Translational Control and mTOR in Cancer

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Rita Seeböck, Nicole Golob-Schwarzl, and Stefanie Krassnig

Contents

Abstract

The mammalian target of rapamycin (mTOR) is a key link between a cell's nutrients and energy sensors and proliferative effector molecules. Essential processes controlled by the activity of mTOR include ribosome biogenesis, protein translation, and cell cycle progression. Antagonizing mTOR activity has shown antitumor effects in preclinical studies, and first clinical trials with mTOR inhibitors gave promising results. Among the broad spectrum of tumor entities that might be sensitive to mTOR targeting, we chose glioblastoma (GBM) and colorectal cancer to report the current state of investigation. In these exemplary tumor types, we review the potential of mTOR pathway components as biomarkers as well as drug targets.

R. Seeböck (✉)
IMC University of Applied Sciences, Krems, Austria
e-mail: rita.seeboeck@fh-krems.ac.at

N. Golob-Schwarzl • S. Krassnig
Institute of Pathology, Medical University Graz, Graz, Austria
e-mail: nicole.golobschwarzl@cbmed.at; stefanie.krassnig@medunigraz.at

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5.1 mTOR Signaling

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase which was identified as the cellular target of rapamycin, a bioactive compound first isolated in the 1970s from soil samples collected on Easter Island [1]. Rapamycin was first used in clinics as an immunosuppressive drug predominantly following kidney transplantations to counteract graft-versus-host disease and acute transplant rejection. Furthermore, it was observed that rapamycin could decrease the frequency of tumor formation in organ transplant experiments and was hence focused on in oncologic research as potential anticancer agent [2–4].

The molecular context of rapamycin was discovered in the early 1990s, with the discovery of mTOR. This elegant genetic study in *Saccharomyces cerevisiae* revealed that FKBP (FK506 binding protein) is a critical component of a rapamycin gain-of-function inhibitory complex with TOR1 and TOR2 [5]. TOR proteins are evolutionarily conserved from yeast to human, with human, mouse, and rat TOR proteins sharing 95% identity at the amino acid level. Soon after the first discovery of TOR, also the mammalian homologue (mTOR) could be purified and ever since is one of the major pathways investigated in molecular oncology [6].

mTOR is active within the cell in two different complexes. mTOR complex 1 (mTORC1) is built up by mTOR, regulatory-associated protein of TOR (Raptor), DEP domain-containing mTOR-inactivating protein (Deptor), mLST8, and PRAS40. mTORC2 is built up by mTOR, rapamycin-insensitive companion of mTOR (Rictor), Deptor, Protor, LST8, GbL, and mSIN1 [7].

The mTOR pathway is regulated by a wide variety of cellular signals, including mitogenic growth factors, hormones, nutrients, cellular energy levels, and stress conditions. Therefore, it could be shown that mTOR is embedded within the PI3K/AKT signal transduction pathway, which is critically involved in the mediation of cell survival and proliferation. Signaling through the PI3K/AKT pathway is initiated by mitogenic signals, triggered by growth factors that bind receptors in the cell membrane. These receptors include insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR), and the ERBB receptor family. Receptor binding activates AKT via PI3K; this process is antagonized by PTEN activity [8]. Akt phosphorylates mTOR directly, but may also work indirectly on mTOR through the actions of the TSC1/TSC2 (tuberous sclerosis complex). The physical association of the proteins TSC1 (Hamartin) and TSC2 (Tuberin) produces a functional complex that inhibits mTOR. This inhibitory effect is understood to act via inactivation of the Ras family small GTPase Rheb by TSC2. GTP hydrolysis of Rheb blocks mTOR activation, which only occurs by Rheb-GTP [9]. Cell surface signals can, next to PI3K/Akt signaling, also trigger the mitogen-activated protein kinase (MAPK) cascade. This phosphorylation cascade of Ras-Raf-MEK and ERK leads to phosphorylation of TSC1/TSC2 and inhibition of its activity [9]. Activation of mTOR results in phosphorylation of several downstream targets. mTORC1 activation by nutrients and availability of cellular energy lead to signals inducing ribosome biogenesis and mRNA translation, all leading to cell growth and proliferation. Rapamycin-insensitive mTORC2 controls the actin cytoskeleton and thereby determines the shape of the cell [8].

The best-characterized effectors downstream of mTOR are two signaling pathways that act in parallel to control mRNA translation. Activated mTOR mediates the phosphorylation of the 4E-BP1 and the ribosomal protein S6 kinase (S6K1). 4E-BP1 represses the activity of the eIF4F complex by blocking its essential, mRNA cap-binding component eIF4e. In its unphosphorylated state, 4E-BP1 binds tightly to eIF4e. Phosphorylation of 4E-BP1 by mTOR reduces its affinity for eIF4e, and the proteins dissociate, releasing eIF4e which is then able to associate with the other components of eIF4F and act in translation initiation. Growth factor deprivation or inhibition of mTOR results in the dephosphorylation of 4E-BP1, followed by its reassociation with eIF4e and a reduction in cap-specific translation [10]. Besides 4E-BP, S6K1 is the most important downstream effector of mTOR. By phosphorylation of mTOR, S6K1 is activated. This leads to a downstream activation of the ribosomal S6 protein, essential for 40S ribosomal subunit recruitment [6].

To sum up these anabolic regulations by mTOR, mTORC1 is mainly activated by AKT, which itself can be regulated by mTORC2. AKT is activated by PI3K, which is antagonized by the tumor suppressor PTEN. Downstream of mTORC1, S6K1, and 4E-BP1 both regulate mRNA translation at the levels of translation initiation and ribosome biogenesis [4].

5.2 Eukaryotic Translation Initiation Factors (eIFs) in Cancer

Major players in translation initiation are the eukaryotic translation initiation factors (eIFs), comprising eIF1, eIF1a, eIF2, eIF2b, eIF3, eIF4a, eIF4e, eIF4g, eIF4b, eIF4h, eIF5, and eIF5b [11]. Many of these have been described to have an implication in carcinogenesis and tumor progression. We wanted to highlight this group as they are physiologically related to mTOR signaling, and their impact in cancer research has dramatically increased over the last few years. Relevant facts for eIFs in cancer are summarized below.

eIF1 is differentially expressed throughout the body and has also been described in association with genotoxic stress situations, including ionizing radiation and heat shock. This connection revealed a dependence of eIF1 on the potent tumor suppressor p53 [12].

Investigations on eIF2 subunits mainly deal with their role in stress response, but overexpression or increased activity was also linked to cancer types including various lymphoma subtypes, gastrointestinal disease, lung cancer, and melanoma [13–17].

eIF3 is the largest and most complex initiation factor with a molecular mass of 600–700 kDa and 13 described subunits, known as eIF3a-m. The subunits can form modules and complexes of varying compositions [18]. The exact contribution of individual eIF3 subunits in translation initiation is not completely refined; however, in carcinogenesis, they are individually described. The largest subunit eIF3a interacts with all other eIF3 subunits and eIF4b, which establishes a direct link to mTOR signaling. Overexpression of eIF3a was correlated to several human cancers, including breast [19], cervix [20], colon [21], lung [22], urinary bladder [23],

esophagus [24], and oral squamous cell carcinoma [25]. Beyond its hypothesized interaction with mTOR [26], eIF3a was discovered as a negative modulator of the extracellular signal-regulated kinase (ERK) pathway, via interaction with SHC and Raf-1 [27].

Upregulation of other eIF3 subunits was also identified in tumors, but it is not yet known if their differential expression is a cause or consequence of carcinogenesis [11]. One eIF3 subunit that has to be dealt with separately is eIF3f, because it is the only eIF3 core subunit which was shown to be downregulated in cancer. This was shown in patients suffering from melanoma and pancreatic cancer [28, 29]. In agreement with that, overexpression of eIF3f led to proliferation inhibition and apoptosis induction in vitro [28, 29]. Similar to eIF3a, eIF3f is suggested to interact with and eventually regulate mTOR and its downstream cascade [30].

Among eIF4 proteins, there are three subunits of the eIF4F complex, namely, eIF4a, eIF4e, and eIF4g, and the independent subunit eIF4b [31]. eIF4b is a downstream target of mTOR which, when phosphorylated, binds tighter to eIF3, thus increasing translational efficiency [32]. eIF4e functions in protein translation initiation by its cap-binding activity. eIF4e and especially its phosphorylated form were intensively studied in different cancer types and found upregulated in breast [33], colon [34], head and neck [35], and ovarian carcinoma [36] and non-Hodgkin's lymphoma [17, 36]. eIF4e availability is regulated by the 4e-binding protein 1 (4E-BP1). 4E-BP1 responds to extracellular stimuli like increased insulin levels or binding of growth factors to cell surface receptors. mTOR phosphorylates 4E-BP1, thereby inactivating it and releasing eIF4e, which can consequently interact in the eIF4F complex in order to initiate translation [37].

5.3 Targeting the mTOR Pathway in Glioblastoma

Glioblastoma multiforme (GBM) is a brain tumor deriving from glial cell origin and belongs to the most common malignant brain tumors [38]. Current treatment strategies combine surgical resection, adjuvant radiotherapy, and chemotherapy [39]. Nevertheless, the outcome with a median survival of 12 months is still very poor [40]. One reason for the poor treatment response is the highly infiltrative nature of GBMs, which leads to frequent recurrences [41]. Thus, there exists an immediate need for novel treatment strategies in glioma therapy.

In glioblastoma, the PI3K/AKT/mTOR signaling has already been extensively studied as many known mutations in GBM patients lead to a constitutive activation of this important pathway. Hyperactivation of this signaling cascade can be induced by the deletion of the tumor suppressor PTEN, overexpression of EGFR, as well as mutations in PI3K [42]. Genetic alterations in the PI3K/AKT/mTOR pathway have been detected in 88% of gliomas [43]. As a result, it was demonstrated that deregulation of the AKT/mTOR signaling seems to be one of the key players driving gliomagenesis [44]. Therefore, it has become an auspicious target for potential novel GBM treatments [45, 46].

The receptor tyrosine kinase (RTK) member EGFR offered itself as promising candidate for the downstream inhibition of the whole PI3K/AKT/mTOR pathway. Nevertheless, the EGFR inhibitors gefitinib [47] and erlotinib [48] exhibited only a reasonable performance during clinical trials.

Besides the regulation of protein translation, mTOR was shown to have diverse functions in the brain such as long-term potentiation, memory formation, and synaptic plasticity [49, 50]. In GBM therapy, rapamycin (sirolimus) and rapalogues, e.g., everolimus (RAD001) and temsirolimus (CCL-779), have been evaluated in clinical trials so far [46]. Although rapamycin has been shown to effectively inhibit glioma cell growth [51], clinical trials were not successful [52]. The fact that rapamycin predominantly inhibits mTORC1, but not mTORC2, and the presence of various feedback loops might explain the failure in clinical trials. Afterward, the focus in mTOR-mediated glioma therapy shifted to combination treatments (e.g., mTOR/PI3K or EGFR) [53].

PI3K-directed therapy has improved since the first-generation inhibitor Wortmannin did not pass the preclinical phase due to clinical toxicity [54]. PX-866 revealed only minimal toxicity, reduced GBM proliferation, increased apoptosis, and prolonged survival in murine xenograft models [55]. In clinics, BKM120 successfully finished phase I and is continuing in phase II [56], whereas the PX-866 trail was completed due to a low overall response rate in phase II [57].

The protein kinase AKT, a key player in the PI3K/AKT/mTOR pathway, has also been targeted as GBM treatment approach. Perifosine inhibits the activation of AKT by preventing its phosphorylation and translocation to the plasma membrane [58]. In murine animal models, perifosine revealed promising results especially in combination with the mTOR inhibitor temsirolimus [59] and temozolomide [60]. Nevertheless, probably also due to several drawbacks (e.g., limited penetrance through the blood-brain barrier), the success in a clinical phase II trial of recurrent GBMs was only moderate [46].

Due to the rather modest results in targeting only one signaling molecule of the mTOR cascade, approaches combining two or even more targets have become improved options in glioma therapy [61]. The PI3K/mTOR inhibitor PI-103 was one of the first dual inhibitors. PI-103 induced cell cycle arrest in glioma cells without revealing neurotoxic properties [62]. However, it never endured the preclinical phase due to its weak pharmacological properties. Other dual inhibitors, e.g., NVP-BEZ235 and XL-765, have been more successful and even completed phase I trials [46]. The EGFR inhibitor BKM120 is also tested clinically in combination with radiation or the monoclonal anti-vascular endothelial growth factor (VEGF) antibody bevacizumab [63]. However, combination therapies of erlotinib and temozolomide failed to improve GBM patient prognosis [64, 65].

To conclude, the PI3K/AKT/mTOR survival pathway plays a crucial role during gliomagenesis and lends itself therefore to be investigated in more detail as potential therapeutic approach. Although mTOR signaling belongs to one of the most investigated pathways in GBM, further efforts are needed to elucidate the exact

mechanism of this complex pathway during gliomagenesis and to use it as potential therapeutic target in advance.

5.4 mTOR Targeting in Colorectal Cancer

Colorectal cancer (CRCs) is the third most common cause of cancer and the fourth most cancer-related death worldwide [44]. Screening strategies and enhancements in treatment have resulted in the decrease in the morbidity and mortality associated with CRC [66]. Treatment of CRC includes a multidisciplinary approach that comprises surgery, radiation, chemotherapy, and targeted therapy [67]. Cancer cells can spread to nearby and remote lymph nodes, as well as to other organs, such as the lung and liver. The prognosis and survival rate depends on the stage of the disease and tumor location. Surgical removal of tumor tissue and nearby lymph nodes is the most common treatment strategy for early stage (stage I and II) CRC. Chemotherapy and/or combinations with radiation therapy are the treatment strategy for late stage CRC [68].

Targeted therapy strategies include monoclonal antibodies, for example, bevacizumab (Avastin, an VEGF-A inhibitor) and cetuximab (anti-EGFR), regorafenib (multiple RTK inhibitor), and aflibercept (anti-VEGF agent) [69, 70]. It is suggested to reconsider the existing examples for the selection of agents in the adjuvant treatment of CRC [71].

Genetic mutations and chromosomal instability can arise either hereditarily or sporadically. A large proportion of these aberrations involve oncogenic pathways converging on the translational machinery. These pathways are MAPK and PI3K/AKT/mTOR cascades that include components and regulators strongly associated with the CRC carcinogenesis, such as PIK3CA, K-RAS, BRAF, PTEN RTKs, and others [72–74]. Mutations of PIK3CA and decreased function of PTEN are also often found in CRC, directed to the activation of the PI3K/AKT/mTOR pathway [74, 75]. EIF4e is one of the most studied translation factors and is associated in the cancer biology in general and in CRC in particular. eIF4e is also involved in regulation by different signaling cascades, including MAPK and PI3K/AKT/mTOR. The PI3K/AKT/mTOR pathway controls 4E-BP, a tumor suppressor, which, when phosphorylated by an activated mTOR, dissociates from eIF4e and facilitates translation. Overexpression and activating phosphorylation of eIF4e, as well as inactivating phosphorylation and downregulation of 4E-BPs, are key notes in CRC. The 4E-BPs/eIF4e axis is a predictive and prognostic biomarker in the therapy of CRC.

Conclusions

Over the past few years, PI3K/AKT/mTOR signaling has been shown to be a key player driving tumorigenesis in various tumor entities [76–78]. Thus, much effort was put into targeting this major survival pathway, regrettably with moderate success. The complexity of the PI3K/AKT/mTOR signaling with its various feedback loops and cross-talk signaling seems to be one of the major challenges in a pathway-directed therapy [79, 80]. Many clinical trials have already been started to target the mTOR cascade via multiple inhibitors [53]. Future cancer

therapeutic approaches will even turn more into the direction of multiple targeting and combination therapy to solve the Sisyphean task of curing cancer.

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