling

Core components

mTOR is a kinase that forms the catalytic subunit of two distinct protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 consists of three core proteins; mTOR, Raptor (regulatory protein associated with mTOR), and mLST8 (mammalian lethal with Sec13 protein 8) as seen in figure 1. Raptor has several functions, first of all it is essential for localization of mTORC1 and the recruitment of substrates of mTORC1 by binding to TOR motifs that are present on several substrates for the complex[6]. Raptor also functions as a scaffold for PRAS40 (proline-rich AKT substrate 40 kDa), which is together with DEPTOR (DEP domain containing mTOR interacting protein) an inhibitor of the mTORC1[7,8]. mLST8 is hypothesized to stabilize the kinase activation loop of mTORC1, however studies have shown that depletion of mLST8 does not influence the function of mTORC1[9]. As mentioned earlier, in addition to these three activating components of the complex, mTORC1 also consists of two inhibitory subunits, PRAS40 and DEPTOR[7]. Similarly to mTORC1, mTORC2 also consists of mTOR and mLST8. Unlike in mTORC1, depletion of mLST8 disrupts the assembly and activity of mTORC2 and suppresses mTORC2 dependent tumour cell proliferation and tumour growth in vivo, showing it is essential for the functioning of the complex[10]. Instead of Raptor, mTORC2 contains Rictor (rapamycin insensitive companion of mTOR). Rictor binds MAPK-interacting protein 1 (mSIN1), protein associated with rictor 1 or 2 (PROTOR1/2), and DEPTOR, which is the same as in mTORC1. mSIN1 has several functions; it facilitates mTORC2 assembly on the plasma membrane, it interacts with Ras, an upstream regulator of mTORC2, and it allows mTORC2 to phosphorylate Akt, which is a downstream target[8,11,12]. The function of PROTOR1 and PROTOR2 is not completely clear. Depletion of PROTOR1 and/or PROTOR2 does not influence the expression of other mTORC2 proteins, and does not change the assembly and function of the complex. The only effect visible is that PROTOR1 knockout mice showed reduced phosphorylation of SGK1, a downstream target of mTORC2, which indicates the possible role of PROTOR1 in enabling mTORC2 to efficiently activate SGK1, particularly in the kidney[13]. Another difference between mTORC1 and mTORC2 is the response to rapamycin. mTORC1 is acutely inhibited by the rapamycin/FKBP12 complex, while mTORC2 is only inhibited by prolonged treatment with rapamycin. This difference can be explained by the fact that RICTOR blocks the rapamycin/FKBP12 binding site on mTOR, which makes the mTORC2 insensitive[14, 8] . It has also been shown that the relative expression of FKBP12 and FKBP51 is key to the response of mTORC2 in a cell to inhibition by rapamycin. In a case of relative high expression of FKBP12 compared to other FKBP's, rapamycin/FKBP12 will inhibit the formation of mTORC2. If other FKBP's compete with FKBP12 to bind with rapamycin, this inhibition of mTORC2 formation decreases. The same study also found that FKBP51 competes with FKBP12 for acute inhibition of mTORC1, while only FKBP12 is required for the chronic inhibition of both mTORC1/2[15].

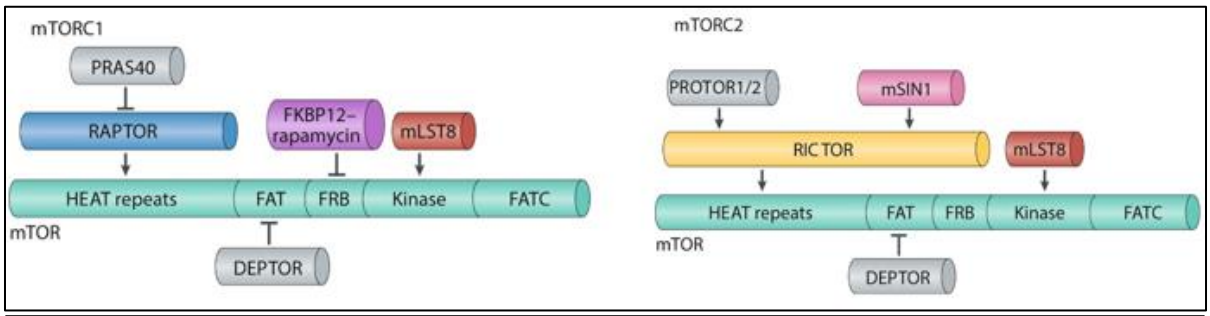

Figure 1. Core components of mTORC1 and mTORC2.
Source: Lieu et al. [8]

Upstream/Regulators of mTORC1

mTORC1 integrates many different signals ranging from growth factors, nutrients, oxygen and stress levels, which can be seen in figure 2A. To be able to collect all these signals, the mTOR pathway uses two G-proteins, Rheb and Rab GTPases. Activation of kinase activity of mTOR is determined by the nucleotide-loading state of Rheb, and the localization of mTOR by the nucleotide-loading status of Rag GTPases[16,17]. In this way, sufficient signals for both Rheb and Rag GTPases are required for mTOR signalling to remain active, which ensures that cell growth and proliferation is only activated when conditions can support this[8].

Growth factors

Growth factors inhibit a key inhibitor of the mTORC1, namely the Tuberous Sclerosis Complex (TSC). The TSC comprises three components; TSC1, TSC2 and TBC1D7, and functions as a GTPase activating protein for Rheb, conversing active Rheb-GTP into inactive Rheb-GDP[18]. TSC is inhibited by numerous growth factors, including insulin growth factor-1 (IGF-1), which inhibits TSC2 by Akt-dependent phosphorylation and thereby dissociating TSC from the lysosomal membrane[19,20]. Insulin mediated regulation of TSC also comprises a feedback loop, since S6K1, a substrate of mTORC1, blocks further activation of the PI3K-Akt pathway[21]. In addition, Wnt and TNF-α regulate mTOR signalling by inhibition of TSC1. However, activating mutations in Ras and PI3K-Akt pathways is common in many cancers, regulation of mTORC1 activity via TSC is often lost in cancers resulting in activation without the presence of the required growth factors[7].

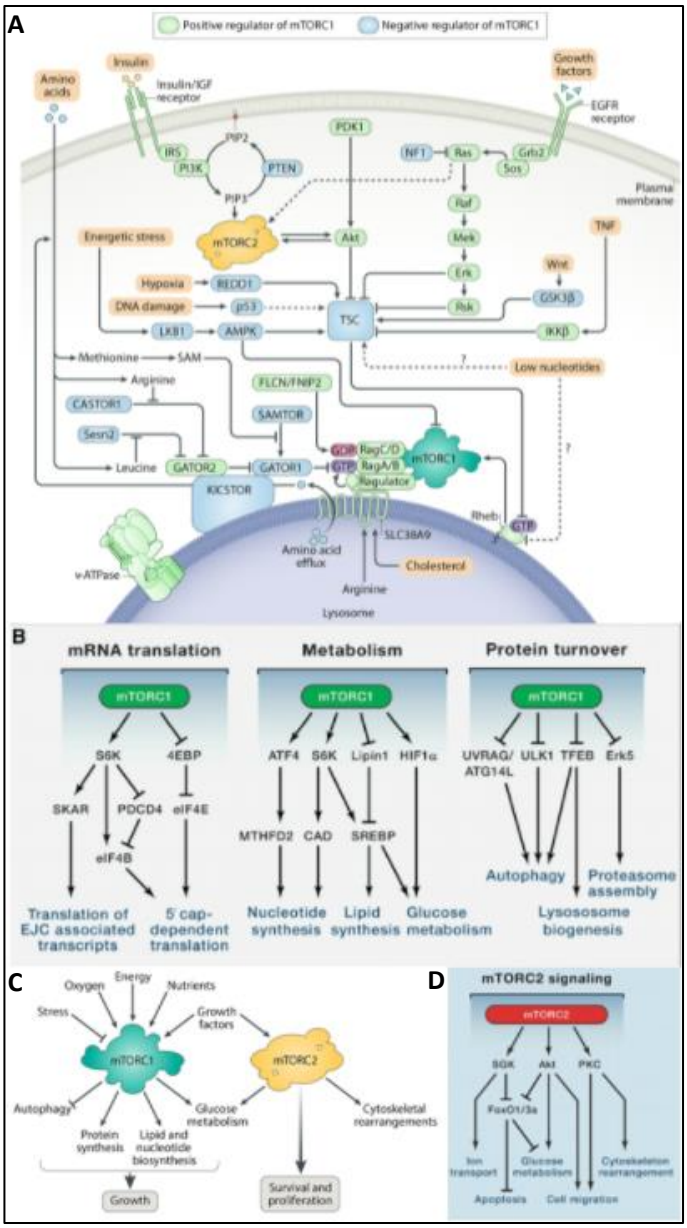

Figure 2. A; Upstream/Regulators of mTORC1/2. B; Effects of mTORC1 on mRNA translation, metabolism, and protein turnover. C; Regulators and downstream effects of mTORC1/2. D; Downstream effects of mTORC2 signalling. Source: Saxton et al. [7], Liu et al. [8]

Oxygen, stress and energy levels

Another regulator of mTORC1 activation is scarcity of oxygen or energy and increased stress. Depleted ATP levels activate the AMP-activated protein kinase (AMPK) complex, which phosphorylates Raptor or activates TSC2, thereby inhibiting mTORC1 activity[22,23]. Increased cellular stress levels in the form of oxidative stress can also inhibit mTORC1 activity. This happens via upregulation of a small protein called REDD1, which activates TSC[24]. In addition, mTOR is inhibited by recruitment of TSC2 as a universal response to presence of stress, and by induction of p53 target genes, which include a subunit of AMPK, PTEN and TSC2 itself, which all activate TSC activity[25]. Diet is also a key regulator of mTOR activity, with amino acids being the main regulator among nutrients. It was already discovered in 1998 that leucine and arginine are essential for mTORC1 activation[26]. However, how communication between amino acids and mTOR worked was only discovered in 2008, with the discovery of the Rag-GTPases[27]. Rags are always heterodimers, with either RagA/B bound to RagC/D. In an active state RagA/B is bound to GTP and RagC/D to GDP, and the

other way around in an inactive state. The Rags are stimulated to be in their activated state by amino acids, this allows them to bind to Raptor and recruit mTORC1 (only activation of mTORC1 when both Rheb and Rags are activated)[7]. As mentioned above, leucine and arginine are essential for mTORC1 activation. These amino acids signal through GATOR1 and GATOR2, which stands for GAP activity towards the Rags 1/2[28]. In low amino acids conditions, GATOR1 hydrolyses the GTP bound to RagA/B and thereby inactivates the heterodimer, and thereby inhibiting mTORC1[29]. In turn, the GATOR1 complex is regulated by the KICSTOR complex, which is required for sensitivity towards amino acids, and by GATOR2, which is a positive regulator of GATOR1[8,30] . Another regulating protein in this pathway is Sestrin2, which was shown to bind and inhibit GATOR2 upon acute leucine depletion, and thereby preventing mTORC1 recruitment[31,32]. When the leucine pool was restored, leucine binds to Sestrin2, releasing the protein from GATOR2, and thus releasing mTORC1 inhibition[33]. In addition, Sestrins are upregulated by transcription factor AFT4, indicating that Sestrins have a role in sensing an acute depletion of amino acids as well as an indirect role in mediating prolonged amino acid starvation[7,34]. Arginine is also essential for mTORC1 activation by binding to CASTOR1 (Cellular Arginine Sensor for mTORC1). It has a similar mode of action as Sestrin2: in absence of arginine, CASTOR1 binds and inhibits GATOR2, and separates in presence of arginine enabling mTORC1 function[35]. There is still a lack of understanding about how other metabolic inputs affect mTORC1 signalling. It is known that glucose deprivation inhibits mTORC1 through AMPK activation and signalling via Rag-GTPases[36]. Cholesterol and phosphatidic acid have also been shown to activate mTORC1 activity, where phosphatidic acid is used as a representation for fatty acid availability[37,38] .

Upstream/Regulators of mTORC2

The primary regulator of mTORC2 is the insulin/PI3K pathway, which can be seen in figure 2A. mTORC2 contains a small subunit called mSin1, which is critical for this integrating this regulation. In absence of regulation, the PH domain of mSin1 binds to mTORC2 and autoinhibits the kinase[39]. This autoinhibition is released by binding of PIP3, which is a product of PI3K after activation by insulin[40]. mSIN1 can also be phosphorylated by Akt, a downstream target of mTORC2, indicating the presence of a positive feedback loop[41]. Another feedback loop influencing mTORC2 activity occurs from mTORC1. This is because mTORC1 activates Grb10, which downregulates the insulin/PI3K/Akt pathway, which in turn inhibits mTORC2 activation[42,43] . This feedback link between mTORC1 and mTORC2 has impact on the pharmacological targeting of mTOR in disease, which will be further discussed below.

Downstream mTORC1

The effect of mTOR signalling is the activation of cell growth and proliferation, only when enough resources make this possible. To enable this growth and proliferation, the mTOR pathway exerts several downstream effects, as seen in figure 2B; activation of protein, lipid and nucleotide synthesis, and the inhibition of catabolism and autophagy. These will be further discussed down below.

Protein synthesis

An essential part of cell growth is of course protein synthesis, and therefore is tightly regulated by mTORC1. mTORC1 phosphorylates the eukaryotic initiation factor 4E-binding proteins (4E-BPs) and the p70 S6 kinase 1 (S6K1). Unphosphorylated 4E-BP1 inhibits translation by binding to eIF4E, which is needed for 5' cap-dependent translation of mRNAs. Upon phosphorylation, this inhibition is released[44]. mTORC1 also phosphorylates S6K1 on its T389 motif to activate its kinase activity[45]. S6K1 then phosphorylates ribosomal protein S6, which is part of the 40S subunit. The full effect of this phosphorylation is still not certain, however, it has been suggested that S6 phosphorylation may promote ribosomal biogenesis[46]. S6K1 also phosphorylates, and thereby activates eIF4B, which as mentioned earlier promotes 5' cap-dependent translation of mRNAs. In addition, S6K1 also enhances protein synthesis by activating RNA polymerase 1 and 3 by phosphorylating regulatory factors UBF, TIF-1A and Maf1, as well as enhancing translation efficiency of spliced mRNAs via recruitment of SKAR[47,48,49,50].

Lipid and nucleotide synthesis and glucose metabolism

mTORC1 regulates lipid synthesis by regulating the transcription factors SREBP1/2 and PPARγ. In the case of low sterol levels, mTORC1 promotes the translocation of SREBPs to the nucleus, where it activates genes enhancing lipid and cholesterol synthesis[51]. mTORC1 accomplishes this by either phosphorylating and

inhibiting lipin 1, which is an inhibitor of SREBPs, or through S6K1 signalling[52,53]. mTORC1 also induces synthesis of nucleotides for DNA replication. This is done by increasing expression of MTHFD2, which is a component of the cycle that provides carbon units for purine synthesis[54], and by phosphorylating CAD, a key enzyme in pyrimidine biosynthesis[55]. In addition, mTORC1 also upregulates expression of glycolytic enzymes and glycolysis by upregulating HIF1α. The upregulation of SREBPS that also induced lipid and cholesterol synthesis, also increases activity of the pentose phosphate pathway, increasing the amount of NADPH and precursors for further synthesis of lipids and nucleotides[53]. To prevent that the newly synthesized building blocks that are made by induction by mTORC1 are broken down again by catabolism, mTORC1 tries to suppress autophagy and catabolism. This is done via inhibitory phosphorylation of ULK1 and ATG13, which induce autophagy[56,57]. By inhibiting these two proteins, mTORC1 also inhibits the formation of the autophagosome, which prevents degradation of proteins and organelles[8].

Downstream mTORC2

Instead of regulating cell growth and metabolism like mTORC1, mTORC2 controls proliferation and survival by phosphorylating members of the AGC family of protein kinases (PKA/PKG/PKC) as seen in figure 2D. mTORC2 also phosphorylates and activates the oncogene Akt, a downstream effector of the insulin/PI3K pathway. Activation of Akt promotes cell survival, proliferation, and cell growth through several substrates including FoxO1/3A transcription factors, and NAD kinase[58,59] . Akt also mediates a feedback loop between mTORC1 and mTORC2, by inactivating TSC2, an inhibitor of mTORC1, and by phosphorylating mSin1[60, 61].

Current therapies (FDA approved)

Rapamycin and Rapalogs: Sirolimus, Everolimus & Temsirolimus

An obvious option for inhibition of mTORC1 signalling is rapamycin. Soon after discovery the anti-fungal, immunosuppressive and anti-tumour activities were observed[1,2,3]. However, rapamycin had both poor solubility and pharmacokinetic properties, driving the search for alternatives. This resulted in the first-generation rapamycin analogues, rapalogs, which included Sirolimus, Everolimus and Temsirolimus. As mentioned above, rapamycin binds as a complex with FKBP12 to mTORC1, and thereby inhibits kinase activity. Rapalogs use this same mechanism of action, but with better pharmacokinetics due to their added side groups as compared to rapamycin (see figure 3). However, the rapalogs were not as successful in clinic as the pre-clinical studies promised them to be. In addition, rapamycin and rapalogs also show side effects, including hyperglycaemia, elevated cholesterol levels, stomatitis, rash and even death due to bowel perforation[62,63,64]. Sirolimus, Everolimus and Temsirolimus are already FDA approved for treatment of renal cell carcinoma, however, the rapalogs were not as successful in clinic as the pre-clinical studies promised them to be. Recent studies show promising new rapalogs, among which DL001. DL001 is a rapalog which is 40x more selective for mTORC1 compared to rapamycin, which should reduce the side effects that are seen with rapamycin. However, this study only uses in vivo studies with mice and cell culture experiments and has not performed a pharmacokinetic study to characterize the metabolism and elimination of DL001[65]. There are also already existing drugs that inhibit mTORC1 signalling, but that were designed for other usages, for example metformin. Metformin is worldwide the most prescribed anti-diabetic drug, and has also been shown to suppress mTORC1 activity via several mechanisms. In addition, a large study showed that type 2 diabetic patients treated with metformin had reduced risk of cancer[66]. Metformin can first of all activate the inhibitors AMPK and TSC, and via the IGF1 and insulin pathway. Metformin blocks IGF and insulin, and thereby releases the inhibition on TSC, which in turn inhibits mTORC1. P53 can also be activated by metformin, which also activates AMPK[67].

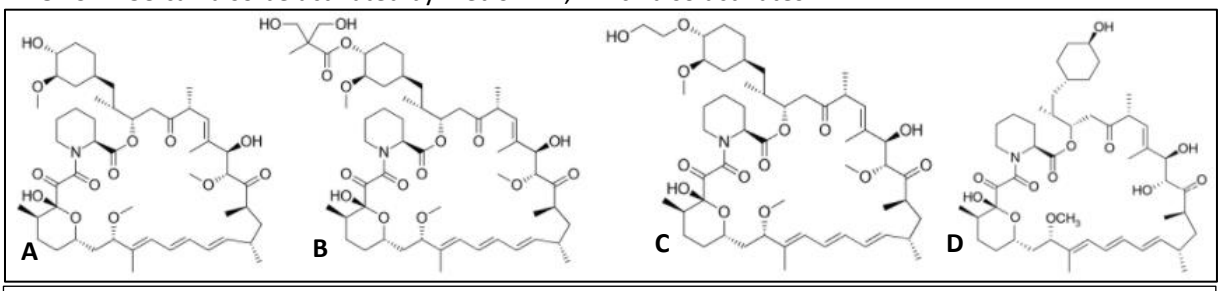

Figure 3. Molecular structure of A; Sirolimus/Rapamycin, B; Temsirolimus, C; Everolimus, D; DL001.

Current trials

ATP-competitive mTOR kinase inhibitors

Because of the limitations that rapamycin and the rapalogs show, new second-generation mTOR inhibitors are being developed, among which ATP-competitive mTOR inhibitors (TKIs). This class of drugs does not only target mTORC1, but also mTORC2 which should give a more complete therapy. They work by directly targeting the mTOR kinase domain and inhibiting its activity in both mTORC1 and mTORC2. This also results in blocking the positive feedback between the complexes via the PI3K/Akt pathway[68]. It has been shown that mTORC2 activity plays a vital role in cancer growth, which should contribute the ATP-competitive mTOR kinase inhibitors in their efficacy compared to rapalogs. It has also been shown that treatment with several different TKIs suppressed protein synthesis by 50% compared to rapalogs, which showed almost no effect. This occurred probably because TKIs can more effectively inhibit translation compared to rapalogs[69]. In addition to this promising effect, TKIs can also reduce aerobic glycolysis in human tumour cells compared to rapalogs. This reduced glycolysis starves the cancer cells, increasing the anti-tumour effect of TKIs[70]. In line with rapalogs, TKIs also inhibit the expression of HIF-1 and HIF-2. Inhibition of these factors reduce VEGF signalling and angiogenesis. Because both rapalogs and TKIs inhibit HIF-1 in a similar manner, this indicates that this is primarily regulated by mTORC1[71]. A limitation of TKIs is the fact that they do inhibit Akt signalling via inhibition of mTORC2, however this also releases inhibition of RTK and subsequently on PI3K, which in turn phosphorylates Akt. So there is initial inhibition of Akt, but this is eventually overturned[72]. A solution would be to add additional RTK inhibitors, which would prevent subsequent activation of Akt and will be further discussed below. TKIs do show major drawbacks due to the fact that there is potential higher toxicity due to the broader inhibition of mTOR. Side effects include weight loss, depression, skin rash, and mucositis.

Dual PI3K/mTOR inhibitors

A solution for late Akt activation after treatment with ATP-competitive mTOR kinase inhibitors is to inhibit both the PI3K pathway together with mTOR. Because there are similarities between these two, some chemicals can inhibit both at the same time, these are called dual PI3K/mTOR inhibitors[73]. Examples are NVP-BEZ235, LY3023414, voxtalisib, PQR309, and gedatolisib. NVP-BEZ235 showed promising effects by inhibiting activity of multiple isoforms of PI3K and mTOR, resulting in potent anti-cancer activity[74]. NVP-BEZ235 also showed to be able to penetrate the blood-brain barrier, showing potential for treatment of brain tumours[75]. It has also potential in treating gastric cancer with resistance to Paclitaxel, which is widely used in treatment. Because this type of cancer shows increased expression of PI3K/Akt/mTOR pathway activity, NVP-BEZ235 can be a potential alternative[76]. Other dual PI3K/mTOR inhibitors like LY3023414 and voxtalisib also show additive anti-tumour activity when given in combination with standard chemotherapeutics[76]. PQR309 is an oral, brain-penetrable PI3K/mTOR inhibitor which is shown to effectively inhibit lymphoma both in monotherapy and combination therapy with other drugs[77,78]. Another promising candidate is gedatolisib which is shown to inhibit tumour growth in breast, lung, colon and glioma cancers, but yet only in xenograft models[79,80]. Additionally, gedatolisib is shown to increase effectiveness of radiation therapy and targeted therapy (cetuximab) in head, neck and nasophageal carcinomas[81,82].

Rapalink-1

Rapalink-1 is a third-generation mTOR inhibitor, which combines rapamycin to MLN0128, an ATP-competitive mTOR kinase inhibitor. Compared to MLN0128 and rapamycin alone, Rapalink shows increased ability to block 4EBP1 compared and its prolonged residence time compared to MLN0128. In addition, Rapalink-1 is also able to pass the blood-brain barrier, showing potential for treatment of brain cancers[83].

Limitations mTOR inhibitors in cancer therapy

Biomarkers to predict efficacy mTOR therapy

Because mTOR inhibition does not work as well in some patients compared to others, using biomarkers to predict therapy efficacy may be a solution. Both genetic and protein markers can be used. For example, mutations in PTEN, PIK3CA, and HER2 are common in solid malignancies and result in increased expression of the PI3K pathway[84]. Expression of S6K1 and S6K2 also predicts efficacy of certain treatments. S6K2 gain was linked with increased benefit from tamoxifen treatment, and S6K1 amplification was linked with reduced benefit from radiotherapy[85,86]. As mentioned earlier, relative high expression of FKBP12 predicts that rapamycin/rapalogs will also inhibit mTORC2 in addition to mTORC1. This double inhibition of mTOR would be preferable in cancer treatment. Therefore, this high expression of FKBP12 is a possible additional biomarker for treatment with rapamycin/rapalogs.

Resistance

Resistance against mTOR inhibitors is a growing problem in treatment, with multiple mechanisms contributing to this problem. Rapamycin and the dual PI3K/mTOR inhibitor NVP-BEZ235 are substrates of the ATP binding cassette transporters ABCB1 and ABCG2 respectively, which decreases intracellular levels of drugs resulting in poorer response to treatment[87]. In addition, some ATP binding cassettes transporters are overexpressed in certain types of cancers, resulting in increased resistance. A solution would be combined treatment of mTOR inhibitors together with inhibitors of these transporters, which would enhance the efficacy of the mTOR inhibitors. Another possible mechanism of resistance in tumours are cancer stem cells, which have hyperactivated mTOR signalling. However, it has been shown that expansion of cancer stem cells increases the resistance to mTOR inhibitors, but is context and cell type dependent[73]. The status of the translation machinery and modes of protein translation and certain mTOR mutations also impact the efficacy of mTOR inhibitors.

Discussion

New insights about the mTOR pathway have been uncovered in the last few years, both about the downstream targets, and the upstream regulators. However still many questions are unanswered, for example about the difference in mTOR signalling in different organs and tissues, and which regulators are dominant in the general regulation of mTOR. The answers to these questions will be important for further development of new mTOR inhibitors, and for future treatment guidelines. Because of the broad effect the mTOR pathway has on cell growth and proliferation, and the fact that mTOR is hyperactivated in many cancers, mTOR inhibitors are on paper a great possibility for cancer treatment. However, this potential is not always translated into real life. There are cases where patients have a great respond to these inhibitors, but not in most cases. Take for example rapalogs, which are today the only FDA approved mTOR inhibitors, and are proved to show more cytostatic than cytotoxic activity, meaning that when the treatment is lifted, the tumour is again able to start growing and proliferating. This can be explained by the fact that not all downstream substrates of mTORC1 are inhibited by treatment of rapalogs, in particular 4EBP. In addition, the feedback between mTORC1 and mTORC2 ensures that when mTORC1 is inhibited, the negative feedback on the insulin/PI3K/Akt is released, which would promote increased signalling of the Akt oncogene. Also, rapalogs release the inhibition of mTORC1 on autophagy, which can actually be beneficial for certain cancers that grow in a vascularly comprised microenvironment[88]. A solution will be combined treatment of rapalogs together with autophagy inhibitors, with phase 1 and 2 trials currently being done[89]. To predict whether a patient is responsive to mTOR inhibitors, certain biomarkers can be used. For example, mutations in TSC, K-Ras, and BRAF are known to be resistant against mTOR inhibitors, while PIK3CA and GATOR mutations are sensitive to treatment[90,91]. However, to apply a screening step to the treatment with mTOR inhibitors greatly increases the cost and subverts the accessibility of this treatment. For the second- and third-generation mTOR inhibitors, toxicity and side effects is a real problem. Although they show promising effects in preclinical studies, some new mTOR inhibitors have shown serious adverse effects. Another limitation of mTOR inhibitors is the occurring resistance. All in all, the mTOR pathway shows great possibilities in new cancer therapy, due to the broad effect it has on cell growth and proliferation. However,

this broad effect also warrants adverse side effects and increased toxicity in unwanted areas, which is a major limitation of this approach. Further research is needed to prevent this from happening, perhaps targeting regulators or downstream substrates from the mTOR pathway to prevent toxicity.

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