

Review

mTOR and the differentiation of mesenchymal stem cells

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The mammalian target of rapamycin (mTOR), an evolutionarily conserved serine–threonine protein kinase, belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family, which contains a lipid kinase-like domain within their C-terminal region. Recent studies have revealed that mTOR as a critical intracellular molecule can sense the extracellular energy status and regulate the cell growth and proliferation in a variety of cells and tissues. This review summarizes our current understanding about the effects of mTOR on cell differentiation and tissue development, with an emphasis on the lineage determination of mesenchymal stem cells. mTOR can promote adipogenesis in white adipocytes, brown adipocytes, and muscle satellite cells, while rapamycin inhibits the adipogenic function of mTOR. mTOR signaling may function to affect osteoblast proliferation and differentiation, however, rapamycin has been reported to either inhibit or promote osteogenesis. Although the precise mechanism remains unclear, mTOR is indispensable for myogenesis. Depending on the cell type, rapamycin has been reported to inhibit, promote, or have no effect on myogenesis.

Keywords mTOR; differentiation; mesenchymal stem cells; adipogenesis; myogenesis; osteogenesis

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Introduction

The evolutionarily conserved checkpoint protein kinase, mammalian target of rapamycin (mTOR), has emerged as a major effector of cell proliferation and differentiation via the regulation of protein synthesis. Studies in the last decade clearly demonstrate that mTOR controls protein synthesis through a variety of downstream targets. Emerging evidences show that mTOR also participates in the lineage determination of mesenchymal stem cells

(MSCs). In this review, we summarize recent advances in this fast-evolving field. We discuss the components of the mTOR signaling pathways and the downstream targets that affect MSCs proliferation and differentiation.

Biochemical features of mTOR

The mTOR protein, also known as FK binding proteins (FKBP)-rapamycin-associated protein, rapamycin and FKBP target, or rapamycin target [1–4], is discovered biochemically based on its FKBP rapamycin binding properties. mTOR contains 2549 amino acids and comprises several conserved structural domains, including the C-terminal domain, the adjacent FKBP-rapamycin binding (FRB) domain, the central toxic effector domain, the putative negative regulatory domain, and the N-terminal Huntingtin, elongation factor 3, a subunit of protein phosphatase 2A (PP2A) and Tor1p (HEAT) repeats.

The C-terminal region of mTOR has strong homology to the catalytic domain of phosphoinositide 3-kinase (PI3K), and therefore, mTOR functions as a protein kinase [5,6]. Indeed, it belongs to a family of serine–threonine (Ser–Thr) protein kinases that are more similar to the lipid kinase PI3K than to members of the larger family of Ser–Thr or tyrosine (Tyr) protein kinases. Adjacent to the catalytic kinase domain is the FRB domain that interacts with the FKBP–rapamycin complex, leading to the inhibition of mTOR function. The N-terminal region of mTOR contains up to 20 tandem repeated HEAT motifs grouped in two blocks, and is important for protein–protein interaction [7]. Each HEAT motif comprises ~40 amino acids that form a pair of antiparallel helices. Two other domains, called FARP-ATM-TRAP (FAT) and FATC (FAT C terminus), have been proposed to mediate interactions in multi-protein complexes [8,9]. Occurring only in combination with the FAT, the FATC domain is absolutely necessary for mTOR activity. Deletion of even a single amino acid from the FATC domain abrogates the activity of mTOR.

mTOR binds to other regulatory components to form two distinct multi-protein complexes [10,11]. The first complex, mTORC1, contains mTOR, regulator-associated protein of mTOR (Raptor), and G protein β subunit-like protein (G β L). The second complex, mTORC2, contains mTOR, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase-interacting protein 1 (mSin1), and G β L. The adaptor proteins Raptor and Rictor determine the substrate specificity of mTORC1 and mTORC2, respectively. mTORC1 specifically phosphorylates ribosomal protein S6 kinase 1 (S6K1, also known as p70S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP) in a rapamycin-sensitive manner. In contrast, mTORC2 phosphorylates Akt at Ser473 and promotes Akt activation.

The first component of the mTOR signaling pathway is the tuberous sclerosis complex (TSC), which comprises two interacting proteins, hamartin (TSC1) and tuberlin (TSC2) that form a stable heterodimeric complex [12–14]. TSC functions as a negative regulator of cell growth (in other words, as a tumor suppressor protein). TSC also integrates information from the insulin-signaling cascade and the adenosine monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway. The immediate downstream target of TSC is Ras homolog enriched in brain (Rheb), a member of the Ras super-family of small guanosine triphosphate (GTP)-binding proteins that functions to activate mTOR kinase [13,15]. This protein can be converted to a lipophilic protein through the enzymatic addition of a farnesyl group, and this modification appears to be functionally important because farnesyltransferase inhibitors can block insulin-mediated activation of the mTOR signaling pathway [15].

A search for readouts of mTOR activity *in vivo* and *in vitro* has revealed that mTOR can be autophosphorylated via its intrinsic Ser/Thr kinase activity [16]. mTOR regulates protein synthesis through the phosphorylation and inactivation of the repressor of mRNA translation-4E-BP1, and through the phosphorylation and activation of S6K1. These two downstream effectors of mTOR, whose phosphorylation is inhibited by rapamycin *in vivo*, can be phosphorylated by recombinant mTOR *in vitro* [5]. Moreover, substitution of aspartic acid (Asp) 2338 with alanine (Ala) in the catalytic domain of mTOR is sufficient to inhibit mTOR kinase activity toward S6K1 and 4E-BP1 [5,16]. Thus, S6K1 or 4E-BP1 phosphorylation is often used as an *in vivo* readout of mTOR activity. However, the question of whether the intrinsic kinase activity of mTOR is sufficient for its full activity *in vivo* has not been resolved. Furthermore, it is not clear as to whether mTOR also serves as a scaffold for other proteins with catalytic activity, such as kinases and phosphatases that may regulate its overall activity *in vivo*.

mTOR is a central signal integrator that receives signals arising from growth factors, nutrients, and cellular energy metabolism [17,18] to regulate cell growth, cell-cycle progression, as well as cell differentiation. mTOR is therefore recognized as an evolutionarily conserved central coordinator of these fundamental biological processes [19–21]. In this review, we summarize some of the important advancements in this fast-evolving field, especially in cell differentiation.

Lineage determination of MSCs

MSCs are a versatile group of non-hematopoietic stem cells with high potency of proliferation and pluripotency of differentiation, derived from many adult tissues, which can differentiate into cells of the mesodermal lineage, such as adipocytes, myocytes, osteocytes, as well as other embryonic lineages [22–25]. Endogenous MSCs circulate in the blood until being called to sites of inflammation or tumors, where they could differentiate into bone, cartilage, fat, or muscle cells to repair or replace damaged tissue [25].

The differentiation of MSCs is induced by culturing with specific induction media. Differentiation can be confirmed using the histological and immunohistological assays. MSCs treated with adipogenic induction medium, containing cyclic AMP (cAMP) agonists and induction agents such as isobutyl-methylxanthine, indomethacin, insulin and dexamethasone, develop lipid-containing droplets that accumulate the lipid dye Oil Red-O [23,26,27]. Glucocorticoids play a direct role in initiating the early phase of adipogenesis through the induction of peroxisome proliferators-activated receptor- γ (PPAR- γ) which is dependent on elevated levels of CCAAT/enhancer binding protein- β (C/EBP- β) [28,29]. The consecutive increase in intracellular cAMP mediates the rapid induction of C/EBP- β and - δ . In response to insulin, cells become able to transport large amounts of glucose, to synthesize fatty acids and to store triacylglycerols. Differentiation of MSCs into osteoblasts is induced *in vitro* by treating cells with low concentrations of ascorbic acid, β -glycerophosphate, and dexamethasone [23,30,31]. Dexamethasone treatment induces morphological transformation of these cells from an elongated to a more cuboidal shape, increases their alkaline phosphatase (ALP) activity and cAMP responses to parathyroid hormone and prostaglandin E₂, and therefore, dexamethasone is essential for mineralization of the extracellular matrix [30]. Cheng *et al.* [30] have reported that dexamethasone results in a population of mature osteoblasts. Early differentiation of MSCs into immature osteoblasts is characterized by ALP enzyme activity [32]. Another later osteogenic-specific marker is the formation of a calcified extracellular matrix [33]. Myogenesis is characterized by a period of myoblast proliferation, followed by the expression of muscle-specific proteins and

fusion of cells to form multinucleated myotubules. Early myogenic differentiation is characterized by the expression of several myogenic regulatory factors including myogenic determination factor 1 (MyoD1) [34–36]. Terminally differentiated myoblasts can be characterized by the expression of myosin and the presence of multiple nuclei [37].

Commitment of stem cells to different lineages is regulated by many cues in the local tissue microenvironment, such as plating density, cell shape, cytoskeletal tension, and integrate adhesive, structural, mechanical, or soluble cues in the microenvironment. Mesenchymal condensations are characterized by increased cell density and cell–cell adhesion [38]. Lower cell densities seem to support osteoblast differentiation of MSCs whereas higher cell densities cause the cells to condense and become adipocytes. Cell shape regulates the adipogenic–osteogenic switch in lineage commitment by modulating endogenous Rho GTPases (RhoA) activity [39]. Expressing dominant-negative RhoA commits hMSCs to become adipocytes, while constitutively active RhoA causes osteogenesis. A chondrogenic-smooth muscle cell fate decision is also mediated by cell shape, Rac1, and N-cadherin [40]. Rac1 activation stimulates smooth muscle cell differentiation, inhibits chondrogenesis, and regulates N-cadherin expression, which is required for robust SMC differentiation. Studies by McBeath *et al.* [38] have shown that myosin-generated cytoskeletal tension that follows spreading of MSCs leads to higher levels of RhoA, Rho kinase (ROCK), and myosin light-chain phosphorylation. Both continuous (10%, 1 Hz) and rest inserted (10%, 1 Hz, 10 s rest) cyclic tensile strain accelerate hMSCs osteodifferentiation and increase calcium accretion [41]. By changing the stiffness of the underlying matrix in MSCs culture, Gao *et al.* [42] have shown that elasticity is a powerful mechanical cue directing MSCs fate. Stiff matrices lead to enhance cytoskeletal tension and osteogenesis, while softer matrices direct MSCs toward alternative lineages. Electromagnetic fields (EMFs) increase ALP activity and mineralized nodule, and stimulate osteoblast-specific mRNA expression [43]. In contrast, EMF decreases adipogenesis and inhibits adipocyte-specific mRNA expression. Thymosin beta-4 (T β ₄), a major G-actin sequestering peptide, known to regulate the cytoskeleton [44], decreases F-actin formation, reduces the F-actin/G-actin ratio, and inhibits osteogenic differentiation. Besides, T β ₄ reciprocally facilitates adipogenic differentiation. In the presence of bone morphogenetic protein-2 (BMP-2), both parathyroid hormone-related protein (PTHrP) and phorbol ester (a protein kinase C stimulator) increase the expression of indexes of the osteoblast phenotype, including ALP, type I collagen, and osteocalcin, while inhibits adipogenesis [45]. Cheng *et al.* [46] have shown that arsenic trioxide, as an anti-tumor drug,

induces MSC senescence and inhibits its adipogenic differentiation but promotes osteogenic differentiation by PPAR- γ . Fei *et al.* [47] have shown that LIM mineralization protein-1 (LMP-1), a novel intracellular osteoinductive protein, can pluripotent myoblastic cells to become osteoblastic cells as measured by altered morphology and osteoblast-specific gene expression.

Intracellular signaling molecules are critical for MSC differentiation. One of the most important signaling pathways is Wnt signaling cascade. Wnt10b shifts cell fate toward the osteoblast phenotype by up-regulating the expression of core binding factor alpha 1 (Cbfa1/Runx2), Distal-less homeobox 5 (Dlx5) and osterix, while down-regulating the expression of C/EBP- α and PPAR- γ [48]. Canonical Wnt signaling supports osteogenic differentiation from both precursor lines and stem cell lines *in vitro* [49]. Studies by Logan and Nusse suggested that catenin blocks the differentiation of MSCs into skeletal precursors [50]. Mitogen-activated protein kinase (MAPK) family members such as extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, modulate the commitment of MSCs [51]. Jaiswal *et al.* [51] showed the activation of ERK and JNK during the osteogenic differentiation. Chemical inhibitor or genetic knockout of the MAPK signaling pathway blocks the osteogenic differentiation, while results in the adipogenic differentiation of the stem cells and the expression of adipose-specific mRNAs. The TWIST family of basic helix-loop-helix transcription factors, Twist-1, and Dermo-1 are potential mediators of MSC self-renewal and lineage commitment in postnatal skeletal tissues [52]. By over-expressing Twist-1 and Dermo-1, MSCs display a decreased capacity of osteo/chondrogenic differentiation and an enhanced capacity of adipogenesis. Spry1 is critical for adipocyte differentiation and MSC lineage allocation, potentially acting through the regulation of C/EBP- β and transcriptional coactivator of PSD-95, DLG, ZO-1 (PDZ)-binding motif (TAZ) [53]. Nishikawa *et al.* [54] demonstrated that the basic leucine-zipper transcription factor Maf (also known as c-Maf) is essential to osteoblast lineage commitment. Quach *et al.* [55] showed that Zinc-finger protein 467 (Zfp467) stimulates adipocyte formation and inhibits osteoblast commitment. pRb (the protein product of the retinoblastoma tumor suppressor gene) promotes cell-cycle exit through inhibition of the E2F transcription factors and the transcriptional repression of genes encoding cell-cycle regulators. Depending on the differentiation factor and cellular context, pRb can either suppress or promote their transcriptional activity. For example, pRb binds to Runx2 and potentiates its ability to promote osteogenic differentiation *in vitro*. In contrast, pRb acts with E2F to suppress PPAR- γ [56], and therefore decreases the adipocyte differentiation.

In summary, major transcriptional factors critical for the lineage determination of MSCs include C/EBP- α and PPAR- γ for adipocytes [57,58], Runx2 and osterix for osteoblasts [59,60], and MyoD, Myf5, myogenic regulatory factor 4 (MRF4), and myogenin for myocytes [61,62].

Effect of mTOR on the differentiation of MSCs

Emerging evidence has indicated that mTOR may modulate cell proliferation and differentiation in a wide variety of cell types. *In vitro* studies suggested that overexpression of mTOR induces the differentiation of several types of cells, including osteoblasts, adipocytes, and neurons. In addition, mTOR stimulates proliferating myoblast cells to differentiate and fuse into multinucleated myotubes. Since osteoblasts, adipocytes, and myocytes are derived from common precursor cells, the MSCs, it is likely that mTOR acts to determine the lineage differentiation of these stem cells (Fig. 1).

mTOR and adipogenesis

Adipocyte differentiation is a developmental process critical for metabolic homeostasis and nutrient signaling. Adipose tissue plays major roles in energy homeostasis, lipid metabolism, and insulin actions. It also functions as an endocrine organ to secrete a wide range of factors and cytokines such as leptin, adiponectin, tumor necrosis factor- α (TNF- α), some of which are key regulators of energy homeostasis [63]. In adipocytes, mTOR is proposed to regulate protein synthesis [64], adipose tissue morphogenesis [65,66], and leptin synthesis/secretion [67].

Studies by Kim and Chen [66] suggested that mTOR signaling is critical for adipocytes to sense nutrient availability and modulate the activity of PPAR- γ . This finding revealed the adipogenic function of mTOR for the first time. The mTOR signaling pathway specifically regulates the activity of PPAR- γ , which is essential for a positive feedback control of C/EBP- α expression as well as the adipogenic gene expression program and thus critical for both initiating and maintaining adipogenesis. Furthermore, mTOR signaling may serve to transduce nutrient availability signals to control the activity of PPAR- γ .

While insulin and Akt signaling have been previously found to be essential for adipogenesis [68–70], it is now generally believed that insulin activates mTORC1 via the PI3K/Akt pathway [71,72], suggesting that insulin may regulate the adipogenesis by the mediation of mTORC1. Further studies have discovered that TSC1/TSC2 and Rheb may function as the intermediate molecules bridging the Akt signaling and the activity of mTORC1 [72,73]. However, mTOR signaling alone is sufficient to regulate adipogenesis, because activation of mTORC1 causes a robust increase in the mRNA and protein expression of PPAR- γ despite severe insulin resistance and the absence of Akt signaling [72].

Inhibition of mTOR with rapamycin has negative effects on adipocyte differentiation and insulin signaling [74–77]. Rapamycin significantly reduces the expression of most adipocyte marker genes including PPAR- γ , adipsin, aP2, adipocyte determination and differentiation factor-1/sterol regulatory element-binding protein-1c (ADD1/SREBP1c) and fatty acid synthase (FAS), and decreases the

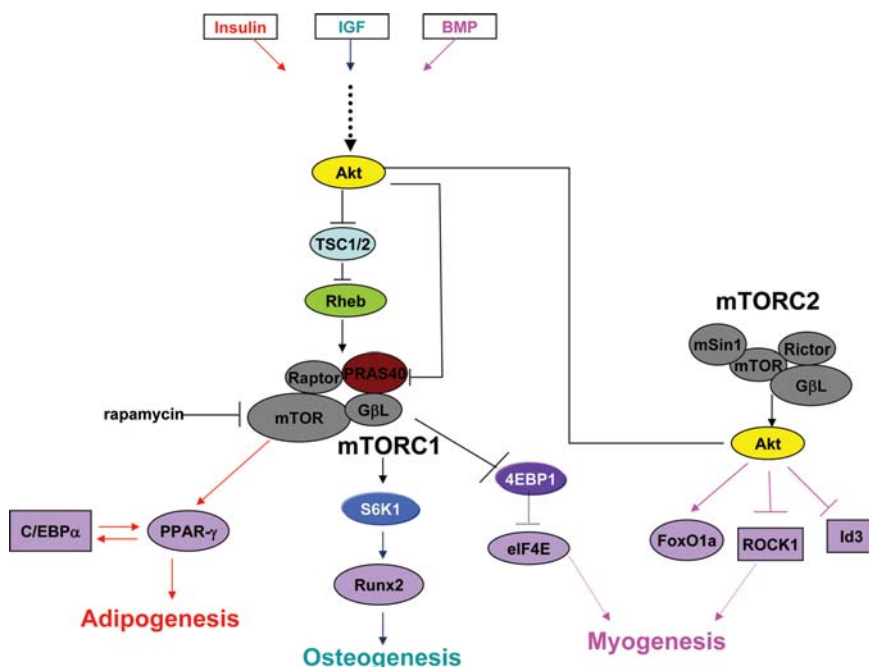


Figure 1 The mTOR signaling pathway regulates the differentiation of MSCs

intracellular lipid accumulation in 3T3-L1 and 3T3-F442A cells [75]. Rapamycin does not equally inhibit the insulin-stimulated phosphorylation of S6K1 (and S6 phosphorylation) compared with that of 4E-BP1 (and eIF4E phosphorylation), even though both proteins are regulated by mTOR [77]. The partial inhibition of insulin-induced 4E-BP1 phosphorylation, compared with the complete absence of S6K1 phosphorylation is consistent with the effect of rapamycin on insulin-induced adipogenesis and Rb phosphorylation.

Differentiation of brown adipocytes employs signaling pathways distinct from white adipocytes, with AMPK-mTOR cross-talk as a central mediator of this process [78]. Sequential activation of p38-MAPK and lexical knowledge base 1 (LKB1)-AMPK-TSC2 as well as significant attenuation of ERK1/2 and mTOR-S6K1 activation were observed throughout the brown adipocyte differentiation process, showing that mTOR activity is essential in the first stages of differentiation. Nevertheless, subsequent inhibition of this cascade by AMPK activation is also necessary at later stages, since an *in vivo* study has shown that prolonged activation of AMPK by 5-aminoimidazole-4-carboxamide ribonucleoside, a specific activator of AMPK, increases the expression of uncoupling protein 1 (UCP 1) and induces an accumulation of brown adipocytes in white adipose tissue.

The mTOR signaling pathway is also involved in the regulation of high glucose-induced intramuscular adipogenesis in porcine muscle satellite cells [79], possibly through the activation of SREBP-1c protein expression to enhance the expressions of C/EBP- α and FAS protein.

In summary, it is generally believed that mTOR can promote adipogenesis of white adipocytes, brown adipocytes, and muscle satellite cells. Rapamycin inhibits the adipogenic function of mTOR.

mTOR and osteogenesis

During bone formation, multipotential mesenchymal cells proliferate and differentiate into osteoblasts which synthesize and deposit a mineralizing extracellular matrix. Mature osteoblasts eventually become either osteocytes, bone lining cells, or vanish because of apoptosis. Bone is important in mammalian survival, calcium phosphorus metabolism, and energy homeostasis.

While the role of mTOR signaling in the regulation of cell growth and proliferation have been extensively studied in many types of cells in species from *Drosophila* to human, surprisingly, there is limited information available in the literatures regarding its independent actions in control of osteoblast proliferation, differentiation, and bone formation. mTOR/S6K1 signaling pathway is required for osteoblast proliferation and differentiation [80,81]. mTOR also regulates the function of osteoblasts. S6K1 has been

reported to regulate TNF- α -induced interleukin-6 synthesis [82], platelet-derived growth factor-BB-induced interleukin-6 synthesis [83], fibroblast growth factor (FGF) 2-stimulated interleukin-6 synthesis [84], BMP-4-stimulated vascular endothelial growth factor (VEGF) synthesis [85], and FGF-2-stimulated VEGF release [86], which in turn regulate osteogenesis.

Effect of rapamycin on osteogenic differentiation is controversial. Depending on cell type, rapamycin either stimulates or inhibits osteogenesis. In MC3T3-E1 subclone 4 (MC-4) cells and primary mouse bone marrow stromal cells (BMSCs), rapamycin inhibits osteoblast differentiation by targeting osteoblast proliferation and the early stage of osteoblast differentiation [81]. Rapamycin blocks osteoblast-specific gene expression, ALP activity, and mineralization of extracellular matrix in differentiating but not differentiated osteoblasts. In rat BMSCs, rapamycin does not demonstrate any spontaneous osteogenesis of MSCs, but inhibits osteogenic differentiation induced by dexamethasone [87]. In primary rat calvarial osteoblasts, inhibition of S6K1 kinase by rapamycin blocks osteogenic protein-1-induced ALP activity [88]. On the other hand, rapamycin has been reported to promote osteoblastic differentiation of human embryonic stem cells (ESCs) by blocking the mTOR signaling pathway and stimulating the BMP/Smad pathway [80]. Likewise, rapamycin treatment slightly increases osteopontin and osteocalcin mRNA expression in the presence of vitamin D3 in rat osteoblast-like ROS17/2.8 cells, a terminally differentiated osteosarcoma cell line expressing high levels of osteocalcin mRNA in the absence of ascorbic acid [89]. BEZ235, a newly developed dual PI3K and mTOR inhibitor, strongly promotes osteogenic differentiation in hMSCs. In addition, BEZ235 enhances *de novo* bone formation in calvarial organotypic cultures [90]. A role for PI3K/AKT/mTOR signaling in the maintenance of ESC pluripotency and survival has been demonstrated both in mice [91–94] and humans [95,96]. An essential role for mTOR in cell growth and proliferation was demonstrated in both early mouse embryos and ESCs by gene targeting technology [91]. Disruption of mTOR's kinase domain and treatment with rapamycin both result in decreased proliferation of mouse ESCs [91,92]. Studies have shown that the mTOR pathway plays important roles in proliferation, survival, and the maintenance of preadipocyte [97,98], osteoblast [81,99,100], and myoblast [101–103]. As to ESCs, rapamycin also inhibits the proliferation of MSCs [104].

While mTOR signaling may function to affect osteoblast proliferation and differentiation, conflicting results have been reported about whether inhibition of mTOR signaling by rapamycin decreases or increases osteogenesis.

mTOR and myogenesis

Most rat and mouse skeletal muscle cell lines proliferate under serum conditions and spontaneously differentiate after several days under low serum due to the autocrine expression of insulin-like growth factor (IGF) acting through the IGF receptor, as demonstrated by the alignment, elongation, and fusion of mononucleated myoblasts into multinucleated myotubes as well as by the induction of creatine kinase activity and caveolin-3 protein. This spontaneous differentiation can be accelerated by insulin included in the culture medium, IGF-I or IGF-II. Activation of the PI3K/S6K1 pathway is essential for IGF-stimulated differentiation [105,106]. In muscle cells, mTOR has been reported to be involved in the regulation of protein synthesis [107], vascular smooth muscle cell (VSMC) migration [108,109], VSMC phenotypic switching [110], and skeletal myocyte hypertrophy [111,112].

As a master regulator of cellular processes from growth and proliferation to survival and differentiation, mTOR has been found to be indispensable for the differentiation of C2C12 myoblasts [113,114]. Whether the kinase function of mTOR is required for myogenic differentiation is still controversial. It is reported that both the kinase function of mTOR and the N terminus (residues 11–91, containing part of the first HEAT domain) are essential for myogenic differentiation [115]. However, it has been demonstrated that neither S6K1 nor mTOR kinase activity is required for initiation of myogenic differentiation [116], although mTOR catalytic activity is required for a second-stage fusion that results in mature myotubes [117]. The initiation of differentiation of myoblasts as marked by expression of myogenin and the formation of nascent myotubes is independent of mTOR kinase activity, while a second-stage myocyte fusion that leads to formation of mature myotubes requires kinase activity of mTOR. An unknown secreted factor is proposed to mediate the function of mTOR in the second-stage fusion. Shu and Houghton [118] have shown that downregulation of rictor, a component of the mTORC2 complex, prevents terminal differentiation of C2C12 myoblasts, believing that mTORC2 signaling to AKT appears necessary for the downregulation of ROCK1 that occurs during myogenic differentiation.

Micro-RNAs (miRNAs) are a class of small non-coding RNAs that regulate protein expression mainly by targeting the 3' untranslated region of messenger RNAs. Several miRNAs have been recognized as important modulators in the development of skeletal and cardiac muscle [119]. mTOR controls MyoD-dependent transcription of miRNA-1 through its upstream enhancer, most likely by regulating MyoD protein stability. Moreover, a functional pathway downstream of mTOR and miR-1 is delineated, in which miR-1 suppression of histone deacetylase 4 results in the production of follistatin and subsequent myocyte

fusion [120]. A novel myogenic miRNA, miR-125b, declines during myogenesis and negatively modulates myoblast differentiation in culture and muscle regeneration in mice [121]. mTOR negatively control miR-125b biogenesis both *in vitro* and *in vivo* as a part of dual mechanism by which mTOR regulates the production of IGF-II, a master switch governing the initiation of skeletal myogenesis.

The effect of rapamycin on myogenesis has been examined in several muscle cell culture systems, and conflicting results have been reported. Depending on the cell types, rapamycin inhibits the differentiation of myoblasts [116,122,123] such as C2C12 or L6A1 myoblasts. Promotion of myogenesis by rapamycin in mouse BC3H1 muscle cells has also been reported [124], while rapamycin demonstrates no effect in rat L6E9, mouse Sol8 myoblasts, and human myoblasts [125]. It is worth noting that most data about myogenesis are based on the studies of precursor cells instead of the MSCs. Future studies should focus on the direct mechanism of mTOR involved in lineage determination of MSCs during development.

In summary, mTOR is indispensable for myogenesis. However, the precise mechanism of mTOR function remains to be explored. Dependent on the cell types, rapamycin has been reported to inhibit, promote, or have no effect on myogenesis.

Conclusions

By integrating signaling from extracellular growth factors and nutrients, mTOR is a central molecule in the regulation of cell growth in a wide variety of cells including adipocytes, osteoblasts, and myocytes. Future studies should aim to reveal the direct mechanism involved in the lineage determination of MSCs during development.

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