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Characteristic of mTOR signaling and its involvement in the regulation of cell movements through remodeling the cytoskeleton architecture

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mTOR kinase is one of the basic links at the crossroad of several signal transduction pathways. Deregulated mTOR kinase signaling accompanies the progress of cancer, diabetes, neurodegenerative disorders and aging. Implication of mTOR inhibitor rapamycin decreases migration and invasion of malignant cells, and metastasis formation. However, a precise mechanism of the regulation of cellular locomotion by mTOR kinase is not fully understood. This article focuses on the recent findings that demonstrated a possible role of mTOR kinase in the regulation of cytoskeleton remodeling and cell migration properties. Detailed studies on this non-canonical mTOR function will extend our knowledge about cell migration and metastasis formation and might improve anti-cancer therapeutic approaches.

Keywords: mTOR signaling, rapamycin, cytoskeleton remodeling, intermediate filaments, microtubules, cancer metastasis.

Introduction

The mTOR (mammalian target of rapamycin) kinase is a central link of several signaling pathways that integrates the signals from growth factors, hormones, stress, energy status, and amino acids to control the organism growth, homeostasis and aging. mTOR acts through two functionally and structurally distinct complexes, named mTORC1 and mTORC2 (mTOR complex 1 and 2).

Taken together, active mTOR complexes stimulate the cellular growth and proliferation by positive regulation of transcription, translation, ribosome biogenesis, cell survival, inhibition of autophagy and apoptosis [1]. Overactivation of the mTOR kinase was found in a range of human diseases, such as different types of cancer, type 2 diabetes, obesity, and neurodegenerative disorders. Therefore, mTOR is considered as a perspective target of anti-cancer and anti-aging therapies [2].

One of the most dangerous stages of oncogenesis is the metastasis formation. At this stage the tumor is considered malignant. It is known, that the primary tumor causes death only in 10 % of the patients, whereas 90 % of deaths are caused by metastases [3]. The metastasis formation is directly dependent on the cell motility and invasion, which allow the cells to change a position within the tissues. It was shown, that known mTOR inhibitor rapamycin and its synthetic analogs can demonstrate not only cytostatic effects, but a decrease in the motility of cancer cells as well [4, 5]. However, the mechanism of the regulation of cell migration and invasion by mTOR kinase is not fully understood.

The cytoskeleton, a cytoplasmic system of fibers, is critical to sustain cell motility. Intermediate filaments, actin-containing microfilaments and microtubules are the three main cytoskeletal systems of vertebrate and many invertebrate cells. The rearrangements of the cytoskeleton architecture are the main

reason of the cell locomotion [6]. Early studies revealed that mTORC2 regulates the actin cytoskeleton polarization in yeasts. Moreover, further research showed that inactivation of both mTOR complexes impairs the movement of normal and cancer cells [7]. This article focuses on the recent studies revealing the role of mTOR kinase in the cytoskeleton remodeling and cell locomotion.

Structure and functions of mTOR kinase complexes

TOR (Target of Rapamycin) is a serine/threonine protein kinase, the activity of which is inhibited specifically by macrolide rapamycin (the other name sirolimus). Rapamycin, produced by filamentous bacterium *Streptomyces hygroscopicus*, was initially found in the soil sample of the Easter Island (Rapa Nui) in 1970s and subsequently discovered to have antifungal, immunosuppressive and cytostatic effects. Biochemical studies and genetic screening in the yeast mutants, resistant to the growth-inhibitory properties of rapamycin, led to the identification of TOR kinase [8]. Interestingly, rapamycin does not directly inhibit TOR, but it forms a complex with cytosolic protein FKBP12 (FK506 binding protein 12 kDa, the other name FKBP1A). The rapamycin-FKBP12 complex binds to the C terminal part of TOR molecule, termed FRB (FKBP12-rapamycin binding domain), thereby inhibiting TOR kinase activity and functions [9]. Further studies revealed the homologues of yeast TOR in the flies (*Drosophila melanogaster)*, worms (*Caenorhabditis elegans)*, fungus *Cryptococcus neoformans*, plants (*Arabidopsis thaliana)* and mammals. That indicates a high evolutionary conservatism of the kinase, and hence its important role in the regulation of intracellular processes.

It should be noted that although mTOR originally stood for 'mammalian TOR', it is now also used officially as an abbreviation for 'mechanistic TOR'. Unfortunately, sometimes the expression 'mechanistic TOR' is used to indicate TOR kinase from any species that brings some confusion in the field [10]. To prevent further confusion we will use the term mTOR when discussing kinase in mammalian organisms.

In mammalian cells mTOR is a catalytic compound of two different complexes mTORC1 and mTORC2, which coordinates anabolic and catabolic processes in response to growth factors and nutrients. The mTOR-containing complexes have different sensitivities to rapamycin as well as upstream regulators and downstream targets.

Components and substrates of mTOR complex 1

The most studied complex is mTORC1. It consists of mTOR, Raptor (regulatory-associated protein of mTOR), mLST8 (mammalian lethal Sec13 protein 8), PRAS40 (proline-rich kt substrate 40 kDa), Deptor (DEP-domain-containing mTOR-interacting protein), and the Tti1/Tel2 complex. Raptor and PRAS40 are unique components of mTORC1. The known functions of mTOR partner proteins are listed in the Table 1 [11]. Cryo-electron studies revealed that mTORC1 is an obligate dimer with an overall rhomboid shape and a central cavity. It was shown that the dimeric interfaces were formed by interlocking interactions between the mTOR and Raptor subunits. It was also proposed that some mTORC1 substrates with multiple phosphorylation sites could shuttle between the two mTOR active sites within the dimer [12].

mTORC1 acts as a signal integrator for four major regulatory inputs: nutrients, growth factors, energy and stress. Growth factors and hormones regulate mTORC1 through several different signaling pathways, such as PI3K/Akt network, Ras–Raf–MAPK/Erk signaling and Wnt pathway. The implication of multiple growth factor-initiated pathways in mTORC1 regulation is likely to allow mTOR to participate in many developmental and physiological processes [1].

The most studied upstream regulators of mTORC1 are the elements of PI3K/Akt/mTOR signaling network: PI3K (phosphatidylinositol-3-kinase), Akt (the other name PKB, protein kinase B), TSC1–TSC2 (tuberous sclerosis complex 1 and 2) and small GTPase RHEB (Ras homolog enriched in brain). Binding growth factors to the receptor tyrosine kinases initiates the production of the second messenger PIP3 (phosphatidylinositol 3,4,5 triphosphate) by PI3K. This lipid serves as plasma membrane docking site for Akt. Recruiting Akt to the membrane induces its

phosphorylation and activation. In turn, activated Akt phosphorylates TSC2 (also known as tuberin), a large protein that, together with TSC1 (also known as hamartin), forms the TSC1–TSC2 complex. TSC1–TSC2 acts as a GTPase activating protein (GAP) for RHEB and promotes its loading with GDP. Akt-mediated phosphorylation of TSC2 inhibits GAP activity of the TSC1–TSC2 complex and induces RHEB to bind GTP. The GTP-bound form of Rheb directly interacts with mTORC1 and strongly stimulates its kinase activity [13, 14].

Nutrients activate mTORC1 through amino acids availability. Import of the amino acids causes small Rag GTPases to switch to the active conformation. The active Rag heterodimer physically interacts with Raptor, causing mTORC1 to cluster onto the surface of late endosomes and lysosomes, where the Rag GTPases reside. This relocalization enables mTORC1 to interact with its activator RHEB [15, 16].

In mammalian cells the most extensively studied substrates of mTORC1 are S6Ks (ribosomal protein S6 kinases) and 4E-BP1 (eIF4E- binding protein 1). The main function of these proteins is the regulation of mRNA translation initiation and progression, thus controlling the rate of protein synthesis. Previous studies have shown that both proteins contain TOS (TOR signaling) motifs. Mutations in the amino acid sequence of the TOS motif significantly reduces the level of phosphorylation of S6K and 4E-BP1 under *in vitro* conditions, due to the impaired ability of these proteins to interact with Raptor. Besides the canonical function of the mentioned mTOR targets, they are involved in the regulation of cell viability, migration, cytoskeleton remodeling etc. [17–19].

Components and substrates of mTOR complex 2

Compared to mTORC1, less is known about mTORC2. It is insensitive to amino acids, but responds to growth factors through a poorly defined mechanism.

mTORC2 is formed by mTOR, Rictor (rapamycin-insensitive companion of mTOR), mLST8, Deptor, mSIN1 (mammalian stress-activated protein kinase interacting protein) and Protor-1 (protein observed with Rictor-1, also known as PRR5). Rictor, mSin1 and Protor-1 are unique components of mTORC2. The known functions of mTORC2 proteins are listed in the Table 1. In yeasts, TORC2 is

Table 1. **The known functions of mTORC1 and mTORC2 proteins**

mTOR	Serine/threonine kinase, catalytic subunit of the complex
	mTORC1
Raptor	Scaffold protein, regulates the assembly, substrates binding and localization of mTORC1. Unique component of mTOR complex 1.
PRAS40	mTOR inhibitor. Unique component of mTORC 1.
Deptor	mTOR inhibitor
mLST8	Unknown function. The loss of mLST8 does not affect mTORC1 activity towards its substrates
Tti1/Tel2 complex	Scaffold proteins, which regulate mTORC1 assembly and stability
	mTORC2
mTOR	Serine/threonine kinase, catalytic subunit of the complex
Rictor	Scaffold protein, regulates the assembly and substrates binding of mTORC2. Unique component of mTOR complex 2.
mSin1	Scaffold protein regulating the assembly, stability of mTORC2 and its interaction with SGK1. Unique component of mTOR complex 2.
Protor-1	Increases mTORC2-mediated activation of SGK1. Unique component of mTOR complex 2.
Deptor	mTOR inhibitor
mLST8	Unknown function. The loss of mLST8 does not affect mTORC1 activity towards its substrates
Tti1/Tel2 complex	Scaffold proteins, which regulate mTORC1 assembly and stability

oligomeric and forms homodimers, but whether mTORC2 can form dimers/multimers in mammalian cells is unknown. mTORC2 is insensitive to acute rapamycin treatment. However, chronic treatment with rapamycin inhibits mTORC2 functions in many cell lines, possibly, by sequestration of all mTOR molecules and, therefore, prevention of the *de novo* mTORC2 assembly [20].

It was revealed, that mTORC2 substrates are the AGC kinase family members, such as: Akt, cPKCs (conventional protein kinases C) and SGK (serum- and glucocorticoid-regulated kinase). Through these kinases mTORC2 takes part in the regulation of cell survival, cell cycle progression and anabolism [1, 20].

Participation of mTOR kinase in the regulation of cytoskeleton reorganization

The initial characterization of mTORC2 led to the discovery of the participation of mTOR signaling in actin cytoskeleton polarization and cell movements [21]. Recent studies revealed that both complexes: mTORC1 and mTORC2 play a crucial role in the processes of cell motility and invasion through regulation of cytoskeleton remodeling [22].

Table 2. **Classification of the intermediate filaments**

Type of IFs	IFprotein	Tissue distribution
Type I	Acidic keratins	All types of the epithelia
Type II	Basic keratins	
Type III	Vimentin	Mesenchymal cells
	Desmin	Muscle
	Glial fibrillary acidic protein	Glial cells, astrocytes
	Peripherin	Neurons
Type IV	NF(Neurofilaments)-L	Neurons
	NF-M	Neurons
	NF-H	Neurons
	Nestin	Neuroepithelial stem cells
	Internexin	Neurons
	Syncolin	Muscle cells
Type V	Lamins A, C, B1, B2	Nuclear lamina of all types of the cells
Beaded filaments	Phakinin	Intermediate filaments of the ocular lens
	Filensin	

Interplay between mTOR kinase and intermediate filaments

Intermediate filaments (IFs) form an extensive cytoskeletal network within the cell (Fig. 1, *A*).

The subunits composing intermediate filaments constitute a superfamily of α-helical proteins that are found in the cytoplasm of different tissues and on the nuclear membrane. In humans, there are at least 67 genes that encode IF proteins, which makes this gene family one of the largest in the human genome. The various members of the intermediate filament protein family are expressed differentially in complex patterns during embryonic development and in the terminally differentiated tissues. So, this superfamily has been divided into five distinct types on the basis of similarities in sequence and their patterns of expression in cells (Table 2) [6, 23, 24].

Phosphorylation rate plays an important role in the assembly and disassembly of the intermediate filaments. Hyperphosphorylation of multiple sites of the IFs during mitosis causes rapid disassembly of the filaments and their separation to the daughter cells. Recent studies also showed high level of flexibility of the IFs even in stationary interphase cells. These findings suggest that dynamic of IF cytoskeleton remodeling is under the control of kinases and phosphatases [23, 25].

For a long time, IFs have been considered as components of the cell that maintain the cellular shape and provide resistance to mechanical stress. However, a lot of recent studies revealed novel non-canonical functions of intermediate filament proteins. For example, it was shown that keratins mediate localization of the hemidesmosomes and desmosomes in the human keratinocytes. Depletion of all keratins by genome engineering caused altered distribution of the hemidesmosomal proteins, which resulted in a faster adhesion and migration of keratin-free cells [26]. Moreover, analyses of vimentin −/− mice have revealed that loss of vimentin leads to impaired wound healing due to defects in the capacity of fibroblasts to migrate [27]. These findings support a hypothesis that intermediate filaments play important role in cell motility and that altered regulation of

IFs assembly could be involved in cancer cell spreading. Also, intermediate filaments take part in the apoptosis regulation and cell signaling.

Studies on the keratin 17 (K17)-null mouse skin keratinocytes revealed that K17 regulates cell growth and size through mTOR signaling. Keratin 17 is an intermediate filament protein rapidly induced in wounded stratified epithelia that alters cellular viscoelastic properties and optimizes tissue repair. Mouse skin keratinocytes lacking K17 show depressed protein translation and are of smaller size, correlating with decreased Akt/mTOR signaling activity. It was discovered that K17 regulates mTOR activation through binding to the adaptor protein 14-3-3σ. Two amino acid residues located in the amino-terminal head domain of keratin 17 are required for the serum-dependent relocalization of 14-3-3σ from the nucleus to the cytoplasm, and for the stimulation of mTOR activity and cell growth [28].

Another evidence of the cooperation between IFs and mTOR kinase comes from the research of the transgenic mice lacking the entire keratin multiprotein family. All keratin-null embryos die from severe growth retardation at embryonic day 9.5. Embryonic epithelia suffer no cytolysis but display mislocalized desmo- somes and glucose transporters GLUT1 and GLUT3. An altered localization of glucose transporters subsequently activates the energy sensor adenosine monophosphate kinase (AMPK). AMPK is a negative mTORC1 regulator, it inactivates mTOR signaling, thereby represses protein biosynthesis in keratin-null embryos [29].

Treatment of a human HaCaT keratinocyte cell line with mTOR inhibitors (rapamycin, temsirolimus or everolimus) resulted in selective keratin 6a (K6a) repression. Furthermore, treatment of the HaCaT cell line with the siRNAs targeting components of the mTOR pathway altered the levels of K6a expression. Oral rapamycin administration also improves the symptoms in pachyonychia congenita patients, suggesting mTOR inhibitors may be a therapeutic option for people with mutations that disrupt the intermediate filaments formation. These results show a possible bidirectional interplay between mTOR kinase and intermediate filament proteins [30].

It is known that the site-specific phosphorylation of IF proteins induces the disassembly of the filament structures. During mitosis, the hyperphosphorylation of intermediate filaments by Cdk1 (Cyclin-dependent kinase 1), Plk1 (Polo-like kinase 1), Rho- and Aurora-B kinases is essential for the efficient segregation of IF networks into daughter cells [31]. However, it was revealed that IF network is also a highly mobile structure in the interphase cells and its remodeling is under the control of protein kinases and phosphatases, such as protein kinase C (PKC) and protein kinase A (PKA) [25].

Regulation of actin cytoskeleton reorganization by mTOR kinase

A globular protein actin forms microfilaments, which are 7 nm in diameter polar fibrils that organize an extensive network in the cytoplasm of all eukaryotic cells (Fig. 1, *B*). Actin can be present as either a free monomer G-actin (globular) or a part of a linear polymer microfilament called F-actin (filamentous). All actin subunits in the microfilament point toward the same end of the filament. Actin filament exhibits polarity: the end that possesses an actin subunit that has its ATP binding site exposed is called the «(-) end», whereas the opposite end where the cleft is directed at a different adjacent monomer is called the «(+) end». The assembly of G-actin into F-actin is accompanied by the hydrolysis of ATP. Actin participates in many important cellular processes, including muscle contraction, cell motility, cell division, vesicle and organelle traffic, and the establishment and maintenance of cell junctions [6, 32].

Early studies on TOR kinase revealed that deletion of TOR2 disrupted the polarized organization of the actin cytoskeleton in yeasts. In mammalian cells mTORC2 also seems to regulate the remodulation of actin cytoskeleton [21]. Knockdown of mTOR, Rictor or mLST8 in the serum-starved NIH 3T3 fibroblast cells resulted in the defective F-actin fibres formation in response to serum, whereas knockdown of raptor did not affect actin polymerization and cell spreading. Additionally, disruption of mTORC2 reduced phosphorylation of the focal adhesion proteins, as well as F-actin reorganization and cell motility [33, 34]

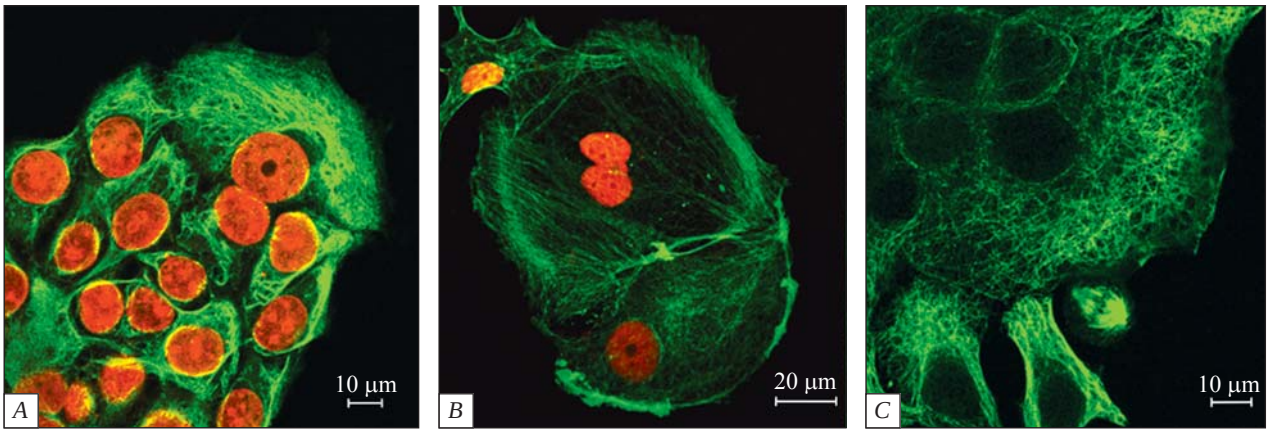

Fig. 1. The architecture of different types of the cytoskeleton fibers in the human breast adenocarcinoma MCF-7 cell line: intermediate filaments (*A*), microfilaments (*B*), microtubules (*C*)

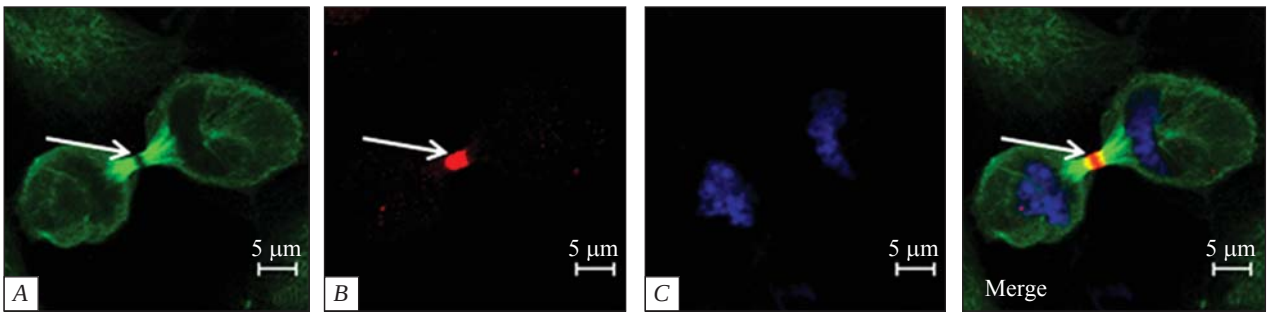

Fig. 2. Immunofluorescent analysis of the phospho-mTOR Ser2481 (*B*) colocalization with tubulin β (*A*) during cytokinesis in the human breast adenocarcinoma MCF-7 cell line. Nuclei were counterstained with Hoechst 33342 (*C*)

The actin cytoskeletal rearrangements are regulated by intracellular signaling pathways directed by Rac (Ras-related C3 botulinum toxin substrate), Rho (Ras homolog gene family), and Cdc42 (Cell division control protein 42 homolog), all Ras-like molecules belonging to the GTPase superfamily of switch proteins [6]. So, it was interesting whether mTOR could influence actin cytoskeleton architecture through these proteins. Indeed, further research showed that in yeasts TOR2 activated Rho1 and Rho2 via their exchange factor ROM2 (Rho1 guanine nucleotide exchange factor 1). However, the actual mechanism by which TORC2 regulates the Rho1 GTPase pathway is not well studied [35, 36].

Depletion of mTOR and Rictor, but not Raptor, impairs actin polymerization, leading edge establishment, and directional migration in neutrophils stimulated with chemoattractants. It was shown that depletion of Rictor inhibits Rac and Cdc42 activities, supposing that they are the target of mTORC2. Interestingly, depletion of mSin1, an integral component of mTORC2, caused no detectable changes in neutrophil polarity and chemotaxis [37, 38].

Several recent studies pointed to the mTORC2 involvement in the formation of long-term memory by regulating and stabilizing the actin cytoskeleton in the dendritic spines of neurons. Rictor-deficient mice showed a reduction in the ratio of fibrilar actin (F-actin) to actin monomers, as well as a reduction in the expression of a number of upstream positive regulators of actin polymerization. These data suggested that mTORC2 is required for the long-term memory formation by increasing the F-actin important for dendritic spine growth and remodeling [39, 40].

Current research revealed that mTORC1 also could be implicated in the actin cytoskeleton reassembly in different cells. It was shown that rapamycin treatment induced S6K inactivation, inhibited actin stress fiber formation and cell migration in a wide range of mammalian cell lines. Further studies discovered that S6K, Akt, PDK1, and activated mTOR were localized to the actin arc of the Swiss 3T3 fibroblasts [10]. Rapamycin treatment blocked the epidermal growth factor (EGF)-induced actin arc formation in these cells, supporting a hypothesis, that mTORC1/S6K axis is also important for the cytoskeleton regulation [41]. It was observed that rapamycin inhibited IGF-I-induced F-actin reorganization and phosphorylation of the focal adhesion proteins, such as FAK (Focal adhesion kinase), paxillin and p130Cas, by inhibition of the S6K1 activity [7, 33]. Knockdown of mTORC1 and mTORC2 induced a mesenchymal-epithelial transition in the colorectal cancer cells, due to increased cell-cell contacts as well as decreased actin cytoskeletal remodeling and decreased activation of the small GTPases, RhoA and Rac1 [36]. It supports the idea that mTOR could regulate cytoskeleton rearrangement through phosphorylation of the actin-remodulating proteins.

The present study showed that activated PI3K-Akt-mTOR signaling pathway promotes invasion and metastasis in hepatocellular carcinoma though up-regulation of MMP-9 (Matrix metalloproteinase 9), though, indicating that mTORC1 could influence cellular locomotion by several distinct directions [42, 43].

The crosstalk between mTOR kinase and microtubules

A microtubule is a polymer of globular tubulin subunits, which are arranged in a cylindrical tube measuring 25 nm in diameter – the thickest fibrils of the cytoskeleton (Fig. 1, *C*). Similar to F-actin a microtubule is polarized and has (+) end and (-) end. Polymerization of the tubulin subunits requires the hydrolysis of the GTP molecules. In addition to regulation of the cell motility, microtubules play a major role in organization of the cell polarity through a special structure called the microtubule-organizing center (MTOC). Located near the nucleus, the MTOC directs the assembly and orientation of microtubules, the route of vesicle trafficking, and the orientation of organelles. Microtubules play a crucial role during mitosis by the formation of mitotic spindle, which is used to separate eukaryotic chromosomes. A large number of proteins influences the assembly and stability of microtubules and their association with other cell structures. These proteins are collectively called microtubule-associated proteins (MAPs) [6, 32].

Involvement of TOR1 and TOR2 in the control of various aspects of microtubule dynamics was reported in yeasts [44]. However, the role of TORs in the regulation of microtubule dynamics has not been fully elucidated yet.

In mammalian cells mTOR was found to bind directly to and phosphorylate cytoplasmic linker protein of 170 kDa (CLIP-170), which is a MAP that binds to the (+) end of the microtubule and stabilizes it [45]. However, the exact function of this phosphorylation is not fully understood. It was revealed that TSC2 knockout resulted in a greater abundance of stabilized microtubules underneath the cellular cortex. Time-lapse imaging of dynamic microtubules also revealed disorganized movements of the growing microtubule plus-ends in the cellular cortex region, including growth in a direction that is parallel to the cortex. The authors suggested that the functional mTOR-CLIP-170 interaction helps microtubules grow to the cellular cortex [46].

It was shown that mTOR kinase is also involved in the regulation of intracellular transport associated with microtubules. Inhibition of the expression of TSC2, which is involved in the activation of mTOR, leads to disruption of the caveolin (scaffold protein, involved in the endocytosis) transport to the plasma membrane. Instead, it was detected in the vesicles, randomly located in the cytoplasm. Incubation of rat fibroblasts in the media that contain high concentrations of mTOR inhibitor rapamycin led to the same result, and chaotic arrangement of microtubules in the cortical zone was observed in the cells [47].

Interestingly, mTORC1–tubulin relations were observed to be bidirectional: the mTORC1 activation requires dynein-dependent transport to a position in the cell where it can be activated [48]. The association between dynein and mTOR was shown by coim-

munoprecipitation. Inhibition of dynein function using RNAi hinders the mTORC1 activity in the human fibroblasts and the human glioblastoma–astrocytoma cell line U373-MG [48].

Moreover, the phosphorylated form of mTOR kinase (phospho-mTOR Ser2481) was observed to localize at the cleavage furrow of different cell lines during cytokinesis. Inhibition of the polymerization of microtubules by nocodazole leads to the loss of phospho- mTOR (Ser2481) ability to target the spindle midzone and the cleavage furrow during cytokinesis. At these conditions phospho-mTOR was randomly dispersed across the entire mitotic cytoplasm, indicating that mitotic traveling of phospho-mTOR (Ser2481) requires dynamic microtubules [49].

Using anti-phospho-mTOR (Ser2481) antibodies (Merck Millipore) we revealed the colocalization of phospho-mTOR (Ser2481) and tubulin β at the cleavage furrow that has not been demonstrated earlier (Fig. 2). Immunofluorescent analysis was performed as described [50]. Our findings support a hypothesis that mTOR phosphorilated at Ser 2481 interacts with microtubules during cytokinesis. However, further studies are needed to understand the mechanism of this process.

Conclusion

Novel findings in the mTOR signaling field shed light on the non-canonical functions of the mTOR kinase. The bidirectional crosstalk between mTOR and all three types of the cytoskeleton points to the important role of mTOR signaling pathway in the normal cell locomotion during embryonic development, wound healing, and chemotaxis as well as in the cancer cells spreading. Further detailed investigation of this new aspect of the mTOR activity might lead to the optimization of the current anti-cancer therapeutic approaches.

REFERENCES

1. *Zoncu R, Efeyan A, Sabatini DM*. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol*. 2011;**12**(1):21–35.
2. *Lamming DW, Ye L, Sabatini DM, Baur JA*. Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J Clin Invest*. 2013;**123**(3):980–9.
3. *Chambers AF, Groom AC, MacDonald IC*. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer*. 2002;**2**(8):563–72.
4. *Hong SM, Park CW, Cha HJ, et al.* Rapamycin inhibits both motility through down-regulation of p-STAT3 (S727) by disrupting the mTORC2 assembly and peritoneal dissemination in sarcomatoid cholangiocarcinoma. *Clin Exp Metastasis*. 2013;**30**(2):177–87.
5. *Feng W, Jia S*. Rapamycin inhibits the invasive ability of thyroid cancer cells by down-regulating the expression of VEGF-C *in vitro*. *Cell Biochem Funct*. 2012;**30**(6):487–91.
6. *Lodish H, Arnold B, Kaiser Chris, Monty K*. Molecular Cell Biology. New York: W. H. Freeman, 2012; 973 p.
7. *Zhou H, Huang S*. Role of mTOR signaling in tumor cell motility, invasion and metastasis. *Curr Protein Pept Sci*. 2011; **12**(1):30–42.
8. *Laplante M, Sabatini DM*. mTOR Signaling. *Cold Spring Harb Perspect Biol*. 2012;**4**(2): a011593.
9. *Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP*. mTOR kinase structure, mechanism and regulation. *Nature*. 2013;**497**(7448):217–23.
10. *Hall MN*. Talks about TORCs: recent advancesin target of rapamycin signalling. On mTOR nomenclature. *Biochem Soc Trans*. 2013;**41**(4):887–8.
11. *Laplante M, Sabatini DM*. mTOR signaling in growth control and disease. *Cell*. 2012;**149**(2):274–93.
12. *Yip CK, Murata K, Walz T, Sabatini DM, Kang SA*. Structure of the human mTOR complex I and its implications for rapamycin inhibition. *Mol Cell*. 2010;**38**(5):768–74.
13. *Manfredi GI, Dicitore A, Gaudenzi G, Caraglia M, Persani L, Vitale G*. PI3K/Akt/mTOR signaling in medullary thyroid cancer: a promising molecular target for cancer therapy. *Endocrine*. 2015;**48**(2):363–70.
14. *Follo MY, Manzoli L, Poli A, McCubrey JA, Cocco L*. PLC and PI3K/Akt/mTOR signalling in disease and cancer. *Adv Biol Regul*. 2015;**57**:10–6.
15. *Efeyan A, Zoncu R, Sabatini DM*. Amino acids and mTORC1: from lysosomes to disease. *Trends Mol Med*. 2012;**18**(9): 524–33.
16. *Bar-Peled L, Chantranupong L, Cherniack AD, et al.* A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science*. 2013;**340**(6136):1100–6.
17. *Filonenko VV*. PI3K/mTOR/S6K signaling pathway – new players and new functional links. *Biopolym Cell.* 2013;**29** (3):207–14.
18. *Lyzogubov V, Khozhaenko Y, Usenko V, et al.* Immunohistochemical analysis of Ki-67, PCNA and S6K1/2 expression in human breast cancer. *Exp Oncol.* 2005;**27**(2): 141–4.
19. *Filonenko VV, Tytarenko R, Azatjan SK, et al.* Immunohistochemical analysis of S6K1 and S6K2 localization in human breast tumors. *Exp Oncol.* 2004;**26**(4): 294–9.
20. *Oh WJ, Jacinto E*. mTOR complex 2 signaling and functions. *Cell Cycle*. 2011;**10**(14):2305–16.
21. *Jacinto E, Loewith R, Schmidt A, et al.* Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*. 2004;**6**(11):1122–8.

22. *Sen B, Xie Z, Case N, et al.* mTORC2 regulates mechanically induced cytoskeletal reorganization and lineage selection in marrow-derived mesenchymal stem cells. *J Bone Miner Res.* 2014;**29**(1):78–89.
23. *Magin TM, Vijayaraj P, Leube RE*. Structural and regulatory functions of keratins. *Exp Cell Res*. 2007;**313**(10): 2021–32.
24. *Chang L, Goldman RD*. Intermediate filaments mediate cytoskeletal crosstalk. *Nat Rev Mol Cell Biol*. 2004;**5**(8): 601–13.
25. *Sihag RK, Inagaki M, Yamaguchi T, Shea TB, Pant HC*. Role of phosphorylation on the structural dynamics and function of types III and IV intermediate filaments. *Exp Cell Res.* 2007;**313**(10):2098–109.
26. *Seltmann K, Roth W, Kröger C, et al.* Keratins mediate localization of hemidesmosomes and repress cell motility. *J Invest Dermatol*. 2013;**133**(1):181–90.
27. *Ivaska J, Pallari HM, Nevo J, Eriksson JE.* Novel functions of vimentin in cell adhesion, migration, and signaling. *Exp Cell Res*. 2007;**313**(10):2050–62.
28. *Kim S, Wong P, Coulombe PA*. A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature*. 2006;**441**(7091):362–5.
29. *Vijayaraj P, Kröger C, Reuter U, Windoffer R, Leube RE, Magin TM*. Keratins regulate protein biosynthesis through localization of GLUT1 and -3 upstream of AMP kinase and Raptor. *J Cell Biol*. 2009;**187**(2):175–84.
30. *Hickerson RP, Leake D, Pho LN, Leachman SA, Kaspar RL*. Rapamycin selectively inhibits expression of an inducible keratin (K6a) in human keratinocytes and improves symptoms in pachyonychia congenita patients. *J Dermatol Sci*. 2009;**56**(2):82–8.
31. *Izawa I, Inagaki M*. Regulatory mechanisms and functions of intermediate filaments: a study using site- and phosphorylation state-specific antibodies. *Cancer Sci*. 2006; **97**(3):167–74.
32. *Lewin B*. Cells. MA:"Jones & Bartlett Learning" 2007. 863p.
33. *Liu L, Chen L, Chung J, Huang S*. Rapamycin inhibits F-actin reorganization and phosphorylation of focal adhesion proteins. *Oncogene*. 2008;**27**(37):4998–5010.
34. *Goncharova EA, James ML, Kudryashova TV, Goncharov DA, Krymskaya VP*. Tumor suppressors TSC1 and TSC2 differentially modulate actin cytoskeleton and motility of mouse embryonic fibroblasts. *PLoS One*. 2014;**9**(10): e111476.
35. *Ohsawa M, Kobayashi T, Okura H, Igarashi T, Mizuguchi M, Hino O.* TSC1 controls distribution of actin fibers through its effect on function of Rho family of small GTPases and regulates cell migration and polarity. *PLoS One*. 2013;**8** (1): e54503.
36. *Gulhati P, Bowen KA, Liu J, et al*. mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res*. 2011; **71**(9):3246–56.
37. *Byles V, Covarrubias AJ, Ben-Sahra I, et al.* The TSC-mTOR pathway regulates macrophage polarization. *Nat Commun*. 2013; **4**:2834.
38. *He Y, Li D, Cook SL, et al.* Mammalian target of rapamycin and Rictor control neutrophil chemotaxis by regulating Rac/Cdc42 activity and the actin cytoskeleton. *Mol Biol Cell.* 2013;**24**(21):3369–80.
39. *Josselyn SA, Frankland PW*. mTORC2: actin on your memory. *Nat Neurosci.* 2013;**16**(4):379–80.
40. *Angliker N, Rüegg MA*. *In vivo* evidence for mTORC2-mediated actin cytoskeleton rearrangement in neurons. *Bioarchitecture*. 2013;**3**(4):113–8.
41. *Berven LA, Willard FS, Crouch MF*. Role of the p70(S6K) pathway in regulating the actin cytoskeleton and cell migration. *Exp Cell Res*. 2004;**296**(2):183–95.
42. *Mendes Sdos S, Candi A, Vansteenbrugge M, et al*. Microarray analyses of the effects of NF-kappaB or PI3K pathway inhibitors on the LPS-induced gene expression profile in RAW264.7 cells: synergistic effects of rapamycin on LPS-induced MMP9-overexpression. *Cell Signal.* 2009; **21**(7): 1109–22.
43. *Zhou HY, Wong AS*. Activation of p70S6K induces expression of matrix metalloproteinase 9 associated with hepatocyte growth factor-mediated invasion in human ovarian cancer cells. *Endocrinology*. 2006;**147**(5):2557–66.
44. *Choi JH, Adames NR, Chan TF, Zeng C, Cooper JA, Zheng XF*. TOR signaling regulates microtubule structure and function. *Curr Biol*. 2000;**10**(14):861–4.
45. *Malik AR, Urbanska M, Macias M, Skalecka A, Jaworski J.* Beyond control of protein translation: what we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). *Biochim Biophys Acta*. 2013;**1834**(7):1434–48.
46. *Zhou Q, Wong CH, Lau CP, et al.* Enhanced antitumor activity with combining effect of mTOR inhibition and microtubule stabilization in hepatocellular carcinoma. *Int J Hepatol.* 2013;**2013**:103830.
47. *Barnes EA, Kenerson HL, Jiang X, Yeung RS*. Tuberin regulates E-cadherin localization: implications in epithelial-mesenchymal transition. *Am J Pathol*. 2010;**177**(4):1765–78.
48. *Clippinger AJ, Alwine JC*. Dynein mediates the localization and activation of mTOR in normal and human cytomegalovirus-infected cells. *Genes Dev*. 2012;**26**(18):2015–26.
49. *Vazquez-Martin A, Sauri-Nadal T, Menendez OJ, et al.* Ser2481-autophosphorylated mTOR colocalizes with chromosomal passenger proteins during mammalian cell cytokinesis. *Cell Cycle.* 2012;**11**(22):4211–21.
50. *Khoruzhenko A, Kukharchuk V, Cherednyk O, et al.* Monoclonal antibodies to Ki-67 protein suitable for immunohistochemical analysis. *Hybridoma (Larchmt).* 2010;**29**(4):301–4.

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Характеристика mTOR сигнального шляху та його залучення до регуляції клітинної рухливості шляхом реконструкції архітектури цитоскелету

mTOR кіназа є однією з основних ланок, розташованих на перетині кількох шляхів внутрішньоклітинної передачі сигналу. Дерегуляція сигналінгу mTOR кінази супроводжує розвиток онкологічних захворювань, діабету, нейродегенеративних розладів і старіння. Застосування інгібітора mTOR рапаміцина знижує рівень міграції та інвазії злоякісних клітин і утворення метастазів. Однак, точний механізм регуляції рухливості клітин mTOR кіназою повністю не зрозумілий. Дана стаття присвячена останнім дослідженням, які демонструють можливу роль mTOR кінази в регуляції ре моделювання цитоскелету та міграції клітин. Докладні дослідження цієї неканонічної функції mTOR кінази дозволить розширити наші знання про міграцію клітин і утворення метастазів і може привести до поліпшення протиракових терапевтичних підходів.

Ключові слова: mTOR сигналінг, рапаміцин, перебудова цитоскелету, проміжні філаменти, мікротрубочки, метастазування.

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Характеристика mTOR сигнального пути и его привлечения к регуляции клеточной подвижности путем реконструкции архитектуры цитоскелета

mTOR киназа является одним из основных звеньев, расположенных на пересечении нескольких путей внутриклеточной передачи сигнала. Дерегуляция сигналинга mTOR киназы сопровождает развитие онкологических заболеваний, диабета, нейродегенеративных расстройств и старения. Применение ингибитора mTOR рапамицина снижает уровень миграции и инвазии злокачественных клеток и образование метастазов. Однако, точный механизм регуляции подвижности клеток mTOR киназы полностью не изучен. Эта статья посвященна последним исследованиям, которые демонстрируют возможную роль mTOR киназы в регуляции ремоделирования цитоскелета и миграции клеток. Подробные исследования этой неканонической функции mTOR киназы позволят расширить наши знания о миграции клеток и образование метастазов и может привести к улучшению противораковых терапевтических подходов.

Ключевые слова: mTOR сигналинг, рапамицин, перестройка цитоскелета, промежуточные филаменты, микротрубочки, метастазирование.

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