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Role of mTOR Signaling in Tumor Cell Motility, Invasion and Metastasis

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Abstract

Tumor cell migration and invasion play fundamental roles in cancer metastasis. The mammalian target of rapamycin (mTOR), a highly conserved and ubiquitously expressed serine/threonine (Ser/Thr) kinase, is a central regulator of cell growth, proliferation, differentiation and survival. Recent studies have shown that mTOR also plays a critical role in the regulation of tumor cell motility, invasion and cancer metastasis. Current knowledge indicates that mTOR functions as two distinct complexes, mTORC1 and mTORC2. mTORC1 phosphorylates p70 S6 kinase (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), and regulates cell growth, proliferation, survival and motility. mTORC2 phosphorylates Akt, protein kinase C α (PKC α) and the focal adhesion proteins, and controls the activities of the small GTPases (RhoA, Cdc42 and Rac1), and regulates cell survival and the actin cytoskeleton. Here we briefly review recent knowledge of mTOR complexes and the role of mTOR signaling in tumor cell migration and invasion. We also discuss recent efforts about the mechanism by which rapamycin, a specific inhibitor of mTOR, inhibits cell migration, invasion and cancer metastasis.

Keywords

Rapamycin; mTOR; cell motility; invasion; metastasis

1. INTRODUCTION

Cancer metastasis, one of the characteristics of malignant tumors, is the primary cause of death in most cancer patients. The metastatic process consists of a series of sequential, interrelated steps including: tumor cells detachment from the primary tumor and invasion of adjacent, healthy tissue, intrusion into the blood and lymphatic vessels, circulation through the bloodstream (circulating tumor cells) to other sites and tissues in the body, extravasation from the vessel of delivery, and metastasis growth in specific distant organs and building a secondary tumor [1-3]. Many of these steps are dependent on cell motility and invasion, which allow the cells to change position within the tissues. To spread within the tissues, tumor cells use similar migration mechanisms with those that occur in normal or non-neoplastic cells during physiological processes such as embryonic morphogenesis, inflammatory immune responses, wound healing, and angiogenesis [4]. However, different

from the physiological processes of cell migration, the tumor cell migration seems to be activated by a variety of promigratory factors without counteracting stop signals, including autocrine motility factors produced by tumor cells, as well as the soluble factors present at the secondary site [5,6]. Because of this imbalance of signals, cancer cells become continuously migratory and invasive, resulting in tumor expansion across tissue boundaries and the formation of cancer metastasis [6].

Cell migration through tissues results from highly integrated multistep cellular events that are regulated by various signaling molecules, including integrins, Rho family small GTPases, and focal adhesion kinase (FAK) [7-9]. In recent years, more proteins and pathways were identified to be essential for cancer cell motility and invasion, such as phosphatidylinositol 3' kinase (PI3K), protein kinase B/Akt, mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinases (S6K) [10-12]. mTOR is known as a key regulator of cell growth, proliferation, differentiation and survival [13,14]. The mTOR pathway regulates several processes, including autophagy, ribosome biogenesis and metabolism by integrating signals from growth factors, nutrients, oxygen and energy status [14]. Increasing evidence suggests that mTOR pathway also plays an essential role in the regulation of tumor cell motility, invasion and cancer metastasis [11,15,16]. Recent work identified two structurally and functionally distinct mTOR-containing multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2. The two complexes consist of unique mTOR-interacting proteins which determine their substrate specificity. mTORC1 regulates cell growth, proliferation and survival by promoting anabolic processes, such as protein synthesis, and by limiting catabolic processes such as autophagy [17,18]. mTORC2 controls the actin cytoskeleton via mechanisms involving the small GTPases Rho and Rac, and by promoting protein kinase C α (PKC α) phosphorylation [19,20]. Here, we briefly discuss the insights of mTOR complexes and highlight their roles in the regulation of tumor cell motility, invasion and cancer metastasis. In addition, we summarize recent findings regarding the principal mechanisms by which rapamycin inhibits cell migration, invasion and cancer metastasis.

2. MTOR STRUCTURE AND SIGNALING COMPLEXES

2.1. mTOR Structure

mTOR, also known as FRAP (FKBP12-rapamycin-associated protein), RAFT1 (rapamycin and FKBP12 target), RAPT 1 (rapamycin target 1), or SEP (sirolimus effector protein), is a highly conserved and ubiquitously expressed Ser/Thr kinase [21-24]. mTOR and yeast TOR proteins share > 65% identity in carboxy-terminal catalytic domains and > 40% identity in overall sequence [25]. mTOR belongs to the PI3K-kinase-related kinase (PIKK) superfamily since the C-terminus of mTOR shares strong homology to the catalytic domain of PI3K [26,27]. The different members of this family, such as MEC1, TEL1, RAD3, MEI-41, DNA-PK, ATM, ATR, and TRRAP, are associated with diverse cellular functions, such as control of cell growth, cell cycle and DNA damage checkpoints, recombination and maintenance of telomere length [28-30]. Structurally, mTOR contains 2549 amino acids and its domain structure is depicted in Fig. (1). The N-terminus of mTOR possesses up to 20 tandemly repeated HEAT motifs including Huntingtin, elongation factor 3 (EF3), A subunit of protein phosphatase 2A (PP2A), and TOR. The C-terminus consists of the FAT (FRAP, ATM, and TRRAP, all PIKK family members) domain, the FRB (FKBP12-rapamycin binding) domain which is a unique feature of mTOR, a catalytic kinase domain, a putative auto-inhibitory domain (repressor domain, RD), and a FAT carboxy-terminal (FATC) domain [13,31]. It is speculated that tandem HEAT repeats may form an extended superhelical structure to create multiple large interfaces for protein-protein interaction, the FRB domain serves as the high-affinity binding site for the FKBP12/rapamycin complex, and the FAT and FATC domains may modulate catalytic kinase activity of mTOR [32,33].

2.2. mTOR Signaling Complexes

2.2.1. mTORC1—In mammalian cells, mTOR functions as two physically and functionally distinct signaling complexes, mTOR complex 1 and 2 (mTORC1 and mTORC2), which are evolutionarily conserved from yeast to mammals. These two complexes consist of unique mTOR-interacting proteins that determine their substrate specificity. The subunit compositions, substrates and signaling functions of mTORC1 and mTORC2 are shown in Fig. (2). mTORC1, which consists of mTOR, raptor (regulatory associated protein of mTOR), mLST8 (also termed G-protein β -subunit-like protein, G β L, a yeast homolog of LST8), and two negative regulators, PRAS40 (proline-rich Akt substrate 40 kDa) and DEPTOR, is rapamycin and nutrient-sensitive [34-37]. Raptor is proposed to interact with mTOR through shared HEAT repeats and functions as an essential scaffold protein for mTOR Fig. (1) [31]. mLST8 was speculated to bind to the kinase domain of mTOR and positively regulate the mTOR kinase activity, but its precise role in mTORC1 function has yet to be defined Fig. (1) [35]. PRAS40 and DEPTOR have been identified as distinct negative regulators of mTORC1 [37,38]. It has been described that PRAS40 binds to raptor [36], and DEPTOR binds to the FAT domain of mTOR [37]. The main function of mTORC1 is to regulate cell growth, proliferation and survival by phosphorylation of its downstream effector molecules, S6K1 and 4E-BP1. mTORC1 responds to mitogen, energy and nutrient signals in part through the upstream regulators tuberous sclerosis complex 1/2 (TSC1/2) and Rheb [18].

In mammals, S6K1 and 4E-BP1 are two best characterized downstream targets of mTORC1 Fig. (2). Mammalian cells possess two similar S6 kinase proteins, S6K1 and S6K2 [39,40]. It was demonstrated that both the activation of S6K1 and S6K2 are regulated by mTORC1 [41,42]. S6K1 is ubiquitously expressed and plays a critical role in the control of cell growth and cell motility [10,43]. Among the phosphorylation sites of S6K1, Thr229, Ser371 and Thr389 appear essential for S6K1 activation [44-46]. Thr229, which is located in the activation loop of the kinase domain, can be phosphorylated by the “loop kinase” PDK1 [47,48]. Thr389, the major rapamycin-sensitive site, can be directly phosphorylated by mTOR *in vitro* [49,50].

S6K1 is known as the major ribosomal protein S6 (rpS6) kinase in mammalian cells and is considered to be a key player in the control of cell growth (cell size) and proliferation [51-53]. Early studies showed that inhibition of S6K either by rapamycin or a dominant-interfering myc-p70^{s6k} A229 construct selectively suppressed mitogen-induced translation of 5' terminal oligopyrimidine (TOP) mRNA, the most notably mRNAs encoding ribosomal proteins and elongation factors [54,55]. However, this mode of regulation has been challenged by the findings that neither S6K1 activity nor rpS6 phosphorylation is required for the translational regulation of TOP mRNAs [56,57]. Further studies have demonstrated that mTOR and S6K1 control on and off the eukaryotic initiation factor 3 (eIF3) translation initiation complex in a growth factor- and rapamycin-sensitive manner [58]. When inactive, S6K1 associates with the eIF3 complex, while the S6K1 activator mTOR/raptor does not. Stimulation of insulin or amino acid promotes mTOR/raptor binding to eIF3 complex and the phosphorylation of S6K1, which results in the dissociation of S6K1 from the eIF3 complex and the fully activation. Activated S6K1 then phosphorylates its translational targets, including the 40S ribosomal protein S6 and eIF4B, promoting translation initiation [58].

4E-BP1 is the other well characterized downstream of mTORC1. In mammals, 4E-BPs, a family of translational repressor proteins, consist of three low molecular weight proteins, 4E-BP1, 4E-BP2, and 4E-BP3 [59-61]. 4E-BP1 (also known as PHAS-I) was first identified as an adipocyte protein that underwent phosphorylation at Ser64 by MAP kinase in response to insulin treatment [59,60]. Further studies showed that mTOR and ATM are involved in

phosphorylation of 4E-BP1 as well [62-65]. Immunoprecipitated mTOR phosphorylates 4E-BP1 on Thr37 and Thr46 *in vitro*, indicating that mTOR can directly phosphorylate these two sites [62,64,66]. In addition, mTOR plays a critical regulatory role in the phosphorylation of Ser65 and Thr70, since these residues display a higher degree of rapamycin sensitivity than Thr37 and Thr46 [32,62]. In its non-phosphorylated state, 4E-BP1 binds tightly to the cap-binding protein eIF4E and represses cap-dependent mRNA translation by blocking the interaction of eIF4E with the eIF4G protein [67]. In response to sufficient growth factors and nutrients stimulation, six sites (Thr37, Thr46, Ser65, Thr70, Ser83 and Ser112) of 4E-BP1 can be phosphorylated [68]. The phosphorylation of 4E-BP1 at multiple site releases eIF4E to restore cap-dependent translation, which is critical for the translation of mRNAs with highly structured 5' untranslated regions (UTRs) [67].

2.2.2. mTORC2—Like mTORC1, mTOR and mLST8 are also present in mTORC2. But instead of raptor, rictor (rapamycin-insensitive companion of mTOR) and mSin1 (mammalian stress-activated protein kinase (SAPK)-interacting protein 1) are two unique subunits of mTORC2 [20,69,70] Fig. (2). In addition, PROTOR (protein observed with rictor; also named PRR5, proline-rich protein 5), DEPTOR, and Hsp70 are other novel components of mTORC2 [37,71-73]. Rictor interacts with the HEAT motifs of mTOR [74]. It was assumed that rictor and mSin1 stabilize each other via binding, building the structural foundation of mTORC2 Fig. (1) [31,70,74]. In addition, rictor interacts with PROTOR, but the role of this interaction is unclear [71]. Compared to mTORC1, mTORC2 has a distinctive physical structure and physiological functions. mTORC2 was originally thought to be rapamycin-insensitive [69]. However, recent studies showed that prolonged rapamycin treatment inhibited the assembly and functions of mTORC2 in some cell lines as well [75]. One of the functions of mTORC2 is to regulate the actin cytoskeleton by mediating the PKC α phosphorylation state [20]. mTORC2 also directly phosphorylates Akt on Ser473, adding a new insight into the role of mTOR in cancer [76]. Akt, also known as protein kinase B, belongs to the AGC kinase family, which also includes S6Ks, glucocorticoid-induced protein kinases (SGKs), p90 ribosomal protein S6 kinases (RSKs), and PKCs. Akt was known to be a key regulator of signal transduction processes that control several cellular functions, such as nutrient metabolism, cell survival and motility [77,78]. Most recently, SGK1, a member of the AGC family, was identified as a novel substrate of mTORC2 [79-81]. In MEFs lacking mTORC2 subunits rictor, mSin1 or mLST8, both the hydrophobic motif phosphorylation and activity of SGK1 are abolished [79]. Moreover, immunoprecipitated mTORC2, but not mTORC1, can phosphorylate SGK1 at Ser422 *in vitro*, further confirming that mTORC2 regulates SGK1 [79]. SGK1 is crucial for cell survival and proliferation [82,83]. However, currently little is known about the role of SGK1 in cell motility and invasion, as well as cancer metastasis.

3. mTOR INHIBITORS

So far, rapamycin (sirolimus, Rapamune, Wyeth Ayerst Laboratories, Philadelphia, PA) and its analogs are the most well studied mTOR inhibitors (Table 1) [84]. Rapamycin was first isolated from a soil sample of Easter Island (Rapa Nui) during a discovery program for antimicrobial agents in the early 1970s, and subsequently discovered to have equally potent immunosuppressive and anti-tumor activities [85-89]. As an immunosuppressive drug, rapamycin (rapamune, sirolimus) was approved by the Food and Drug Administration (FDA) in USA in 1999 for prevention of renal allograft rejection [90]. Subsequent studies from many laboratories found that rapamycin can also act as a cytostatic agent, slowing or arresting growth of cell lines derived from different tumor types [91-95]. In the early 1990s, during the yeast genetic screens for mutations that rescue the growth-inhibitory properties of rapamycin, two target genes of rapamycin named TOR1 (the target of rapamycin 1) and TOR2 were identified [96,97]. Further studies revealed the action mechanism by which

rapamycin inhibits mTOR signaling. Upon entering the cells, rapamycin forms a complex with an intracellular receptor, FKBP12 (FK506-binding protein 12 kDa), and then binds to a region in the C terminus of TOR proteins termed FRB (FKB12-rapamycin binding) domain, thereby exerting its cell growth-inhibitory and cytotoxic effects by inhibiting the functions of TOR [98,99]. Subsequent biochemical studies extended this model to mammalian cells, leading to the discovery of the mTOR [21,23,24,100]. *Rapamycin* has also shown potent antitumor effects in several experimental tumor models, but the poor aqueous solubility and chemical stability restricts its clinical development as an anticancer agent. Therefore, several rapamycin analogs (termed rapalogs) with more favorable pharmaceutical characteristics have been developed, such as CCI-779 (Temozolamide, Wyeth, Madison, NJ, USA), RAD001 (Everolimus, Novartis, Novartis, Basel, Switzerland), AP23573 (Deforolimus, ARIAD, Cambridge, MA, USA), 32-deoxorapamycin (SAR943) or zotarolimus (ABT-578, Abbott Laboratories, Abbott Park, IL, USA). Like rapamycin, rapalogs form a complex with the intracellular receptor FKBP12, resulting in potent inhibition on mTORC1 signaling. CCI-779, RAD001 and AP23573, which are currently under clinical trials as anticancer agents, have shown anti-tumor effects against a diverse range of cancer types in preclinical studies [101-103]. Clinical studies have demonstrated promising results of these rapalogs in a subset of cancers [104-106]. As such, Temozolamide was approved by the FDA for advanced renal carcinoma treatment in May 2007.

A class of small molecules related to mTOR kinase inhibition is the mTOR and PI3K dual-specificity inhibitors. These molecules, which include GNE-477, NVP-BEZ235, PI-103 and XL765, simultaneously target the ATP binding sites of mTOR and PI3K with similar potency (Table 1) [107-110]. These inhibitors cannot be used to selectively inhibit mTOR-specific activities in cells, but they may have unique advantages for the treatment of particular cancer because they can target at least three key enzymes in the PI3K signaling pathway. For example, inhibition of mTORC1 activity alone may result in the enhanced activation of the PI3K axis because of the mTOR-p70S6K-IRS1 negative feedback loop [111]. Therefore, the mTOR and PI3K dual-specificity inhibitors, such as NVP-BEZ235, might be sufficient to avoid PI3K pathway reactivation.

Recently, a new generation of mTOR inhibitors, which binds to the catalytic site of mTOR and inhibits the kinase activity of mTORC1 and mTORC2, is being developed (Table 1) [112-115]. Since Akt was demonstrated to be a key target of mTORC2, inhibition of mTORC2 would effectively minimize the feedback activation of PI3K and suppress the activation of Akt, finally preventing malignant progression of tumors. The active-site inhibitor of mTOR, PP242, which suppresses mTORC2 and blocks the phosphorylation of Akt at Ser473, inhibited proliferation of primary MEFs more efficiently than rapamycin [113]. Torin1, another selective ATP-competitive mTOR inhibitor that directly inhibited both mTORC1 and mTORC2, inhibited cell growth and proliferation more completely than rapamycin as well [112].

In addition, several preliminary studies showed that some diet-derived chemopreventive agents (e.g. curcumin, resveratrol, 3, 3-diindolylmethane, epigallocatechin gallate, or genistein) may inhibit mTOR signaling directly or indirectly (Table 1) [116-122]. For example, curcumin, a polyphenol natural product isolated from the rhizome of the plant *Curcuma longa*, showed effectiveness as a chemopreventive agent in animal models of carcinogenesis and is undergoing early clinical trials as a novel anti-cancer drug [123]. Our studies showed that curcumin inhibited cell growth, induced apoptosis and inhibited the basal or IGF-I-induced motility of rhabdomyosarcoma cells [124]. In numerous cancer cell lines, curcumin inhibited phosphorylation of mTOR and its downstream targets, S6K1 and 4E-BP1, suggesting that curcumin may execute its anticancer activity primarily by blocking mTOR mediated signaling pathways [124,125]. Most recently, we further found that

curcumin was able to dissociate raptor from mTOR, leading to inhibition of mTORC1 activity [116].

4. THE ROLE OF MTOR SIGNALING PATHWAY IN CELL MOTILITY AND INVASION

Early studies in the budding yeast *Saccharomyces cerevisiae* have demonstrated that two TORs, participates in the control of many growth-related processes, such as translational initiation and early G1 progression, in response to nutrient availability and favorable environmental conditions [126,127]. Recent work has identified additional functions of TOR, including the regulation of transcription, cytoskeletal organization, cell motility and protein degradation through autophagy [128-130]. The initial characterization of mTORC2 led to discovery of the participation of mTORC2 signaling in cytoskeletal events and cell movement [20, 69, 131]. Recently, more studies revealed that rapamycin-sensitive mTORC1 also plays a crucial role in the regulation of cell motility and invasion [10, 12, 132, 133].

4.1. mTORC1 Signaling in Cell Motility and Invasion

Increasing evidence demonstrated that both mTORC1-mediated S6K1 and 4E-BP1 pathways are involved in the regulation of cell motility and invasion Fig. (3) [10,12,134]. In several cell lines, such as porcine, murine and human aortic smooth muscle cells [133,135,136], canine kidney epithelial cells (MDCKT23) and human colorectal cells (HCT-8/S11) [137], Swiss 3T3 fibroblasts [10], neutrophils [138], ovarian cells [139], human umbilical vein endothelial cells [140], human rhabdomyosarcoma (Rh1 and Rh30), breast carcinoma (MDA-MB-468), cervical adenocarcinoma (HeLa), and SV40 transformed African green monkey kidney fibroblast (COS-1) cells [12], rapamycin showed inhibitory effect on cell motility or invasion.

In Swiss 3T3 fibroblasts, pretreatment of cells with rapamycin inhibited thrombin-induced S6K activity and stress fiber formation, supporting a role for S6K in thrombin-stimulated stress fiber formation [141]. Subsequent study examined the interaction between S6K and actin cytoskeleton, and determined whether S6K pathway plays a role in regulating cell migration. Berven *et al.* showed that S6K, Akt1, PDK1, p85 PI3K and activated mTOR were localized to the actin arc, which is a caveolin-enriched cytoskeletal structure located at the leading edge of migrating Swiss 3T3 cells [10]. Rapamycin treatment blocked epidermal growth factor (EGF)-induced actin arc formation, which is a functional indicator of cell migration. In transwell migration assay, rapamycin effectively suppressed EGF-stimulated cell migration as well, suggesting that activation of the mTOR-S6K signaling pathway is important to cell migration [10]. In either granulocyte-macrophage colony-stimulating factor (GM-CSF)- or IL-8-treated neutrophil, pre-incubation with rapamycin strongly inhibited chemotaxis, chemokinesis and actin polymerization in these cells [138]. Consistently, rapamycin inhibited both the increase of enzymatic activity and the phosphorylation of S6K1 which were induced by GM-CSF. However, FK506, a structural analog of rapamycin and an FKBP12-binding compound that lacks the ability to inhibit mTOR, did not have significant effect on chemotaxis and actin polymerization, suggesting that the effect of rapamycin on neutrophil migration was directly through the mTOR/S6K pathway [138]. Moreover, Sakakibara *et al.* showed that rapamycin markedly diminished chemotaxis of a human arterial smooth muscle cell line E47 cells and rat aortic vascular smooth muscle cells (SMCs) toward the matrix protein fibronectin as well as type I collagen and laminin [133]. Pretreatment of rapamycin completely inhibited fibronectin-induced mTOR and S6K1 phosphorylation, indicating that mTOR and S6K1 play an important role in the rapamycin-sensitive signaling pathway which is responsible for fibronectin-induced migration [133]. The critical role of mTOR-S6K pathway in cell motility and invasion were further explored

in other cell types. Zhou *et al.* showed that hepatocyte growth factor (HGF) selectively induced cell motility and invasive response in human ovarian cancer cells with high levels of the Met receptor [142]. Expression of constitutively active forms of S6K duplicated the motile response and invasive effects of HGF, indicating a crucial role for S6K signaling in the regulation of invasion and cell motility [142]. Furthermore, pretreatment with mTOR inhibitor rapamycin, MAPK inhibitor PD98059 or pan-PKC inhibitor GF109203X, only rapamycin significantly inhibited cell scattering in SKOV-3 and CaOV-3 cells, indicating that mTOR, but not the extracellular signal-regulated kinase 1/2 (Erk1/2) and PKC activity, was required for HGF to activate S6K and finally induce cell migration and invasion in these cells [142]. Notably, this study revealed that the mechanism by which S6K promotes cell migration and invasion may through increased expression and proteolytic activities of MMP-9, a zinc-dependent endopeptidase which plays an important role in the proteolytic destruction of extracellular matrix and basement membranes, and is essential for tumor invasion and metastasis [142]. Consistently, a recent study showed that activated PI3K-Akt-mTOR signaling pathway promotes invasion and metastasis in hepatocellular carcinoma through up-regulation of MMP-9 [11]. Evidence for involvement of mTORC1-S6K signaling in cell motility is further supported by our studies, which showed that rapamycin inhibited IGF-induced motility in a panel of cell lines, and this inhibitory effect is dependent on mTOR kinase activity [12]. In Rh30 cells, expression of mTOR^{rr} (rapamycin resistant mutant of mTOR), but not mTOR-SIDA (kinase-dead mTOR^{rr}), prevented the inhibition of rapamycin on S6K1 and 4E-BP1 phosphorylation, as well as the cell motility. In the single cell motility assay, downregulation of S6K1 by using lentiviral shRNA effectively inhibited IGF-I-stimulated cell motility, and cells expressing constitutively active S6K1 are resistant to rapamycin inhibition of IGF-I-stimulated cell motility. Similarly, ectopic expression of constitutively hypophosphorylated 4E-BP1 dramatically inhibited IGF-I-stimulated cell motility, while downregulation of 4E-BP1 by shRNA attenuated rapamycin inhibition of cell motility. The data strongly suggest that the inhibition of rapamycin on IGF-induced motility may through the suppression of mTORC1-mediated S6K1 and 4E-BP1/eIF4E-signaling pathways [12]. Furthermore, we observed that rapamycin inhibited IGF-I-induced F-actin reorganization and phosphorylation of the focal adhesion proteins by disruption of mTORC1 complex [132]. Both S6K1 and 4E-BP1 pathways, mediated by the mTORC1, are involved in the regulation of IGF-I-stimulated F-actin reorganization, whereas only S6K1 pathway controls IGF-I-stimulated phosphorylation of the focal adhesion proteins [132]. These results suggest that S6K1 regulates cell motility, probably related to its regulation on phosphorylation of the focal adhesion proteins, such as FAK, paxillin and p130^{Cas}, and F-actin reorganization (or lamellipodia formation) Fig. (3) [132]. One recent study on epithelial to mesenchymal transition (EMT) showed that increased cell size and protein synthesis induced by TGF- β during EMT is correlated with the activation of mTOR and phosphorylation of its effectors S6K1 and 4E-BP1 through the PI3K-Akt pathway [134]. Inhibition of mTOR by rapamycin blocked TGF- β -induced increases in cell size and protein synthesis, as well as inhibited the invasive behavior that accompanies EMT, suggesting that mTORC1 plays a role in defining the migration and invasion of cells that have undergone EMT in response to TGF- β [134]. One recent study also revealed the mechanisms by which mTOR regulates human trophoblast invasion [15]. Busch *et al.* showed that rapamycin exposure or siRNA-mediated silencing of mTOR protein expression in human trophoblastic HTR-8/SVneo cells lead to lower invasion *in vitro* [15]. Meanwhile, knockdown of mTOR expression, which leads to the lack of properly assembled and functional mTORC1, resulted in the abrogation of MMP-2, -9, uPA, PAI-1 activity and STAT3 serine phosphorylation [15]. The data suggest that mTOR signaling is involved in a tightly regulated network of intracellular signal pathways including the JAK/STAT signaling and contributes to the invasiveness of human trophoblast cells by regulation of matrix-remodeling enzymes such as MMP-2, -9, uPA and PAI-1.

The metastatic process consists of a series of sequential, interrelated steps. Acquisition of a motile and invasive phenotype is one requirement for a cell to obtain metastatic competence [143]. Besides Akt, mTOR, and FAK, the small GTPases (RhoA, Rac1 and Cdc42), PP2A, Src, and Erk1/2 have also been reported to be involved in this process Fig. (3) [144-149]. For example, activation of Erk1/2 by IGF-I is associated with mitogenesis and cell motility and invasion [144,145]. Inhibition of PP2A activity promotes motility in a number of transformed cells and cancer cell lines [146,147]. mTORC1 mediates phosphorylation of Erk1/2 (Thr202) through direct or indirect regulation of PP2A [150]. Our recent studies further demonstrated that treatment of the cells with rapamycin activated PP2A activity, and concurrently inhibited IGF-I stimulated phosphorylation of Erk1/2 [151]. Inhibition of Erk1/2 with PD98059 did not significantly affect the basal mobility of the cells, but dramatically inhibited IGF-I-induced cell motility. Furthermore, inhibition of PP2A with okadaic acid significantly attenuated the inhibitory effect of rapamycin on IGF-I-stimulated phosphorylation of Erk1/2 as well as cell motility [151]. Consistent with this data, expression of dominant negative PP2A conferred resistance to IGF-I-stimulated phosphorylation of Erk1/2 and cell motility. Expression of constitutively active MKK1 also attenuated rapamycin inhibition of IGF-I-stimulated phosphorylation of Erk1/2 and cell motility [151]. These results suggest that rapamycin inhibits cell motility, in part by targeting PP2A-Erk1/2 pathway.

4.2. mTORC2 Signaling in Cell Motility and Invasion

Early studies on TOR established a role for TORC2 in the actin cytoskeleton rearrangement [130,152,153]. In yeast, deletion of TOR2 disrupted the polarized organization of the actin cytoskeleton, indicating TOR2 is required for organization of the actin cytoskeleton [130]. Further study showed that TOR2 activated Rho1 and Rho2 via their exchange factor ROM2, and the activation of the Rho1 GTPase pathway was required for TOR2-mediated signaling to the actin cytoskeleton [153]. However, the actual mechanism by which TORC2 regulates the Rho1 GTPase pathway is not well understood but might involve Slm1/2 and YPK2, two recently identified substrates of TORC2 [154-156]. Several studies have shown that the members of the Rho family (Rho, Rac and Cdc42) play important roles in the regulation of cell motility, invasion and thus metastasis [143,157-159]. Recent findings demonstrated that like yeast TORC2, mTORC2 also seems to function upstream of Rho GTPases to regulate the actin cytoskeleton Fig. (3) [69]. In mTOR, mLST8 or rictor siRNA-transfected cells, expression of constitutively active form of Rac (Rac1-L61) or Rho (RhoA-L63) restored organization of the actin cytoskeleton [69]. Knockdown of mTOR, rictor or mLST8 in serum-starved NIH 3T3 fibroblast cells resulted in defective F-actin fibres (stress fibres and/or lamellipodia) formation in response to serum, whereas knockdown of raptor did not prevent actin polymerization and cell spreading, indicating that mTORC2, but not mTORC1, may signal to the actin cytoskeleton through Rho and Rac [69]. Previously, the molecular mechanism by which mTORC2 mediates the activation of Rho GTPases and cytoskeletal events is not well understood. However, a recent study provided a novel mechanism by which mTORC2 can control the actin cytoskeleton through the activation of Rho GTPases. Hernandez-Negrete *et al.* demonstrated that P-Rex1, a Rac guanine exchange factor connecting G protein-coupled receptors through GβL and PI3K signaling to Rac activation, links mTOR2 signaling to Rac activation, leading to the regulation of actin cytoskeleton and cell migration [160]. P-Rex family of Rac guanine exchange factors, including P-Rex1, P-Rex2a, and P-Rex2b, are able to interact directly with mTOR through their tandem DEP domains [160]. Dominant-negative constructs and short hairpin RNA-mediated knockdown of P-Rex1 decreased leucine-induced mTOR-dependent activation of Rac and cell migration, indicating that both P-Rex1 and mTOR are required for Rac activation and cell migration induced by leucine [160]. However, further studies are needed

to elucidate whether P-Rex1 and mTOR are involved in part of the same signaling pathway or act through independent pathways.

PKC α is also involved in the regulation of mTORC2 on the organization of the actin cytoskeleton and cell motility Fig. (3) [20,131]. In HeLa cells, knockdown of mTOR or rictor leads to increased F-actin bundles concomitant with increased cytoplasmic paxillin patches that colocalize to the ends of the thick actin fibres [20]. Meanwhile, the reduction in rictor expression decreased the phosphorylation of Ser657 of PKC α as well. In PKC α silencing cells, the thick actin fibres appear more numerous, better organized and connected to the remainder of the cytoskeleton [20]. Masri *et al.* showed that overexpression of rictor resulted in enhanced activities of both mTORC2 and PKC α , and promoted glioma cell proliferation, migration, and invasiveness [131]. The data indicate that mTORC2 regulates the organization of the actin cytoskeleton and that PKC α is a mediator of this function.

Akt also function as a suppressor of tumor cell migration and invasion [161]. Further studies indicate that Akt isoforms, Akt1 and Akt2, have distinct roles in controlling growth factor-stimulated migration and invasion in breast epithelial cells [162]. Irie *et al.* reported that in the non-transformed immortalized breast epithelial cell line MCF10A expressing the IGF-I-receptor (IGF-IR), Akt1 down-regulation enhanced cell migration induced by IGF-I or EGF [162]. Moreover, downregulation of Akt2 reversed the increased migration induced by Akt1 down-regulation [162]. A previous study on Akt2 and migration, showing that overexpression of Akt2 leads to increased invasion and metastasis of human breast and ovarian cancer cells, is consistent with this finding [163]. It has been speculated that the effects of Akt on cell motility and invasion seem to be cell and tissue specific [79]. More studies are needed to explore the underlying mechanisms mediating the effects of Akt on cell motility in different cell types.

In addition, mTORC2 may regulate cell motility by regulation of phosphorylation of the focal adhesion proteins [20,69,132], which are critical for cell adhesion and migration [164]. Disruption of mTORC2 reduced IGF-I-induced phosphorylation of FAK, paxillin and p130^{cas}, as well as F-actin reorganization and cell motility [132]. In rictor-null MEFs, the phosphorylation of paxillin Tyr118 is dramatically reduced and the actin cytoskeleton is defected [69,164]. Currently it is unknown how mTORC2 regulates the phosphorylation of the focal adhesion proteins.

5. RAPAMYCIN AND TUMOR METASTASES

When *in vitro*, rapamycin is effective in blocking the cell growth, migration and invasion of a number of tumor cell lines. When *in vivo*, rapamycin also potently inhibits metastases of transplanted tumors in different mouse models. Guba *et al.* showed that rapamycin inhibited metastatic tumor growth and angiogenesis in *in vivo* mouse models [165]. In BALB/c mice, intraportal injection of syngenic CT-26 adenocarcinoma cells stimulated metastasis of colon cancer to the liver, which was markedly reduced by rapamycin treatment [165]. In addition, the multiple quantitative analyses on the tumors in dorsal skin-fold chambers showed that rapamycin-treated mice had a smaller vascularized tumor area and lower vascular density than control mice, suggesting that rapamycin can suppress tumor growth by inhibiting angiogenesis [165]. It was also suggested in this study that the antiangiogenic effect of rapamycin is related to reduced translational production of vascular endothelial growth factor (VEGF) and blockage of VEGF-induced human umbilicalcord vein endothelial cell (HUVEC) tubular formation [165]. In the human renal cell cancer (RCC) pulmonary metastasis models, rapamycin prevented tumor growth and pulmonary metastasis, and its antitumor efficacy was realized by cell cycle arrest and targeted reduction of tumor promoting cytokines VEGF-A and TGF- β 1 [166,167]. In non-small cell lung cancer cell

(KLN-205) model, rapamycin is also highly efficacious in preventing the formation and development of pulmonary metastases [168]. Ezrin, which is a substrate of tyrosine kinases and binds adhesion molecules such as CD43, CD44, and intercellular adhesion molecule-1, is believed to be involved in intracellular signal transduction related to cell migration and metastasis [169-171]. mTORC1-mediated S6K1 and 4E-BP1 pathways were found to be related to ezrin related metastatic behavior [16]. Wan *et al.* showed that reduction of ezrin expression by RNAi or disruption of ezrin function by transfection of a dominant-negative ezrin-T567A mutant resulted in decreased phosphorylation of both S6K1 and 4E-BP1. In K7M2 cells, rapamycin treatment inhibited mTORC1-mediated phosphorylation of S6K1 and 4E-BP1, leading to decreased cell migration and invasion [16]. In tumor-inoculated SCID beige mice, rapamycin or its analog CCI-779 led to significant inhibition of experimental lung metastasis [16]. These results suggest that suppression of mTORC1/S6K1/4E-BP1 pathway may be a potential strategy to reduce cancer metastasis [16].

Recently, the effect of rapamycin on lymphangiogenesis and lymph node metastasis were investigated by using an experimental metastatic model in nude mice [172,173]. The results showed that rapamycin significantly decreased the number and the area of lymphatic vessels in the primary tumors, and finally reduced the lymph node metastasis in nude mice [173]. Several studies have provided evidence that the VEGF-C/VEGFR-3 signal transduction pathway plays a causal role in tumor associated lymphangiogenesis and lymphatic metastasis [174-176]. Under serum-starved or normal culture condition, rapamycin repressed protein and mRNA expressions of VEGF-C in B13LM cells, indicating that rapamycin inhibits lymphangiogenesis probably by inhibition of VEGF-C expression [173]. In lymphatic endothelial cells, rapamycin suppressed downstream signaling of VEGF-A and VEGF-C through mTOR-S6K1 pathway as well [172]. In another study performed in lymphatic endothelial cells, blockade of Akt or mTOR/S6K signaling pathway inhibited the formation of tubes induced by fibroblast growth factor-2 (FGF-2), further suggesting involvement of mTORC1/2 pathways in lymphangiogenesis [177]. Recently, Hammer *et al.* reported that IL-20 stimulates tube formation in lymphatic endothelial cells (hTERT-HDLEC) in a PI3K- and mTOR-dependent way [178]. In addition, IL-20 induced phosphorylation of Akt and endothelial nitric oxide synthase (eNOS), as well as increased intracellular free calcium concentration and perinuclear nitric oxide (NO) production [179]. However, whether mTOR regulates lymphangiogenesis through calcium/NO signaling remains to be defined. Most recent studies showed that rapamycin treatment decreased formation of metastatic nodules in the lung by B16 cells [180]. This potential anti-metastatic effect of rapamycin is due to its down-regulation of α_v integrin expression and subsequently the phosphorylation of FAK. The up-regulation of apoptosis signaling by rapamycin is also involved in its anti-metastatic effect [180]. The data further suggest that rapamycin might be worthy of clinical evaluations as an anti-metastatic agent.

6. SUMMARY

Cell migration is a highly orchestrated multi-step process, and several signaling pathway and proteins have been found to be essential in this process. mTOR, which functions as two distinct multiprotein complexes, mTORC1 and mTORC2, is known as a central controller of cell growth, proliferation, differentiation and survival. Increasing evidence demonstrates the participation of both mTORC1 and mTORC2 signaling in the regulation of cell motility, invasion and cancer metastasis. mTORC1 phosphorylates S6K1 and 4E-BP1, and regulates cell growth, proliferation, migration and invasion. mTORC2 regulates the actin cytoskeleton via mechanisms involving the small GTPases, focal adhesion proteins, as well as PKC α . However, several questions still remain obscure, such as whether and how other downstream effector molecules of mTOR, such as Akt and SGK, are involved in the regulation of cell motility and invasion; and how mTOR interacts with other signaling pathways or proteins,

leading to the regulation of cell migration and invasion; and how mTOR regulates cell attachment/de-attachment. Obviously, more studies are required to elucidate these questions, which may enhance our understanding of the molecular mechanisms by which metastases are generated, and may provide novel therapeutic strategies to reduce metastatic spread of tumor cells, block metastatic progression and increase patient survival.

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Fig. (1).

Schematic structure of mTOR and its interacting proteins. N-terminus of mTOR contains two tandemly repeated HEAT motifs. Downstream of the HEAT repeat region lies a FAT domain, an FRB domain, a catalytic kinase domain (KD), an auto-inhibitory (repressor domain, RD), and a FATC domain which is located at the C-terminus of the protein. Within mTORC1, raptor interacts with mTOR through shared HEAT repeats and functions as an essential scaffold protein for mTOR. Within mTORC2, rictor and mSin1 stabilize each other via binding, building the structural foundation of mTORC2. mLST8 was speculated to bind to the kinase domain of mTOR. DEPTOR binds to FAT domain and functions as a negative regulator of both mTORC1 and mTORC2.

Fig. (2).

Summary of mTOR signaling complexes, their known effector molecules and main functions. mTOR functions as two distinct signaling complexes, mTORC1 and mTORC2. mTORC1 contains mTOR, raptor, PRAS40 and mLST8; mTORC2 consists of mTOR, rictor, mSin1, protor and mLST8. Both mTORC1 and mTORC2 interact with a negative regulator DEPTOR. Their major effector molecules and functions are shown.

Fig. (3).

Overview of mTOR-mediated signaling pathways in tumor cell motility, invasion and cancer metastasis. mTORC1-mediated S6K1, 4E-BP1, PP2A-Erk1/2 pathways are involved in the regulation of cell motility and/or invasion. mTORC2 regulates the actin organization and cell motility by activating PKC α , GTPases, and/or focal adhesion proteins, such as FAK, p130^{Cas} and paxillin. Arrows represent activation, whereas bars represent inhibition. Dotted lines represent hypothetical signaling pathways.

Table 1**mTOR Inhibitors**

mTOR Inhibitors	Mechanism of Action	References
Rapamycins		
Rapamycin and rapalogs	Functions by binding to the immunophilin FKBP12 Partial mTORC1 inhibitor Cell-type specific mTORC2 inhibitor	[101-106]
mTOR and PI3K dual-specificity inhibitors		
GNE-477	Dual PI3K/mTOR inhibitor	[107]
NVP-BEZ235	ATP-competitive inhibitor of PI3K and mTOR	[108]
PI-103	ATP competitive inhibitor of DNA-PK, PI3K and mTOR	[109]
XL765	ATP-competitive inhibitor of DNA-PK, PI3K and mTOR	[110]
Small molecule inhibitors of kinases		
Torin1	mTOR kinase inhibitor	[112]
PP242	mTOR kinase inhibitor	[113]
PP30	mTOR kinase inhibitor	[113]
Ku-0063794	specific mTORC1 and mTORC2 inhibitor	[114]
WYE-354	ATP competitive inhibitor of mTOR	[115]
Diet-derived chemopreventive agents		
Curcumin	Disrupts the mTOR-Raptor Complex	[116]
Resveratrol	Inhibits PI3K/Akt/mTOR signaling pathway	[117,118]
epigallocatechin gallate (EGCG)	Inhibits PI3K/Akt/mTOR signaling pathway	[119]
Genistein	Inhibits PI3K/Akt/mTOR signaling pathway	[120,121]
3,3-Diindolylmethane (DIM)	Inhibits both mTOR and Akt activity	[122]