

The Biological Role of mTOR in the Pathogenesis of Solid Tumors: An Overview

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Abstract: The mammalian target of rapamycin (mTOR) constitutes an integrator of multiple signals and a master programmer of pivotal cellular functions such as cell growth and proliferation. Due to its complex function, it plays a substantial role in homeostasis at molecular, cellular, tissue and organism level and its aberrant activation is implicated in tumorigenesis and tumor progression. mTOR signaling depends on a number of upstream regulators such as PI3K and Akt, and a number of downstream effectors such as p70 S6 kinase 1 (S6K1) and 4E-BP1. The mTOR pathway seems to be a promising pathway in anticancer treatment and mTOR inhibitors constitute a currently emerging and evaluated class of antitumor agents. Nonetheless, the complexity and multifactorial regulation of this signal transduction pathway make it difficult to determine pivotal parameters such as the optimal therapeutic schedules and the appropriate criteria for the selection of patients most likely to respond, which will enable medical oncologists to proceed to the appropriate use of these agents in clinical setting. The complete dissection of both mTOR signaling and the adjacent pathways will enable experts to develop and implement multi-targeted treatment, which appears to be the most promising approach, due to the persistent and dynamic interaction between different signaling pathways. Under such circumstances, we will be capable of exploiting mTOR signaling and maximizing the benefit of patients. In the present review, we discuss the regulation of the mTOR signaling, pointing out its implication in the pathogenesis of solid tumors as well as its encouraging therapeutic potential.

Keywords: Mammalian target of rapamycin (mTOR), rapamycin, apoptosis, autophagy, targeted therapy.

INTRODUCTION

The mammalian target of rapamycin (mTOR) is a particularly conserved serine-threonine kinase, involved in a number of signal transduction pathways responsible for cell growth, cell proliferation, cell survival and angiogenesis. More precisely, mTOR is an integrator of diverse incoming intracellular and extracellular signals and, due to its pivotal role in cellular and organism homeostasis, its aberrations are related to a number of health disorders such as cancer, cardiovascular diseases, obesity, neurological disorders and diabetes. Moreover, mTOR seems to be a master programmer of life span and implicated in the pathogenesis of a number of age-related diseases. The complex regulation of cellular function by mTOR is mediated *via* the modification of transcription, translation (formation of functional ribosomal units), organization of cytoskeleton and autophagy. Thus the inhibition of these signaling pathways could lead to the inhibition of such events and be useful for the treatment of a lot of disorders, including specific malignancies [1-3]. There are two multiprotein complexes of mTOR: the mTOR complex 1 (mTORC1) and the mTOR complex 2 (mTORC2). The two complexes have different substrates and different physiological functions. Specifically, the mTORC1 consists of TOR1 or TOR2, KOG1 and LST8 and regulates protein synthesis, whereas the mTORC2 consists of TOR2, AVO1, AVO2, AVO3 and LST8 and is involved in

actin organization. A wide range of factors such as growth factors, nutrients, insulin, energy supply and cellular stress (hypoxia, osmotic stress, reactive oxygen species-oxidative stress, viral infection) regulate the mTORC1 pathway, which is sensitive to rapamycin, and they are implicated in essential cellular events. On the contrary, the mTORC2 is not sensitive to rapamycin [4-8]. mTOR forms a multimer in both mTORC1 and mTORC2 [9]. A recently identified component of the mTORC2, called PRR5, appears to be involved in tumorigenesis. The inhibition of PRR5 leads to Akt and S6K1 inhibition *via* a decrease in PDGFR beta expression [10]. The precise regulation mediated by mTOR depends on the proteins binding mTOR. Different protein compounds lead to the development of different complexes with distinct function. The identification of such mTOR-associated proteins and the investigation of their role in the mTOR signaling are necessary for the development of different inhibitory strategies [11]. A number of mTOR inhibitors are evaluated as anti-cancer agents in the treatment of specific malignant tumors [12]. Nonetheless, the results of such trials are ambiguous due to the tremendous complexity of the factors involved. On the one hand, there are a number of signal transduction pathways involved in tumorigenesis and tumor progression and single inhibition is inadequate. On the other hand, mTOR has two different isoforms, and subsequently different regulators, different multi-component protein complexes and different functions [13]. Interestingly, it has been indicated that the pathways involved in the mTOR signaling are several times aberrant in preneoplastic lesions. Thus the inhibition of such pathways could lead to the development of a chemopreventive strategy [14].

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The Mammalian Target of Rapamycin (mTOR) Pathway

The mammalian target of rapamycin (mTOR) signal transduction pathway is of utmost importance in normal cells for the maintenance of homeostasis in response to a number of extracellular stimuli and stressful events. TOR is a serine/threonine kinase with a molecular weight of 289kDa. TOR kinases are highly conserved and identical in up to 60% in humans and other mammalian organisms. The domain structure of TOR consists of about 20 tandem amino acids sequences, called HEAT repeats at the NH₂-terminus domain followed by the conserved FAT domain, the catalytic domain FRB, which is the domain where the rapamycin-FKBP12 complex binds and exerts its inhibitory actions, another FAT domain close to the C-terminus and finally the C-terminus domain. The mTOR protein kinase is an integrator of signals which achieves conjunction between multiple incoming signals and a number of cellular functions such as cell growth and proliferation. The substantial role of the mTOR explicates the reason why this network is amazingly conserved during the years [15, 16]. The essential role of the mTOR pathway in cellular function is also depicted by the fact that its deregulation is implicated in the pathogenesis and progression of a number of diseases including cancer [17]. The mTOR expression is high in a wide range of malignancies, whereas major differences are observed between different types of cancer. mTOR inhibition is effective in approximately 26% of tumors and the exact percentage of sensitivity depends on the tumor type (melanoma 0%, ovarian 41%) [18]. Moreover, it is noteworthy the fact that the activation of the PI3K/Akt/mTOR pathway leads to resistance to a number of therapeutic strategies and this is one of the reasons why patients with such tumors have a poor prognosis [19].

The mTOR exerts two major actions: a. induces the transition of the cell cycle from G1 to S phase (G1/S transition) and b. promotes protein translation and the initiation of cap-dependent translation. Through these mechanisms it is implicated in the initiation, maintenance and progression of tumors and constitutes a promising therapeutic target under investigation and clinical evaluation [20, 21]. The perpetual interaction of mTOR with both cytoplasm and nucleus seems to be necessary for its cytoplasmic action. Moreover, the action of mTOR on ribosomal protein S6 kinase (S6K1) necessitates the translocation of mTOR in nucleus [22, 23]. The mammalian target of rapamycin is a downstream molecule of the PI3K/PTEN-AKT-mTOR pathway. This signaling network plays a crucial role in the translational level, by modifying phosphorylation of pivotal targets such as the translation initiation factor 4E-binding proteins and S6Ks. The interplay between the PI3K and mTOR pathways is mediated through the tumor suppressor proteins tuberous sclerosis 1 (TSC1 or hamartin) and tuberous sclerosis 2 (TSC2 or tuberin) and Ras-homolog enriched in brain (Rheb) [24-26]. The protein product of the tumor suppressor genes TSC1 and TSC2 form a complex which down-regulates the mTORC1 through the G-protein Rheb. Rheb is a member of the Ras superfamily of GTPases, promotes the phosphorylation of mTOR, S6K and 4EBP1 and in this way constitutes a significant regulator of mTOR activation. Specifically, TSC2 has a GTPase activating domain (GAP activity) which acts on Rheb. This complex is responsible for the detection and

integration of multiple growth signals and results in the mTORC1 down-regulation. When stimuli such as nutrient deprivation or stressful events are absent, tuberin is phosphorylated and the TSC1/TSC2 complex is degraded. This results in the activation of mTOR downstream effectors [27-34]. Totally, the TSC1/TSC2 complex has three functions: 1. inhibition of 4E-BP1 phosphorylation and high association of 4E-BP1 with eIF4E 2. inhibition of S6K1 activity and 3. inhibition of the S6K1 activation by amino-acids under nutrient deprivation [35]. It has been importantly demonstrated that TSC2 (tuberin) is a substrate of Akt and AMPK and integrates growth and energy signals mediating mTOR function, while the TSC1/TSC2 complex seems to modulate the activity of beta-catenin and TGFbeta. Interestingly, rapamycin has shown efficacy in preclinical models of TSC and it is currently evaluated [36]. Akt activates mTOR by inhibiting its negative regulator TSC2 in double mechanism: 1. directly phosphorylates and inhibits the TSC2 and 2. inhibits the AMP-activated protein kinase (AMPK) and subsequently the AMPK-mediated phosphorylation of TSC2 as well. The second mechanism seems to be the prevalent one through which Akt activates mTOR *in vivo* [37]. The direct phosphorylation of TSC2 by Akt destabilizes TSC2 and impairs its interaction with TSC1 [38]. The serine/threonine kinase Akt has a number of upstream regulators and downstream effectors, which mediate its multiple and complex actions on cell growth and proliferation, inhibition of apoptosis and neo-angiogenesis [39]. Specifically, among regulators of Akt are two tumor suppressors: an upstream negative regulator, called PTEN and a downstream negative regulator, the complex TSC1/TSC2 [40]. TSC2 is also affected by intracellular energy levels. Energy dearth leads to the activation of AMPK, which phosphorylates and activates TSC2, preventing cells from apoptosis and regulating cell size and translation [41]. Furthermore, mTOR promotes translation, a crucial event for tumorigenesis, by phosphorylating the eIF4E binding proteins, which are repressors of the translation initiation factor eIF4F complex. A number of mutations in the suppressors of mTOR lead to enhanced formation of the eIF4F complex and consequently, to increased initiation of translation [42, 43]. In hypoxia the 4EBP1 and the transporter of eIF4E are activated and lead to the inhibition of translation. On the contrary, the blockage of 4EBP1 results in increased synthesis of S100 calcium-binding protein A4 (S100A4) and transgelin 2, which enhance the motility as well as the invasive and metastatic potential of cancer cells [44]. Tumor hypoxia, known to be a poor prognostic marker, has been shown to diminish protein synthesis in part through mTOR inhibition, which exerts an inhibitory effect on the eukaryotic initiation complex eIF4F [45].

Another upstream regulator of mTOR, apart from the PI3K pathway, seems to be the phosphatidic acid, a metabolic product of the phospholipase D (PLD). PLD is increased in a number of cancer cell lines and promotes both the survival of cancer cells by inhibiting apoptosis and their invasive and metastatic potential. What is more, phospholipase D 1 (PLD1) mediates the activation of the mTOR downstream target, ribosomal S6K1, by Cdc42. This interaction reveals that mTOR is probably an integrator of nutrient and mitogen signals [46-50]. Moreover, lysophosphatidic acid exerts its triggering effect on protein synthesis through

the activation of mTOR by the PLD1-produced phosphatidic acid [51]. However, the PLD isoform which seems to be primarily involved in mTOR activation by mitogens is PLD2. PLD2 triggers mTOR by binding the complex mTOR/raptor with lipase activity [52]. In addition, PLD has been proved to regulate mTOR not only by growth signals but also by mechanical stimuli in skeletal muscle [53]. In addition to the aforementioned data, it has been indicated that the PLD-2-produced phosphatidic acid can stimulate the p70 S6 kinase independently of the mTOR signaling [54]. Interestingly, the stimulation of mTOR by the α_1 adrenergic receptor necessitates an increase in intracellular Ca^{2+} and activated PLD, whereas the PDGF receptor stimulates mTOR regardless of the Ca^{2+} concentration or PLD [55].

It is noteworthy that mTOR has been proved to be a detector of the intracellular level of ATP and even more, the mTOR pathway is influenced by the intracellular ATP level [56]. In detail, the AMP-activated protein kinase (AMPK), which is a regulator of the intracellular energy homeostasis and activated in the case of dearth of energy, is an upstream mediator of the mTOR regulation [57]. AMPK is regulated by AMP levels with the view to modulating cellular metabolism and links the metabolic changes to the regulation of p70 S6K [58]. It has been indicated that the inhibitory effect of AMPK on S6K1 in the case of lack of energy (increased AMP/ATP ratio) is potentiated by the protein tyrosine phosphatase SHP-2 [59]. AMPK is activated through phosphorylation by LKB1, is a negative regulator of mTOR while phosphorylates and activates TSC2. Through these actions AMPK is involved in the pathogenesis of cancer [60]. The interaction between the mTOR pathway and AMPK seems to constitute the basis of the integration between the energy sensing and amino-acid sensing. Specifically, mitochondrial dysfunction activates AMPK, which inhibits the activation of the mTOR downstream effector p70 S6 kinase alpha 1 (p70 alpha or S6K1). This regulation suggests the mechanism of interaction between the mTOR pathway and AMPK. Leucine seems to modulate the mitochondrial function and AMPK and through this mechanism mTOR and constitutes the basis of the amino-acid/energy integration [61]. It has been indicated that amino acid sufficiency and mTOR influence the activation of the p70 S6 kinase (S6K1) *via* a common mediator, associated either directly or indirectly with mTOR [62]. The (Fig. 1) depicts the major mTOR regulators as well as the promoting effect of the activated mTOR effectors on cell survival, growth and proliferation through the enhancement of both the G_1/S transition and protein translation.

In addition to the above described tumor-associated and promoting effects of the mTOR signaling, a number of other mechanisms and implications have been observed. Firstly, the activation of mTOR by the PI3K/Akt pathway mediates the epithelial to mesenchymal transition (EMT) induced by transforming growth factor-beta (TGF-beta) [63]. Furthermore, three-dimensional mesothelioma mass seems to present acquired resistance to apoptosis (programmed cell death type I) due to the mTOR pathway. This observation is of great clinical significance, provided that tumor mass *in vivo* is three-dimensional, and not two-dimensional as occurs in the vast majority of preclinical models, and this provides an explication of the obscure and unfathomable cases of resistance observed in clinical practice [64]. Moreover, the

PI3K/mTOR/ erythroblastosis virus transcription factor 2 pathway is implicated in the anticancer, chemosensitizing and radiosensitizing action of a dietary substance called curcumin, mediating a down-regulation in the oncogene MDM [65]. Another mechanism of the implication of the PI3K/mTOR signaling in tumorigenesis is mediated by the insulin/insulin-like growth factor-I/mTOR pathway, which alters the mitochondrial function by up-regulating the pyrimidine nucleotide carrier PNC1, while the latter promotes cell growth and proliferation [66].

The Role of mTOR Signaling in Angiogenesis

The mTOR signaling pathway is involved in the angiogenesis of both blood and lymphatic vessels. We are going to analyze the implication of the mTOR signal transduction pathway in each of these two crucial tumor promoting effects.

Firstly, tumors with increased activity of mTOR have a high density of blood vessels. This is due to the fact that mTOR activates the transcription factor hypoxia-inducible factor 1 alpha (HIF-1alpha), which promotes the expression of VEGF. In detail, raptor, a component of the mTORC1, that regulates mTOR, demands an mTOR signaling motif which is found in the N-terminus of HIF1a and in this way HIF-1a is triggered [67]. Nonetheless, HIF-1a can also be up-regulated by Akt under both normoxic and hypoxic conditions regardless of mTOR [68]. In addition, a significant part of tumorigenesis and tumor angiogenesis is mediated by inflammatory agents. Tumor-associated macrophages (TAMs) promote tumor progression and angiogenesis through pathways which have not yet been identified. It has been indicated that IKKbeta inactivates the complex TSC1-TSC2 through phosphorylation of TSC1 and in this way activates mTOR signal transduction pathway and subsequently angiogenesis [69]. The above data provide an explication for the antiangiogenic effect of mTOR inhibitors. Phosphatidylinositol 3'-kinase/Akt pathway is responsible to some extent for the regulation of vascular endothelial growth factor (VEGF). Rapamycin has been proved effective at reducing VEGF in normoxic rhabdomyosarcoma cells while the rapamycin-induced decrease in VEGF under hypoxic conditions is limited. Moreover, the double inhibition of the phosphatidylinositol 3'-kinase pathway could lead to the inhibition of VEGF production in pediatric solid tumors [70]. Temsirolimus not only inhibits HIF-1a-dependent VEGF production but also inhibits proliferation of endothelial cells and formation of vessels which are directly mediated by VEGF. A significant part of the action of temsirolimus against breast cancer cells seems to be mediated by its antiangiogenic effect [71]. Due to their inhibitory effect on angiogenesis, mTOR inhibitors seem to act in a synergistic way with radiation through the impairment of tumor vasculature. In other words, mTOR inhibitors radiosensitize the vascular endothelial cells and act as antiangiogenic agents [72, 73].

Secondly, the mTOR signaling mediates the development of lymphatic vessels *via* a number of molecular events. It has been indicated that the fibroblast growth factor-2 (FGF-2) promotes lymphangiogenesis through the Akt/mTOR/p70S6 kinase signal transduction pathway [74]. Rapamycin and its

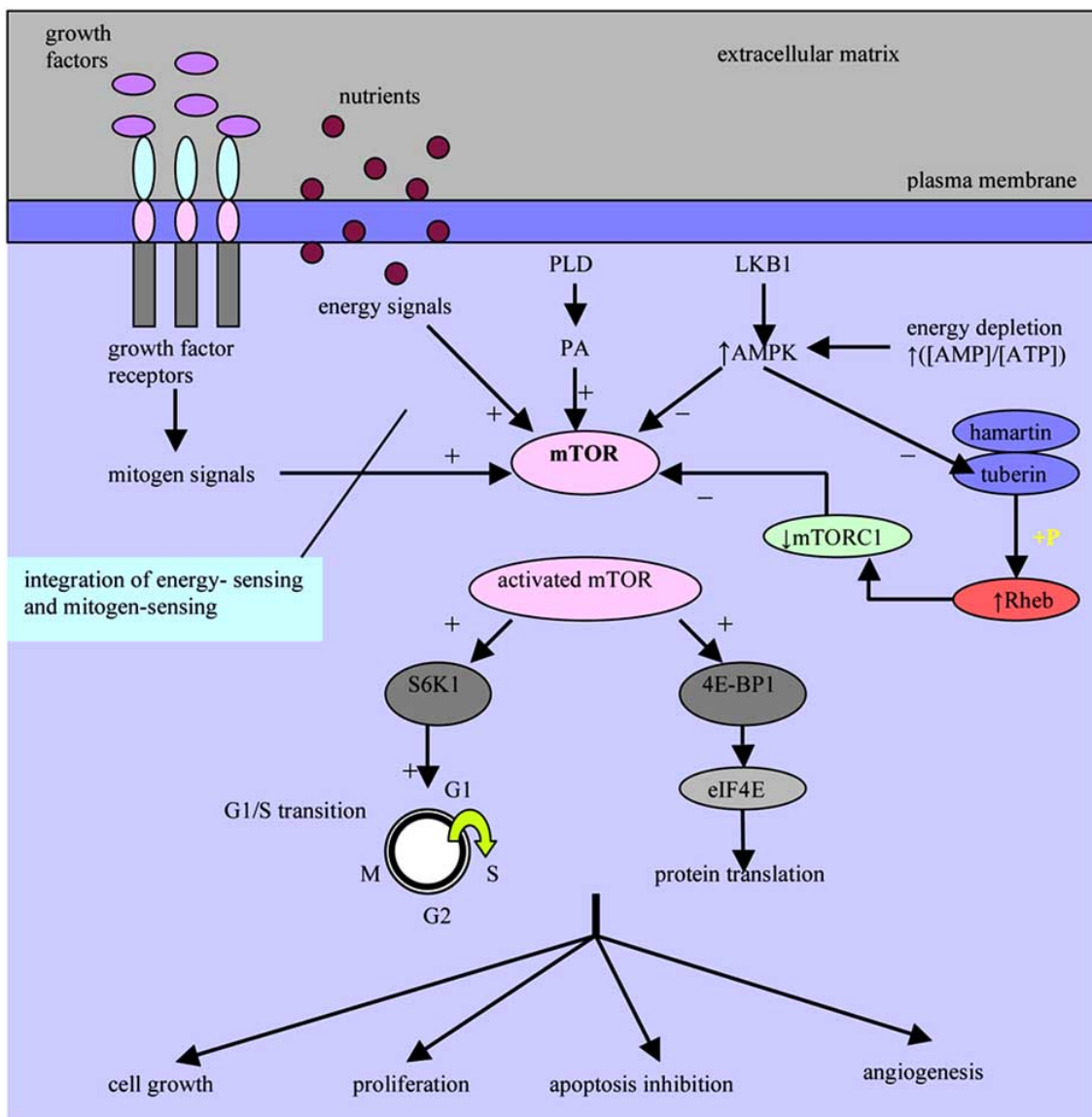


Fig. (1). The upper part of the figure depicts the multiple mTOR regulators and the role of mTOR as an integrator of energy-sensing and mitogen-sensing. The lower part depicts the positive effect of the two major mTOR effectors, S6K1 and 4E-BP1, on cell growth and proliferation, through the promotion of G1/S transition and protein translation.

analogues seem to inhibit lymphangiogenesis and thus lymph node metastasis, by reducing the number and the area of lymphatic vessels in primary tumors, *via* the reduction of the VEGF-C expression. VEGF-C is an isoform of VEGF which promotes the proliferation and migration of lymphatic endothelial cells and in this way, promotes lymphangiogenesis and subsequently lymph node metastases. These data provide a rationale for the usefulness of mTOR inhibitors in the treatment of malignant tumors [75, 76].

THE BIOLOGICAL ROLE OF THE MTOR SIGNALING IN THE PATHOGENESIS AND MANAGEMENT OF SPECIFIC SOLID TUMORS

Renal Cell Carcinoma

The targeting of molecular markers has been proved to be an encouraging therapeutic manipulation for renal cell carcinoma (RCC). Besides the proved utility of the inhibitors of

the vascular endothelial growth factor receptor (VEGFR), mTOR is another promising target for this type of cancer. The inhibition of this kinase is currently evaluated with the view to developing a novel class of drugs for such a hard to cure carcinoma [77]. The most promising approach of the rational use of mTOR inhibitors in clinical practice is the determination and establishment of specific criteria related to patients and tumors, in order for the targeted therapy to be selective and considerably increase the therapeutic ratio [78]. The levels of the molecules implicated in the mTOR pathway are related to the features of the tumors and patients' prognosis. Some components involved in the mTOR signaling such as PTEN, pAkt, p27 and pS6 appear to be useful predictive and prognostic markers [79]. mTOR is frequently up-regulated in RCC. Patients with a poor prognosis seem to take advantage of mTOR inhibition, whereas preclinical data indicate that mTOR inhibition in these patients is mediated through the inhibition of the transcription factor HIF-1. Even

more importantly, cell histology seems to be relevant to the mTOR inhibition, provided that patients with nonclear RCC benefit from targeted therapy against mTOR [80]. Clinical studies support the view that temsirolimus should be used as first-line treatment for patients with advanced RCC and a poor risk [81]. Importantly, the combined inhibition of mTOR and other molecules which act upstream, is a promising therapeutic approach for the treatment of RCC [82]. Temsirolimus improves overall survival in patients with metastatic RCC in comparison with interferon alfa. Moreover, the addition of temsirolimus to interferon alfa does not improve the efficacy of the treatment [83]. Finally, mTOR inhibitors do not diminish the levels of hypoxia-inducible factor-2a (HIF-2a), which is in part responsible for the radioresistance observed in RCC and thus, they do not seem to be useful agents for increasing the sensitivity of RCC to radiation [84].

Gynecologic Malignancies

Cancer cells which depend on estrogen signaling could be sensitive to mTOR inhibition due to the interplay between the phosphatidylinositol 3'-kinase/Akt pathway and the estrogen receptor signal transduction pathway [85]. mTOR inhibitors are useful therapeutic agents in addition to conventional chemotherapy in cell lines with aberrations of the phosphatidylinositol 3'-kinase/Akt pathway. A subset of breast cancer cell lines seems to be especially sensitive to the combination of mTOR inhibitors with chemotherapeutic agents [86]. More importantly, it seems that mTOR signaling is necessary for proliferation of estrogen-dependent breast cancer cells. Moreover, the combination of everolimus and letrozole (aromatase inhibitor), has a potent synergistic effect, inducing proliferation arrest and apoptosis [87]. Interestingly, it has been indicated that breast cancers with high activity of the phosphatidylinositol 3'-kinase/Akt pathway are resistant to tamoxifen. The administration of mTOR inhibitors can make these cancers become sensitive to tamoxifen [88, 89]. The same data are also supported by another study which indicated that the combined administration of the mTOR inhibitor RAD001 and tamoxifen has additive antitumoral effect on both ovarian and breast cancer cell lines [85]. In specific breast cancer cell lines with high PLD activity and suppressed PI3K, mTOR can be alternatively activated by PLD [90]. More importantly, the sensitivity of breast cancer cells to rapamycin seems to depend on the activity of phospholipase D (PLD), and specifically as PLD activity increases, the sensitivity of breast cancer cells to rapamycin decreases [91]. In addition to the abovementioned data, AMPK activation and subsequent inhibition of the mTOR pathway has been suggested as an alternative manipulation in targeted therapy of breast cancer cell lines [92]. A number of factors such as the overexpression of S6K1, expression of phosphorylated Akt and alterations of cyclin D1 levels have been suggested as predictors of response to rapamycin and they should be evaluated in patients with breast cancer who are going to undergo mTOR targeted therapy [93]. It has been indicated that irradiation activates the PI3K/Akt pathway and its downstream signal mTOR. Everolimus has been proved effective at radiosensitizing breast cancer cells, through the prevention of the activation of this pathway in irradiated breast cancer cells and thus enhances the toxicity of irradiation [94].

The mTOR targeted therapy seems to be a promising therapeutic strategy for ovarian cancer as well. Preclinical data strongly suggest that mTOR inhibitors might constitute an effective approach for the prevention of ovarian cancer in women who are at high familial risk of ovarian cancer [95]. In addition to this, everolimus has showed great efficacy in human ovarian cancers with high activity of the Akt/mTOR pathway and moreover, in such cancers everolimus strengthens the therapeutic effect of cisplatin [96]. Interestingly, it has been demonstrated that the simultaneous inhibition of vascular endothelial growth factor receptor (VEGFR) and mTOR is an effective therapeutic strategy in ovarian cancer, which prevents peritoneal carcinomatosis and delays the accumulation of ascetic fluid in patients with secondary peritoneal lesions [97].

Lung Cancer

The targeting of mTOR is currently being evaluated in lung cancer. There is growing evidence suggesting that mTOR inhibitors expand the duration of stable disease and induce tumor regression in patients with non-small cell lung cancer (NSCLC) [98]. However, the inhibition of mTOR in lung cancer does not appear to be equally effective as in other malignancies. Nonetheless, mTOR inhibition could be a substantial part of multi-targeted therapy for lung cancer [99]. Indeed, a phase I trial has indicated that the combination of everolimus, at a daily dose of 5 mg, with gefitinib, at a daily dose of 250 mg, has to some extent encouraging results in patients with advanced NSCLC [100]. Moreover, the activated mTOR pathway, resulting from AKT1 overexpression, has been implicated in the mechanism of resistance of lung cancer cell lines to cisplatin [101]. More importantly, the activation of the Akt/mTOR pathway seems to be an early event in progression of NSCLC, detected in metaplastic and dysplastic lesions, where it enhances the invasive potential of such lesions. Therefore, the therapeutic potential of the mTOR pathway in lung cancer is not limited to the treatment of this type of cancer, but it could also constitute the basis of a chemopreventive strategy, implemented in patients who are at high risk of NSCLC [102, 103]. This is also supported by additional preclinical data indicating that preneoplastic lung lesions induced by smoking have increased activity of the Akt/mTOR pathway and rapamycin inhibits the growth of such tumors [104]. Interestingly, mTOR decreases in hypoxia and mTOR inhibition prevents cancer cells from hypoxia-induced death, through the decrease in metabolism and consequently, increasing the supply of cellular energy and available nutrients [105]. Moreover, the stimulation of NSCLC proliferation by fibronectin necessitates the activation of the Akt/mTOR pathway and the subsequent activation of the p70 S6 kinase 1, which seem to be mediated by the inhibition of their negative regulators LKB1/AMPK [106]. Finally, the combined inhibition of apoptosis and mTOR enhances the sensitivity of NSCLC to radiation [107].

Head and Neck Cancer

Targeted therapy seems to be a promising therapeutic approach for the treatment of squamous head and neck carcinoma (HNSCC). The most extensively evaluated molecular

marker is the epidermal growth factor receptor (EGFR). Besides, *in vitro* analyses have shown that the PI3K/Akt/mTOR pathway is an additional pivotal pathway involved in the pathogenesis of head and neck cancer and it is worthy of further investigation and evaluation [108]. According to the Head and Neck Cancer Tissue Array Initiative, the Akt/mTOR signaling pathway is frequently activated in HNSCC, regardless of the status of other pathways such as EGFR or p53. In addition to this, the mTOR is in certain cases activated in absence of Akt activation, suggesting an additional mechanism of mTOR stimulation [109]. Analyses in oral squamous carcinoma cells have shown that the levels of mTOR and p70 S6 kinase are increased in M phase of the cell cycle, while the 4E-BP1 levels are decreased in M phase. Moreover, the maximum mTOR activity is measured in G2 and M phase [110]. Preclinical data support the usefulness of mTOR inhibitors in the treatment of HNSCC [111]. Analyses of the activation status of the Akt/mTOR/p70 S6 kinase pathway in HNSCC clinical specimens also indicate increased activity of this pathway. This signaling is inhibited by rapamycin, which acts by preventing DNA synthesis and inducing apoptosis of HNSCC cells [112]. Rapamycin inhibits proliferation and induces programmed cell death, especially in cancer cells lacking MDR1 and BCL2. Moreover, the combination of rapamycin with carboplatin or paclitaxel results in a more potent therapeutic effect in comparison with either agent alone [113]. It has been indicated that the main action of rapamycin *in vivo* is the targeting of the squamous cancer cells, whereas the observed antiangiogenic effect seems to be a downstream event [114]. It has been indicated that both mutations of the TSC1/TSC2 complex and HIF-1 α polymorphisms lead to increased HIF-1 α levels in squamous head and neck carcinomas [115]. It is noteworthy that there is a proclivity towards activation of the Akt/mTOR pathway in surgical margins. The activated eIF4E by the Akt/mTOR pathway in residual cancer cells at the seemingly free-tumor surgical margins is an indicator of possible recurrence and the administration of the mTOR inhibitor CCI-779 in adjuvant setting should be further evaluated for such cases of squamous head and neck carcinoma [116, 117].

Malignancies of the Gastrointestinal Tract

There are growing data indicating that patients suffering from malignant tumors of the gastrointestinal tract could take advantage of the targeted treatment based on mTOR inhibitors. To begin with, the mTOR pathway is frequently activated in gastric cancer [118]. Furthermore, the inhibition of the Akt/mTOR signaling pathway could provide a benefit to patients with malignancies of the gastrointestinal tract apart from gastric cancer. Indeed, mTOR activation is detected in 25% of patients with squamous cell carcinoma of the esophagus. For this reason a number of trials evaluating mTOR inhibitors in the treatment of such malignancies are conducted [119, 120]. The abovementioned findings are supported by additional data suggesting that the mTOR signaling is activated in esophageal squamous cell carcinoma and the inhibition of the mTOR/p70S6 kinase by the combination of rapamycin and small interfering RNA against mTOR appears to be an effective therapeutic approach for these patients [121]. It is noteworthy the fact that patients

with esophageal squamous cell carcinoma treated with neoadjuvant chemotherapy, have higher phospho-Akt expression in comparison with patients not subjected to preoperative chemotherapy, and this finding is associated with a poor prognosis [122]. Phosphorylated p70 S6 kinase could be a good biological marker of the activation of the Akt/mTOR pathway and of rapamycin sensitivity in colorectal cancer cells [123]. Interestingly, it has been indicated that the activated PI3K/mTOR pathway is implicated in the K-Ras-induced transformation of intestinal epithelial cells and the inhibition of this pathway results in G1 arrest of transformed cells. Nonetheless, the inhibition of the PI3K/mTOR pathway can sometimes induce the epithelial to mesenchymal transition (EMT) and promote malignant behaviour [124].

Liver Malignant Tumors

According to the 2007 Liver Carcinogenesis Symposium, the mTOR signal transduction pathway is one of the four prevalent tumor suppressor signaling pathways involved in liver carcinogenesis [125]. It is noteworthy that activation of the mTOR signaling in hepatic cell lines leads to impotence for differentiation, damages the hepatic energy homeostasis by affecting pathways involved in lipid homeostasis as well as affects pathways implicated in growth control [126]. The mTOR signaling is also associated with the expression and levels of the amino acid transporter-2 (ASCT2), a system essential for survival and growth of human hepatoma cells. Specifically, the ASCT2 down-regulation leads to inhibition of the mTORC1-mediated translation, whereas stimulates the mTORC2-mediated survival response [127]. According to experimental data, mTOR inhibitors have been proved useful in the treatment of malignant liver tumors [128]. In addition, there is ample preclinical evidence suggesting that mTOR inhibitors can reduce the growth of hepatocellular carcinoma and ameliorate the survival mainly through their antiangiogenic action [129]. According to another analysis, the mTOR is activated in about 5%, whilst phospho-mTOR is overexpressed in about 15% of hepatocellular carcinoma. Moreover, rapamycin decreases cell proliferation of cancer cells and might constitute an effective therapeutic strategy in the treatment of this type of cancer [130]. Encouraging data has also provided a pilot study which has indicated that sirolimus appears to be a promising drug for the treatment of hepatocellular carcinoma and cholangiocellular carcinoma. Nevertheless, its usefulness in such patients should be evaluated and determined by additional studies [131]. Besides mTOR inhibitors, 5-fluorouracil (5-FU) has been indicated to down-regulate the PI3K/Akt/mTOR pathway. Specifically, 5-FU promotes the apoptosis of hepatocellular carcinoma cells *via* telomerase activity inhibitor at both transcription level and post-transcription level. The post-transcription inhibition is mediated by the down-regulation of the PI3K/Akt/mTOR pathway [132]. The usefulness of mTOR inhibitors has also been evaluated in patients with hepatocellular carcinoma who are subjected to orthotopic liver transplantation. Despite the fact that the mTOR pathway is activated in HCC tissues in 40% of these patients, nonetheless it does not seem to have a prognostic significance and the reliance on mTOR activity for the prediction of the antitumor effect of the post-transplantation administration of mTOR inhibitors in such cases should be further evaluated [133].

Brain Tumors

The mTOR signal transduction pathway is of paramount importance for the normal development and function of the nervous system and its implication in both neurophysiology and neuropathology is multifarious. More specifically, mTOR plays a pivotal role in survival and differentiation of neurons as well as in the function of synapses and the analysis and integration of signals [134]. Such a complex and multifactorial implication explicates the reason why mTOR deregulation is responsible for a number of neurological disorders, such as brain tumors and neurodegenerative diseases and its modification could be an effective therapeutic approach for such disorders [135]. Aberrations in the mTOR pathway are responsible for an inherited susceptibility to brain tumors, leading to specific cancer syndromes such as Lhermitte-Duclos disease, neurofibromatosis type 1 and tuberous sclerosis complex. The mTOR signaling is worthy of further investigation in order to provide us with data about the potential for its exploitation in the treatment of brain tumors [136]. The PI3K/Akt/mTOR pathway is frequently activated in brain tumors because of the stimulation by growth factor receptors and Ras signaling [137]. The insight into molecular biology of gliomas will enable experts to exploit the implicated signaling pathways, including the PI3K/Akt/mTOR signaling, so that patients take advantage of molecularly based strategies [138]. Research has revealed the substantial role of the mTOR signaling in the maintenance of malignant brain tumors. Specifically, mTOR signaling seems to be indispensable for the prevention of apoptosis of certain cells in glioblastoma mass as well as for the maintenance of the astrocytic characteristics of the tumor. The latter is depicted by the observation that mTOR inhibition makes astrocytomas be converted into oligodendrogliomas [139]. Furthermore, it has been suggested that the PI3K, Akt and mTOR signaling pathways are some of the intracellular events leading migrating glioblastoma cells to apoptosis resistance. Other pathways with the same effect are the ones of NF-kappaB and autophagy [140]. The mTOR implication in the natural history of gliomas is in part mediated by the mTORC2. The rictor, a component of the mTORC2, is overexpressed in gliomas, leading to increased mTORC2 levels and *via* this mechanism, to tumor progression [141]. Interestingly, it has been indicated that one of the major mechanisms of antitumor action of rapamycin to glioma cells is the induction of autophagy, which could be further enhanced through the inhibition of the mTOR kinase activity [142]. Glioblastomas which are resistant to apoptosis have shown response to proautophagic drugs such as temozolomide. Such a response could be strengthened by inhibitors of the PI3K/Akt/mTOR pathway, provided that this pathway is implicated in autophagy and apoptosis resistance [143]. In addition, Akt inhibitors increase the radiosensitivity of glioma cell lines by inducing autophagy. This is due to the fact that ionizing radiation exerts its action through the induction of autophagy and what is more, the Akt/mTOR pathway is a major negative regulator of the autophagic process [144]. *In vivo* analysis of the PI3K pathway in glioblastomas has indicated that the loss of the tumor suppressor protein PTEN is associated with activation of Akt and phosphorylation of mTOR. Moreover, the mutant EGFR vIII is associated with activation of Akt downstream effectors [145]. These data explicate the clinical observation ac-

ording to which the simultaneous inhibition of EGFR, PI3K and mTOR has a more potent therapeutic effect in comparison with EGFR inhibition alone or with EGFR inhibition with single targeting of mTOR or PI3K in EGFR-driven and PTEN-mutant gliomas [146]. In addition, a pilot study has indicated that the combination of EGFR and mTOR inhibitors is a promising therapeutic schedule for recurrent malignant gliomas [147]. A phase I trial including patients with glioblastoma with loss of PTEN indicated that rapamycin can exert a significant antitumor action in such cases [148]. In addition to the abovementioned data, mTOR inhibitors can sensitize glioblastoma multiforme to fractionated irradiation, *via* inhibition of the G1-specific cyclin-dependent kinases, and their combination seems to be a promising therapeutic strategy [149].

Sarcomas

The mTOR inhibitors appear to be useful in the treatment of sarcomas. Four inhibitors of the mTOR pathway: rapamycin, CCI-779 (temsirolimus), RAD001 (everolimus) and AP23573, are at the moment subjected to evaluation of their efficacy and tolerability in such patients. These molecules exert an inhibitory effect on translation and metabolism [150]. mTOR inhibitors seem also to be useful agents for the treatment of advanced or metastatic sarcomas, which have not responded to previous therapeutic manipulations [151]. A specific mTOR inhibitor, called AP23573, seems to be effective and tolerable in the treatment of patients with sarcomas. However, such novel agents should be further investigated and evaluated in order to lead to the best possible therapeutic outcome. Moreover, different mTOR inhibitors should be separately evaluated in diverse types of sarcomas [152, 153].

The Biological Role of mTOR in other Malignancies

According to preclinical data, rapamycin has showed a potent growth inhibitory effect in neuroendocrine tumors. However, mTOR inhibition seems to be neither additive nor synergistic in combination with octreotide [154]. Another malignancy for the treatment of which mTOR inhibitors might be useful is angiomyolipoma, a perivascular epithelioid cell tumor. It has been indicated that these tumors have high mTOR activity and loss of function of the TSC2. Thus the administration of mTOR inhibitors to these patients could be an effective therapeutic strategy [155]. Phospho-mTOR has been indicated to be a significant independent prognostic marker of cervical cancer. Moreover, rapamycin potentiates the antitumor action of cisplatin in cervical cancer cell lines [156]. Importantly, rapamycin can make paclitaxel-resistant cervical cancer cells become sensitive to paclitaxel [157]. The mTOR pathway seems also to be involved in prostate cancer. Specifically, it mediates cell growth and proliferation as well as invasion and angiogenesis, induced by platelet-derived growth factor-D (PDGF-D) in PDGF-D-overexpressing prostate cancer cells [158].

The mTOR Signaling and Autophagy: A Potent and Dynamic Crosstalk

Autophagy seems to be the crucial event which determines the radiosensitivity of tumor cells and increases when

apoptosis is down-regulated. On the contrary, apoptosis is of little significance for irradiated solid tumors. It is noteworthy that the mTOR inhibition promotes autophagy, while the Bax/Bak activation inhibits autophagy. These data explicate the radiosensitivity observed when such cancer cells are exposed to Bax/Bak inhibitors or mTOR inhibitors [159, 160]. Autophagy constitutes one of the four mechanisms of cell death, which totally are the following: 1. necrosis 2. apoptosis (or programmed cell death type I), 3. autophagy (or programmed cell death type II) and 4. mitotic catastrophe. Autophagy is a biological program characterised by degradation of proteins and organelles of cytoplasm and it is involved in both cell survival and cell death, according to the intracellular and extracellular stimuli. On the one hand, autophagy is activated in cells which lack nutrients and promotes their survival under such conditions. On the other hand, the autophagy genes Atg7 and Beclin 1 are necessary for the death of cells in specific cases [161-163]. The regulation of the autophagic pathway depends on multiple mechanisms and pathways, such as the mTOR signaling, the PI3K-I/PKB signaling, GTPases, calcium and protein synthesis [164]. A major regulator of autophagy is the mTOR signal transduction pathway, due to its ability to detect and integrate nutrient and hormonal signals, which also regulate autophagy [165]. It has been strongly suggested that AMPK activation is necessary for the induction of autophagy [166]. The aberrant regulation of autophagy seems to be responsible for a number of diseases, including some types of cancer [167]. The mTOR inhibitors promote the radiation-induced autophagy and this synergistic combination strengthens the therapeutic ratio [168]. In addition to the induction of auto-

phagy, mTOR inhibitors can lead to radiosensitivity *via* another mechanism. In detail, gamma radiation activates the Akt/mTOR pathway, which leads to phosphorylation of 4E-BP1 and release of eIF4E. The latter induces the expression of the radioresistance kinase TLK1B and through this mechanism the activation of the mTOR signaling pathway can induce radioresistance. Therefore, mTOR inhibitors could thwart the sequence of these radioresistance-related events [169]. The (Fig. (2)) depicts in a diagrammatic way the inhibitory effect of mTOR activation on autophagy as well as the autophagy-induced radiosensitivity resulting from mTOR signaling inhibition.

The Present and the Future of the mTOR Signaling Inhibition

The mTOR signaling pathway is of great significance for tumorigenesis and tumor progression and mTOR constitutes a promising target of antitumor treatment, as it mediates survival signals resulting from a number of upstream regulators, such as PI3K and PLD [170]. The mTOR inhibitors constitute a novel member of the antitumor therapeutic arsenal and appear to be effective in the treatment of a number of solid tumors, either alone or in combination with chemotherapeutic or biological agents. The inhibitors of mTOR include rapamycin (sirolimus) and the analogues of rapamycin: temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573). Their clinical evaluation has indicated that these drugs are characterised by high efficacy and a safe toxicity profile [171]. The most disturbing side-effects of these agents that limit the recommended dose are skin rash and

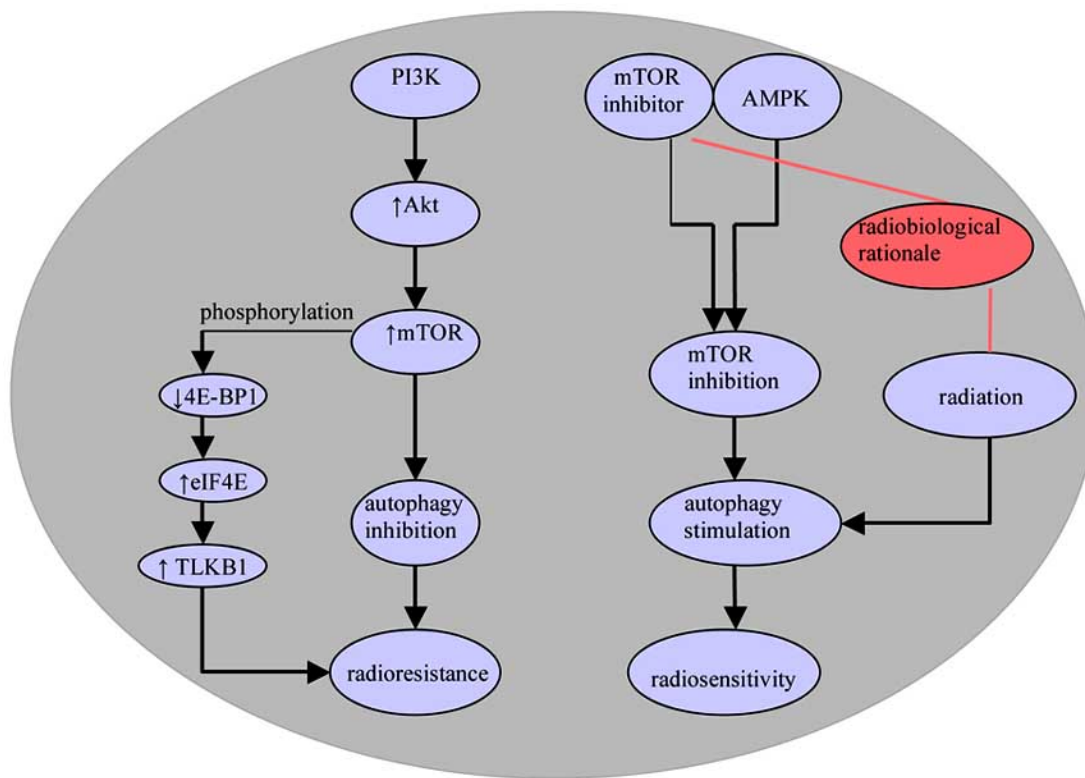


Fig. (2). This figure depicts the potent crosstalk between mTOR signaling and autophagy. The activation of mTOR leads to radioresistance through double mechanism, while mTOR inhibition results in radiosensitivity. The latter reveals the radiobiological synergistic inhibitory effect on tumor growth and could constitute the basis of the radiobiological rationale.

mucositis [172]. The analogues of rapamycin have similar pharmacodynamics and side-effects to those of rapamycin and have attracted a great deal of attention due to their accessibility and tolerability on behalf of patients [173]. Importantly, deforolimus has shown effectiveness against a wide range of tumors and manageable side-effects and it is currently evaluated [174].

The mTOR inhibitors exert their action through two mechanisms: a. a direct action on tumor cells, by inhibiting proliferation and inducing apoptosis and b. an immediate action, exerting a potent antiangiogenic effect, through the down-regulation of the VEGF signal transduction pathway [175]. It has been observed that rapamycin binds an intracellular protein termed FKBP12 and the complex binds mTOR. The mTOR interacts with both the nucleus and cytoplasm and such interplay is necessary for the action of rapamycin [176]. It seems that mTOR inhibitors act through direct inhibition as well as indirect inhibition of mTOR target. Rapamycin is an immunosuppressive agent which acts *via* both the inhibition of protein synthesis and the inhibition of G1/S transition [177]. Rapamycin induces the blockade of G1/S transition by reducing cyclin D1 level through three mechanisms: 1. reduces the formation of the cyclin D1 transcript 2. reduces the stability of the mRNA transcript and 3. promotes the degradation of the synthesized cyclin D1. Thus, the development of Cdk4 complexes is limited [178]. Despite the fact that rapamycin has a major immunosuppressive action, has an even more potent anti-tumor effect. This is depicted by experimental data showing that rapamycin, when combined with cyclosporine, diminishes the incidence of tumors developed in organ transplants in comparison with the respective incidence when cyclosporine is administered alone [179]. The glycogen synthase kinase 3beta has been indicated to enhance the antitumor effect of rapamycin [180]. The mTOR inhibitors normally inhibit the eukaryotic translation initiation factor 4E (eIF4E) by preventing its phosphorylation. Nonetheless, mTOR inhibition leads to phosphorylation and activation of the eIF4E in certain cases. This paradoxical effect seems to be mediated by the Mnk and dependent on PI3K signaling [181]. A number of clinical trials are conducted with the view to optimizing the recommended schedule of the administration of mTOR inhibitors. Specifically, everolimus has been proved effective at a daily dose of 10 mg or at a weekly dose of 50 mg in patients with advanced solid tumors [182]. What is more, a daily dose up to 10 mg or a weekly dose up to 70 mg has been proved both effective and safe [183].

The mTOR inhibitors constitute a novel category of biological agents and, in spite of the encouraging results, their appropriate administration to cancer patients necessitates the elucidation of specific pivotal issues, such as the optimization of the dose-schedule, the possible criteria for the selection of the patients in order for the targeted therapy to be cost-effective as well as the possible synergistic combinations with the view to maximizing the efficacy of the treatment [171]. Unfortunately, we have not yet determined the optimal dosing schedules of such agents and we lack their effectiveness and contribution to antitumor treatment, due to our impotence to effectively determine such parameters [175]. One of the most important difficulties for such a determination is the great complexity of the mTOR signaling

and the particularities required for the design of the appropriate clinical trials [184, 19]. The detailed and consistent insight into mTOR signaling will enable insiders to improve mTOR targeted therapy and further the utility and administration of such agents in clinical setting [185, 186]. The combination of different therapeutic strategies (multi-modality treatment) or the simultaneous administration of different biological agents (multi-targeted therapy), is one of the most promising therapeutic approaches [187]. A molecular interplay of great significance for the establishment of multi-targeted therapy is that between EGFR and mTOR. EGFR regulates to some extent the PI3K/Akt/mTOR pathway and this explicates the reason why the combination of rapamycin and erlotinib enhances the antitumor effect of erlotinib [188]. Interestingly, it has been demonstrated that mTOR inhibitors (temsirolimus) are effective therapeutic agents against squamous cell carcinoma resistant to EGFR inhibitors. Moreover, the simultaneous administration of mTOR and EGFR inhibitors has a synergistic antitumor effect in squamous cell carcinoma sensitive to EGFR inhibitors [189]. In addition, the combination of everolimus with EGFR inhibitors has a synergistic antitumor effect, because everolimus causes a decrease in EGFR-related molecules and VEGF production. More importantly, everolimus partially reverses the resistance of cancer cells to EGFR inhibitors and it is active against EGFR-resistant cancer cells [190]. The combination of gefitinib followed by rapamycin has indicated synergistic antitumor and antiangiogenic effect in pancreatic cancer cell lines [191].

It has been indicated that activated mTOR resulting from TSC mutations enhances the translation of p53 and cells cannot escape genomic damage and apoptosis under stressful conditions as well as in case of nutrient depletion. This interaction between mTOR and p53 may modify to some extent the *in vivo* antitumor action of mTOR inhibitors [192]. The understanding of the mechanism of action of mTOR inhibitors is of utmost importance in order to explicate the mechanism of the emerging resistance and to develop either new mTOR inhibitors or useful therapeutic combinations [193]. A proposed mechanism implicated in the pathogenesis of resistance to mTOR inhibitors is the reactive phosphorylation and activation of Akt or eIF4E through PI3K, resulting from rapamycin administration [194]. Furthermore, mTOR inhibitors could be used in combinations with the view to dealing with the major problem of drug resistance [195]. Moreover, other than rapamycin superfamily drugs could therapeutically modulate the mTOR pathway. A very recent study showed that fluvastatin express antiproliferative activity in renal cell carcinoma cells *in vitro* targeting the Akt/mTOR pathway [196]. One more agent, rosiglitazone has due to mutations or overactivation of upstream pathways. Shown efficacy in NSCLC cell proliferation inhibition *via* up-regulation of the PTEN/AMPK and down-regulation of the Akt/mTOR/p70S6K signal cascades [197]. Curcumin seems to target mTOR and EGFR tyrosine kinases. This inhibition results in tumor suppression [198].

CONCLUSIONS

The mammalian target of rapamycin (mTOR) is involved in a number of signal transduction pathways responsible for

cell growth, cell proliferation, cell survival and angiogenesis. The two main actions that mTOR exerts are transition of the cell cycle from G1 to S phase as well as promotion of protein translation. While in normal cell mTOR controls the load of signals from its effectors, in cancer cell this balance is lost.

Since mTOR has a key position in the cell, which is on the crossroad of various signal pathways towards mRNA, ribosom, protein synthesis and translation of significant molecules has attracted researchers interest in its inhibition as a therapeutic approach for cancer. At the moment, mTOR inhibitors are under evaluation as monotherapy or in combination with other agents in many phase I-III clinical trials in a variety of cancer.

ABBREVIATIONS

AMPK	= Adenosine monophosphate-dependent protein kinase
ASCT2	= Amino acid transporter-2
CCI-779	= Temsirolimus
4EBP1	= 4E binding rotein-1
eIF4E	= Eukaryotic initiation factor 4E
EMT	= Epithelial to mesenchymal transition
FGF-2	= Fibroblast growth factor-2
5-FU	= 5 Fluorouracil
HIF-1	= Hypoxia inducible factor 1
HNSCC	= Head and Neck squamous carcinoma
mTOR	= Mammalian target of Rapamycin
mTORC1	= mTOR Complex 1
mTORC2	= mTOR Complex 2
NSCLC	= Non-small cell lung cancer
PI3K	= Phosphoinositidine 3-kinase
PDGF	= Platelet-derived Growth Factor-D
PLD	= Phospholipase D
PNC1	= Pyrimidine nucleotide carrier
PTEN	= Phosphatase and tensin homologue deleted on chromosome 10.
RAD 001	= Everolimus; Raptor, regulatory associated protein of mTOR
Rheb	= Ras-homolog enriched in brain
S100A4	= S100 calcium-binding protein A4
S6K	= Ribosomal protein 6 kinase
TAMs	= Tumor-associated macrophages
TGF-beta	= Transforming growth factor beta
TSC1	= Tubrous Sclerosis Complex 1: hamartin
TSC2	= Tubrous Sclerosis Complex 2: tuberin
VEGF	= Vascular endothelial growth factor
VEGFR	= Vascular endothelial growth factor Receptor

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