mTOR Pathway as a Target in Tissue Hypertrophy

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Abstract

Recent work has shown that the mTOR (mammalian target of rapamycin) pathway is an integral cell growth regulator. The mTOR pathway involves two functional complexes, TORC1 and TORC2, which have been defined by both their association with raptor or rictor, respectively, and their sensitivity to short-term rapamycin inhibition. Loss of tumor suppressors TSC1 or TSC2 leads to aberrant activation of TORC1, which has been implicated in the control of cell size. As a result, both physiologic and pathologic tissue hypertrophy are associated with TORC1 activation. Some clinical examples include skeletal and cardiac muscle hypertrophy, vascular restenosis, and compensatory nephrotic hypertrophy. Clarification of the mTOR pathway may lead to increased understanding of both the etiology and consequences of aberrant cell size regulation. This review covers some of the biochemical regulation of the mTOR pathway that may be important to the regulation of cell size, and it will present several potential clinical applications where the control of cell size may be biologically significant.

PATHWAY OVERVIEW OF mTOR/RAPTOR REGULATION

mTOR serves as a signal integrator of several upstream signals, including growth factors, nutrients, energy levels, and stress (1). Consequently, one of the critical functions of mTOR is to integrate these signals into a decision to positively or negatively influence cellular growth and proliferation, in other words, the size and rate of replication.

Tumor Suppressors TSC1 and TSC2 on mTOR

Most upstream regulators of mTOR appear to function through the tumor suppressors tuberous sclerosis complex 1 (TSC1) and tuberous sclerosis complex 2 (TSC2). TSC1 and TSC2 form a physical and functional complex, where mutation of either protein is sufficient to release mTOR from negative regulation. Functionally, TSC1 is thought to be the regulatory component, whereas TSC2 is thought to be the catalytic component. TSC1 has no obvious catalytic domain, but it contains a coiled-coiled domain (2). In contrast, TSC2 shows a C-terminal homology with Rap1GAP (3). In $TSC1^{-/-}$ MEFs, TSC2 levels are substantially decreased (4); however, $TSC2^{-/-}$ MEFs do not show significant reductions in TSC1 levels (5). It has been suggested that TSC1 levels are not significantly affected by the loss of TSC2 because TSC1 is capable of forming stable homodimers, whereas TSC2 is not (6). A possible mechanism by which TSC1 may stabilize TSC2 is through exclusion of the ubiquitin E3 ligase HERC1 (7). HERC1 binds to TSC2 and destabilizes it; however, in the presence of TSC1, HERC1 is unable to bind to the TSC1/TSC2 complex. Consequently, the stability of TSC2 is increased in the presence of TSC1. Another potential E3 ligase for TSC2 is protein-associated with Myc (PAM), which associates with TSC1/TSC2 in neurons and contains a ring zinc finger. Although PAM negatively regulates TSC1/TSC2, it has not been shown that PAM specifically modulates TSC2 stability (8). In *Drosophila* S2 cells, knockouts of *TSC1* also demonstrate significant reductions in TSC2 levels; however, unlike the results seen in mice, knockout of *TSC2* also decreases the levels of TSC1 (9). Despite the differences seen in mice and *Drosophila*, it is clear that both proteins are necessary for the proper regulation of mTOR. Therefore, loss of either TSC1 or TSC2 is generally considered to have similar effects on mTOR regulation.

Rheb GTPase

Although mTOR is tightly regulated by TSC1/TSC2, this regulation is indirect. Instead, TSC1/TSC2 regulates mTOR via the Ras-like GTPase, Rheb (Ras homolog enriched in brain). Rheb is a member of the Ras superfamily of GTPases; however, it is unique because it has low intrinsic GTPase activity. Therefore, the majority of Rheb is found in the GTP-bound form. Biochemically, TSC2 negatively regulates mTOR by functioning as a GTPase activating protein (GAP) for Rheb, thereby inactivating it (10–15). However, the relationship between Rheb-GTP and its effector mTOR is unique because both Rheb-GDP and the nucleotide-free form of Rheb bind to mTOR

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more strongly than Rheb-GTP (16–18). Consequently, it is still a matter of debate whether the activation of mTOR by Rheb is direct. Recently, it has been suggested that Rheb may directly activate mTOR. By using mutants of Rheb with different GTP loading percentages, it was shown that although nucleotide-free Rheb bound to mTOR more strongly than wild-type Rheb, the bound mTOR displays low in vitro kinase activity against S6 kinase 1 (S6K1), a direct target of mTOR. However, a Rheb mutant that was almost entirely associated with GTP showed greater in vitro mTOR activity against S6K1 than wild-type Rheb (16). In *Schizosaccharomyces pombe*, it was shown that hyperactive mutants of Rheb with high GTP binding are able to induce a phenotype similar to loss of TSC1 or TSC2. Compared with wild-type Rheb, these mutants had enhanced affinity for tor2, one of the two yeast homologues of mTOR (19). However, it has yet to be demonstrated that these Rheb interactions occur in a similar fashion in vivo in higher eukaryotes. Additionally, a guanine nucleotide exchange factor (GEF) for Rheb still remains to be identified; however, it has also been suggested that on account of Rheb's low intrinsic GTPase activity, it is possible that Rheb may not have an associated GEF (20, 21).

AKT as an Upstream Regulator of mTOR

Both growth factor and energy level stimulation influence mTOR activity through TSC2-Rheb (13, 22). Growth factor stimulation, such as insulin and IGF-1, primarily regulates mTOR signaling through PI3K-AKT. Activation of the insulin receptor leads to the activation of phosphatidylinositol 3-kinase (PI3K), which increases levels of PIP_3 and leads to activation of AKT. Overexpression of either active PI3K or myr-AKT, an active form of AKT, leads to increased phosphorylation of both eukaryotic initiation factor 4E binding protein 1 (4EBP-1) and S6K1, which are the two major targets of mTOR in translation regulation. However, this phosphorylation of 4EBP-1 and S6K1 can be inhibited by rapamycin, which is a specific inhibitor of mTOR. This inhibition by rapamycin can be rescued by coexpression of rapamycin-resistant mTOR mutants (23). The regulation of mTOR by PI3K-AKT occurs primarily through the phosphorylation of TSC2. The loss of the AKT phosphorylation sites on TSC2 increases the ability of TSC2 to inhibit mTOR, and consequently leads to increased S6K phosphorylation (24–26). Phosphorylation of TSC2 by AKT increases mTOR activity, and prevention of TSC2 GAP activity toward Rheb is necessary for the activation of mTOR. However, it remains to be shown that phosphorylation by AKT directly modulates TSC2 GAP activity. A recent report suggests that AKT regulates TSC2 activity by altering its localization (27, 106). In its hypophosphorylated form, TSC2 is associated with TSC1 at the membrane; however, upon phosphorylation by AKT, it is translocated away from the membrane without changing its intrinsic GAP activity toward Rheb. Bound by 14-3-3 proteins, AKT-phosphorylated TSC2 localizes to the cytosol, where physical separation prevents the inactivation of Rheb that is membrane associated (27). It is also interesting to note that conflicting reports exist regarding the role of TSC2 in mediating signaling between AKT and dTOR. In one report, TSC2 does not appear to be important for AKT to regulate dTOR. In *Drosophila* S2 cells, phosphomimetic mutations of the AKT phosphorylation sites on

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TSC2 (AA and DE) had no effect on binding to TSC1 or activation of S6K in response to insulin stimulation as compared with wild-type TSC2. Additionally, mutation of the AKT phosphorylation sites on TSC2 had no effect on *Drosophila* development (28). However, another group has suggested that AKT, TSC2, and dTOR behave more like their mammalian counterparts, where phosphorylation by AKT changes TSC2 localization and affinity for TSC1. Additionally, they also showed that the AKT phosphorylation sites are important for the regulation of cell size in the *Drosophila* eye (25); therefore, more studies are needed to better understand the relationship between AKT and TSC2 in the regulation of the mTOR pathway.

Downstream Targets of mTOR

Downstream of mTOR, two well-characterized targets are S6K1 and 4EBP-1 (1). As a result of tight regulation of these two proteins by mTOR, they are often used as functional readouts of mTOR activity. S6K1, which is phosphorylated and activated by mTOR, phosphorylates the ribosomal S6 protein, which is a component of the 40S ribosomal subunit. Activation of S6 leads to increased ribosomal biogenesis; however, interestingly enough, mutational loss of the S6K1 phosphorylation sites on S6 leads to increased global protein translation without increasing the percentage of ribosomes engaged in the polysomes (29). On the other hand, 4EBP-1 is inactivated by mTOR phosphorylation. 4EBP-1 in its hypophosphorylated form binds to and inactivates eIF4E, which is responsible for CAP-dependent translation (30). Therefore, inactivation/phosphorylation of 4EBP-1 by mTOR increases CAP-dependent protein translation.

Raptor as an Essential Component of the TORC1

For efficient phosphorylation of S6K1 and 4EBP-1, these downstream targets must associate with raptor, a scaffolding protein. However, the precise mechanism by which raptor mediates efficient phosphorylation between mTOR and its downstream targets is still not completely understood. Two models have been proposed to explain the mechanism by which raptor mediates signaling downstream of mTOR. The first model suggests that raptor and mTOR associate in two states with varying affinities, one that binds tightly and one that binds loosely. The loose-binding complex is the active complex and promotes efficient phosphorylation of mTOR targets; however, the tight-binding complex is formed in nutrient-poor conditions and inhibits mTOR kinase activity. Furthermore, overexpression of raptor increases the amount of mTOR found in the tight-binding complex, thereby explaining the observation that overexpression of raptor inhibits mTOR activity. However, it is interesting to note that rapamycin is able to disrupt the raptor-mTOR interaction regardless of nutrient status (31), but it is phosphate dependent. The second model suggests raptor simply acts as a scaffolding protein. However, raptor preferentially binds to unphosphorylated forms of mTOR targets and recruits the substrates to the mTOR complex for phosphorylation. Stimulation by insulin decreases the amount of raptor that can be coimmunoprecipitated with 4EBP-1, and mutation of the mTOR

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phosphorylation sites on 4EBP-1 to alanines increases the binding of 4EBP-1 to raptor, whereas mutation to glutamic acid reduces the binding to raptor (32). Two motifs on the substrates are important for activation by mTOR, the Tor signaling (TOS) motif and the RAIP (named by its amino acid sequence) motif. The TOS motif is believed to be the site by which the mTOR/raptor complex interacts with its downstream target (33–35) However, it appears that the RAIP motif operates via promotion of mTOR-dependent phosphorylation (36, 37).

Disruption of mTOR/Raptor by Rapamycin

The study of the mTOR pathway has been greatly facilitated by the availability of a specific and potent inhibitor, rapamycin. Rapamycin was originally identified in *Streptomyces hygroscopicus*, and to date there are no other known targets for rapamycin. This specificity is conferred by the use of an intermediary to inhibit mTOR. Rapamycin first complexes with the immunophillin FK506 binding protein 12 (FKBP12), and the rapamycin-FKBP12 complex binds and inhibits mTOR (38). As the name suggests, FKBP12 also binds to FK506, an inhibitor of the calcineurin pathway. In the presence of both FK506 and rapamycin, there is competition for binding to FKBP12; therefore, in large excess of FK506, rapamycin is unable to inhibit mTOR. It is believed that the rapamycin-FKBP12 complex prevents the association between mTOR and raptor; therefore, downstream targets that depend on raptor binding are specifically inhibited (31, 32). The downstream targets S6K1 and 4EBP-1 are two such targets that depend on raptor for efficient phosphorylation by mTOR, and thereby their phosphorylation is inhibited in the presence of rapamycin. However, rapamycin has little effect on intrinsic mTOR kinase activity (39).

Regulation of the Translation Initiation Complex Via eIF3

Recently, it has also been suggested that mTOR's role in translation initiation can be mediated through the eIF3 translation initiation complex. eIF3 is one of the largest initiation factors, with at least 12 different subunits (40). eIF3 binds to the 40S ribosomal subunit, to which S6 is a component. Binding of eIF3 to the 40S subunit inhibits premature association with the 60S ribosomal subunit. In addition, eIF3 also enhances initiation by increasing the binding of the ternary complex (41). Under serum starvation or rapamycin inhibition, S6K1 binds tightly to eIF3; however, upon insulin stimulation, S6K1 dissociates from eIF3. This association with eIF3 is disrupted by the phosphorylation of the hydrophobic motif on S6K1 (T389); thus, either phosphorylation by mTOR or phosphomimetic mutation seems to be sufficient to decrease the binding affinity between S6K1 and eIF3 (42). Upon release, S6K1 can be further phosphorylated and activated by PDK1 in a manner dependent on the hydrophobic motif phosphorylation. The fully activated S6K1 is then free to phosphorylate downstream targets (43, 44).

On the other hand, the association between eIF3 and mTOR changes with activation or inhibition of mTOR. Serum starvation and rapamycin treatment reduce the binding affinity between eIF3 and mTOR/raptor, whereas insulin stimulates binding

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between eIF3 and mTOR/raptor. Increases in interaction between mTOR/raptor and eIF3 by insulin stimulation may also help mediate efficient phosphorylation of 4EBP-1 by bringing the translation initiation machinery into proximity of the mTOR complex (42).

Additionally, insulin may stimulate the association of eIF3 with eIF4G in an mTOR-dependent manner. eIF4G is a scaffold protein that helps the formation of the eIF4F complex. The eIF4F complex binds to the 5′CAP on mRNAs to promote efficient translation, and it consists of eIF4E, eIF4A, and eIF4G. Although mTOR regulates eIF4E through 4EBP-1, it appears that binding between eIF3 and eIF4G is independent of eIF4E. Insulin is able to stimulate the binding of eIF3 and eIF4G in the absence of eIF4E binding. Although it has been reported that eIF4G is phosphorylated in a rapamycin-reversible fashion on three phosphorylation sites (S1108, S1148, and S1192) (45), binding to eIF3 is not correlated to the phosphorylation of S1108; however, correlation to the other phosphorylation sites is still unknown (46). Although it appears that eIF3 binds to eIF4G in an mTOR-dependent fashion, the specifics of this regulation are yet to be elucidated. For example, it still remains to be determined the mechanism by which mTOR regulates eIF3 and eIF4G binding, and whether phosphorylation of eIF4G is of any physiological significance.

Negative Feedback of the mTOR Pathway Via Phosphorylation of IRS-1

Regulation of the AKT-TSC2-mTOR pathway has been further complicated by the discovery of feedback inhibition on the pathway by both S6K1 and mTOR on insulin receptor substrate 1 (IRS-1). IRS-1 and IRS-2 are responsible for conveying downstream signaling upon stimulation of the insulin receptor (IR). When fed a high-fat diet, wild-type mice showed increased activation of S6K1 but decreased phosphorylation of AKT in response to insulin; however, in S6K1$^{-/-}$ mice, a high-fat diet did not lead to insulin resistance. In wild-type mice fed the high-fat diet, phosphorylation on IRS-1 was also increased, which was absent in S6K1$^{-/-}$ mice (47). It was also shown that activation of PI3K by insulin was dependent on TSC2. In the TSC2$^{-/-}$ MEFs, S6K1 activity is highly upregulated, and IGF-1 stimulation yields a muted AKT phosphorylation. However, the phosphorylation of AKT in response to IGF-1 could be restored in the TSC2$^{-/-}$ MEFs by prolonged pretreatment with rapamycin (48, 49). IRS-1 was identified as a novel S6K1 target in vitro, and inhibition of IRS-1 phosphorylation could be seen in vivo with the addition of rapamycin or RNAi of S6K1 but not S6K2. Phosphorylation of IRS-1 by S6K1 blocks its function. In addition to phosphorylation of IRS-1 by S6K1, IRS-1 mRNA is also decreased in TSC2$^{-/-}$ MEFs, and treatment with rapamycin or RNAi of either S6K1 or S6K2 can restore IRS-1 mRNA (48). However, IRS-1 protein levels in S6K1$^{-/-}$ and wild-type mice are similar (47). In addition to phosphorylation by S6K1, IRS-1 can also be directly phosphorylated by mTOR/raptor on sites differing from the S6K1 phosphorylation site. However, phosphorylation by mTOR/raptor also decouples IRS-1 from insulin

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signaling. IRS-1 is phosphorylated in vitro by the immunoprecipitated mTOR/raptor complex, which may also contain S6K1. In vivo, this phosphorylation could be inhibited by cotransfection of either kinase-dead mTOR or kinase-dead S6K1. However, even in the presence of rapamycin-resistant S6K1, which does not need mTOR/raptor for activation, phosphorylation of the putative mTOR sites can still be inhibited in a rapamycin-dependent manner. Furthermore, phosphorylation of those sites is eliminated by knockdown of raptor even in the presence of rapamycin-resistant S6K1. Together, this suggests that IRS-1 may also be a direct target of mTOR (22). It is possible that the phosphorylation on the S6K1-dependent site may influence subsequent phosphorylation by mTOR/raptor.

TORC2: AN mTOR/RICTOR COMPLEX

Recently, the understanding of mTOR signaling was greatly enhanced by the discovery of a new mTOR binding partner that displaces raptor and changes its downstream specificities. The identification of rictor (rapamycin-insensitive companion of mTOR) demonstrated new functions of the mTOR pathway that were not originally recognized owing to the insensitivity to rapamycin inhibition; however, a recent report has suggested that this may not necessarily be the complete story (50). It appears that the effect of rapamycin on mTOR-rictor may depend on both cell type and duration of treatment. However, mTOR-rictor is resistant to short-term (<2 h) rapamycin treatment; therefore, unless otherwise noted, the rapamycin effects on mTOR-rictor refer to short-term rapamycin treatment (see below for the exception). Under certain lysis conditions, it was shown that rapamycin can specifically disrupt the mTOR-raptor interaction without disrupting the mTOR-rictor interaction. When treated with rapamycin, immunoprecipitation of FKBP12 is capable of coimmunoprecipitating mTOR/raptor but not mTOR/rictor (51). In addition to rapamycin resistance, the rictor-mTOR interaction is also unaffected by leucine levels and mitochondrial inhibition, which modulate S6K1 and 4EBP-1 phosphorylation and change the raptor-mTOR interaction. Not only does mTOR/rictor not phosphorylate S6K1 and 4EBP-1, but binding of raptor or rictor is mutually exclusive. This implies that there might be a degree of competition between raptor and rictor for mTOR. With the discovery of rictor, mTOR signaling can now draw greater analogy to yeast TOR signaling. In *Saccharomyces cerevisiae*, two distinct *Tors* and TOR complexes (TORCs) exist (52). Either Tor1 or Tor2 can be used to form TORC1, which is sensitive to rapamycin inhibition, but only Tor2 can be used to form TORC2, which is resistant to rapamycin inhibition. Although in mammals there is only one mTOR, it is now understood that mTOR participates in two distinct functional complexes, mammalian TORC1 (mTORC1) and mammalian TORC2 (mTORC2). Both mTORC1 and mTORC2 share mTOR and mLST8; however, their downstream specificities are predicted by association with raptor and rictor, respectively. In the case of mTORC1, raptor is responsible for binding to mTORC1 substrates; however, it is still unreported whether rictor is responsible for mTORC2 substrate specificity.

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Downstream Targets of mTORC2

The downstream functions of mTORC2 are less well characterized than mTORC1. Two such effects are actin cytoskeleton regulation and AKT regulation. However, studies on regulation of the actin cytoskeleton have yielded conflicting reports, as, depending on context, knockdown of rictor can either stimulate or inhibit actin cytoskeleton organization. In yeast, rapamycin-resistant TORC2 regulates the actin cytoskeleton through PKC1. It was reported that RNAi knockdown of rictor in HeLa cells changed the cell morphology and induced actin stress fiber formation. Additionally, knockdown of rictor also changed the localization of paxillin, an adaptor protein found at the actin/plasma membrane junction. However, although knockdown of PKCα, the mammalian homologue of PKC1, yields a similar actin morphology to the rictor knockdown, the stress fibers in the PKCα knockdown appeared better organized (53). It was also shown that reintroduction of serum to serum-starved cells induces the formation of stress fibers and induces cell spreading, which is not preventable by pretreatment with rapamycin. In this situation, knockdown of rictor but not raptor by RNAi reduced tyrosine phosphorylation on paxillin and reduced cell spreading and stress fiber formation. The effect of rictor RNAi could be reversed by active Rac-GTP (51). Similarly, in yeast, disruption of the TORC2 complex leads to actin depolymerization (52).

It has been convincingly demonstrated that mTORC2 acts as the PDK-2 on AKT to allow full activation of AKT. This was shown by decreased phosphorylation of AKT on S473 by RNAi of rictor but not raptor in both mammalian and *Drosophila* cells. Additionally, AKT is phosphorylated in vitro by immunoprecipitated mTOR/rictor complexes but not mTOR/raptor complexes (54). Consequently, this implies that mTOR may influence the growing list of AKT functions, which include antiapoptotic, cell proliferative, and metabolic roles (55). However, because AKT is a one of the important activators of TORC1, it is no longer accurate to think of AKT as an upstream regulator of mTOR. Instead, AKT exists both upstream and downstream of mTOR. The significance of this mutual regulation between AKT and mTOR is still not fully understood and is undergoing further study. Furthermore, knocking down the rictor complex decreases AKT activity but not S6K1 phosphorylation. This provides evidence against the assertion that AKT functions upstream of mTORC1. Although, it is possible that nutrient-dependent activation of mTORC1 can compensate for decreases in PI3K-AKT signaling. Additionally, it is still not clear whether mutual regulation of mTORC1 and mTORC2 is dominated by direct competition for mTOR or dominated by negative feedback via IRS-1.

It has also been proposed that the substrate specificity for mTORC1 and mTORC2 is partially mediated by sequences on the substrate, including the TOS motif and the C terminus, which in the case of S6K1 is different from other AGC family members. S6K1, which is usually an mTORC1 target, can be made resistant to rapamycin by deletion of both the TOS motif and the C terminus after the hydrophobic motif. The TOS motif is thought to facilitate mTORC1 binding, whereas the C terminus protects the substrate from phosphorylation by mTORC2.

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Interestingly, deletion of the C terminus of S6K1 renders the C-terminal end of S6K1 very similar to AKT. It has been proposed that other TORC2 substrates will have C-terminal ends similar to AKT and the truncated S6K1; however, these other targets have yet to be identified in mammals (56). In yeast, Ypk2 has been identified as a direct target of Tor2. Ypk2 is homologous to serum/glucocorticoid kinase (SGK), which is closely related to AKT and a member of the AGC kinase family. The AGC kinase family also includes S6K1. Truncation of the autoinhibitory domain of Ypk2 is able to suppress both the actin polymerization defects and lethality associated with loss of Tor2. However, Ypk2 is unable to restore defects conferred by loss of Tor1. Together, this suggests that Ypk2 may be an important downstream target of TORC2 in yeast; but, it has yet to be shown that SGK plays a similar role in higher eukaryotes (57).

It is apparent that rictor is critical for TORC2 function, but it is still unclear how rictor influences TORC2 activity. Unlike raptor, it has yet to be shown how rictor mediates the phosphorylation of downstream substrates by mTOR. In addition, rictor is also phosphorylated in a PI3K-dependent manner; yet, it is unknown what role phosphorylation of rictor may play in the mTOR pathway (53).

Long-Term Rapamycin Treatment Negatively Regulates TORC2

Recently, it was reported that prolonged treatment with rapamycin inactivates TORC2 in addition to TORC1. Rapamycin inhibition of TORC1 occurs within 30 min. This inhibition is associated with decreases in phosphorylation of TORC1 targets, such as S6K1, and disruption of the mTOR/raptor complex. However, in certain cell types, rapamycin can also inhibit TORC2, as seen by decreased phosphorylation of the TORC2 target AKT and disruption of the mTOR/rictor complex. This inhibition occurs on the order of 24 h, which suggests that the mechanism by which rapamycin inhibits TORC2 may not be identical to the mechanism for TORC1 inhibition. FKBP12/rapamycin is unable to bind to mTORC2; however, it is capable of binding free mTOR. It has been proposed that association of free mTOR with FKBP12/rapamycin may preclude formation of mTORC2; yet, FKBP12/rapamycin does not disrupt the intact mTORC2. Therefore, inhibition of mTORC2 by rapamycin may require the turnover of existing mTORC2 before mTORC2 levels drop below the threshold necessary for AKT phosphorylation; however, this model has yet to be conclusively shown. Alternative explanations for this effect include transcriptional or translational regulation of an integral TORC2 component or the induction of an inhibitor of rictor by rapamycin treatment, but this all is merely speculation. Interestingly, both HeLa cells and HEK293 cells, which are commonly used for many experiments in the field, are resistant to inhibition of TORC2 by prolonged rapamycin. A variety of cell types are sensitive, including lymphocyte cells lines (BJAB, U937, Jurkat, SKW3), a glioblastoma cell line (U87), a melanoma cell line (UACC-903), a muscle tubule cell line (C2C12), an endothelial cell line (HUVEC), and a prostate cancer cell line (PC3). The mechanisms for this cell-type specificity are still unknown and would be of great interest (50).

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CELL SIZE CONTROL MECHANISMS

Cell Size Versus Cell Cycle Control

An interesting idea that has been suggested is that the role of mTOR on cell growth is independent of its role in proliferation. In other words, cell cycle and cell size may be controlled independently. PTEN is a tumor suppressor that is commonly mutated in many hamartoma syndromes, such as Cowden disease, Lhermitte-Duclos disease, Bannayan-Zonana syndrome, and *Proteus* syndrome, and several malignant cancers, such as glioblastomas, endometrial carcinomas, melanomas, and advanced prostate adenocarcinomas. Biochemically, PTEN functions as a lipid phosphatase and dephosphorylates PIP_3. Consequently, PTEN negatively regulates AKT, which in turn can lead to negative regulation of mTOR, thereby providing a possible etiology for the hamartoma syndromes. It was observed that in human cancer cells where PTEN was knocked out ($PTEN^{-/-}$), cells underwent a rapid increase in size after irradiation as compared with wild-type counterparts ($PTEN^{+/+}$). Interestingly, irradiation led to cell cycle arrest in both $PTEN^{+/+}$ and $PTEN^{-/-}$ cells via P53-dependent pathways; however, cell size was only increased in the $PTEN^{-/-}$ cells. Implication of mTOR's role in the decoupling of cell size and cell cycle in PTEN mutants is shown by pharmacological recovery of cell size control. Both inhibition of PI3K via wortmannin and inhibition of mTOR via rapamycin lead to decreases in cell size in the irradiated $PTEN^{-/-}$ cells. It is worth noting that wortmannin treatment was able to reduce cell size to the wild-type levels; however, rapamycin only led to a partial recovery in cell size. This may imply that the sensing necessary for PTEN-mediated cell size control may be predominantly due to PIP_3 regulation; however, execution of cell size control is only partially mediated through rapamycin sensitive mTOR targets. Furthermore, it was also shown that cells subjected to TSC2 RNAi were of similar size to cells subjected to PTEN RNAi; however, the reduction in TSC2 and PTEN levels were not dramatic enough to draw firm conclusions (58). In the brain, it appears that the relationship between mTOR and PTEN in cell size regulation is variable. In $PTEN^{-/-}$ mice, both the soma in the dentate gyrus and the cerebellum show increased cell size compared with $PTEN^{+/+}$ mice; however, low-dose treatment with a rapamycin analogue reduced the cell size in the dentate gyrus to wild-type levels, but it had little effect on the cell size in the cerebellum. Further treatment at high doses helped reduced the size of cerebellar soma, but not to the size of wild-type mice. This variability may be the consequence of differences in bioavailability, as levels of S6 phosphorylation were not reduced as significantly in cerebellum (59). On the other hand, in *Drosophila*, knockdown of either dPTEN or dTSC1 is sufficient to increase cell size; however, a double knockdown of dPTEN and dTSC1 has additive effects on cell size regulation. This further suggests that in *Drosophila*, the pathways may have independent components in the regulation of cell size (60). It may also highlight the differences in the regulation of TSC2 by AKT in *Drosophila* as seen by mutations of the AKT phosphorylation sites on TSC2 (28). Loss of either dPTEN or dTSC1 can lead to increases in cell size; however, a report has suggested that only knockdown of dTSC1 leads to increases in dS6K (61), whereas other reports have also seen increases

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in dS6K with the knockdown of dPTEN (54, 62). It is possible that dTSC1 regulates cell size in a dTOR-dependent manner, whereas dPTEN partially regulates cell size in a dTOR-independent manner (61).

Cell Size Control Downstream of mTOR

Roles of the mTOR-S6K pathway in cell size regulation. It is quite clear that mTOR is important for cell size regulation, as seen by both genetic perturbation of mTOR and pharmacologic inhibition of mTOR. Shown more directly, loss of dTOR leads to a decrease in larvae size; however, the larvae fail to mature and die before reaching adulthood. In mosaic *Drosophila*, loss of dTOR leads to a decrease in cell size while maintaining the general organization of the tissue (63, 64). However, it is less clear how cell size is regulated downstream of mTOR. One of the most potent candidates in this regulation is S6K. In *Drosophila*, knockout of *S6K* results in high rates of embryonic lethality. In the surviving adults, however, there is a decrease in body size. On the other hand, two S6K homologues exist in mammals, namely S6K1 and S6K2. Although the two homologues seem to be regulated in a similar fashion, including rapamycin sensitivity, they exhibit different localizations; S6K1 is primarily cytoplasmic, whereas S6K2 is primarily nuclear (65). Either homologue of S6K is sufficient for phosphorylation of S6; however, full phosphorylation of S6 requires both S6K1 and S6K2. Additionally, knockout of either *S6K1* or *S6K2* also has different effects on animal size. Knockout of *S6K1* results in animals of decreased cell size as compared with wild type; however, knockout of *S6K2* results in animals slightly larger than wild type. Double knockouts of *S6K1* and *S6K2* yield animal sizes similar to the single knockouts of *S6K1*; however, double knockouts also experience high levels of embryonic lethality. Surprisingly, in the double knockouts for *S6K*, phosphorylation of the S6K sites on S6 still can be seen. This may be due to redundant regulation by the RSK pathway on S6 (66).

Role of downstream proteins of S6K in cell size control. Characterization of S6 has also yielded more information about the regulation of cell size. Mice with a knockin of S6 to which the phosphorylation targets of S6K were mutated to alanines ($S6^{P-/-}$) were characterized. When compared with wild-type MEFs, $S6^{P-/-}$ MEFs were significantly smaller; however, unexpectedly, these cells also showed increased rates of protein synthesis and cell division. Additionally, to distinguish between the possibilities that the reduction in cell size could be due to either a failure to grow or a secondary effect of accelerated cell cycle progression, cells were arrested by blocking DNA synthesis with aphidicolin. Cell cycle arrest was unable to eliminate the difference in cell sizes. From this data it was apparent that the decrease in cell size was independent of cell cycle progression; thus, this implies that elimination of the S6 phosphorylation sites affected the ability of the cells to grow. To further separate the effects of cell growth and proliferation, the $S6^{P-/-}$ cells were treated with rapamycin. The $S6^{P-/-}$ cells failed to undergo any further reduction in cell size when treated with rapamycin; however, they experienced a decreased rate of cell

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cycle progression. These experiments imply that mTOR-mediated cell size regulation functions primarily through S6 (29). However, the role of S6 in mediating ribosome biogenesis may be distinct from its role in regulating cell size. In mouse T cells with only one copy of the S6 allele, ribosome biogenesis is inhibited; however, stimulation of the T cells by anti-CD3 and anti-CD28 leads to no difference in the increases in cell size in T cells with one copy of S6 versus wild type. However, loss of one copy of S6 leads to a failure to proliferate by activation of a P53-dependent checkpoint (67). It is not yet clear whether this phenomenon will also be seen in other cell types.

Although reports suggest S6 plays a major role for controlling cell size regulation by mTOR, SKAR (S6K1 Aly/REF-like target), another target of S6K, is also involved in cell size regulation. RNAi of SKAR leads to a reduction in cell size; however, the reduction in cell size is not as dramatic as RNAi of S6K1. It has not yet been determined whether this difference in cell size is due to efficiencies in RNAi or due to other molecules being involved in cell size signaling. As mentioned earlier, knockouts of *S6K1* are smaller; however, knockouts of *S6K2* show little change in cell size. It was also shown that SKAR binds and is phosphorylated by S6K1 but not S6K2, which provides circumstantial evidence that it might be involved in mediating S6K1's regulation of cell size (68).

Despite these studies, it is still not fully understood how the downstream targets of S6K regulate cell size. It is likely that both SKAR and S6 play a role in cell size regulation; however, SKAR probably still needs to be further characterized to better understand its functional role in cell size regulation. On the other hand, it still remains to be reconciled how phosphorylation of S6 by S6K1 yields a phenotypic outcome different from phosphorylation by S6K2.

CLINICAL CORRELATIONS

As a mediator of cell size and cell growth, the mTOR pathway has many functions in cellular homeostasis. However, it is also quite interesting that mTOR has a functional role in the physiological modulation of cell size. The physiological manipulation of cell size is perhaps most apparent in the development of muscle to which loads and strains often lead to increases in muscle mass to compensate for increases in demand.

Skeletal Muscles

In skeletal muscle models, it has been shown that IGF-1 has a role in myogenic induction that is independent of IGF-1's role in maturation (69). Additionally, IGF-1 also plays a role in the prevention of skeletal muscle atrophy induced by angiotensin II (70). However, only more recently has the molecular mechanism for this induction been better characterized. Because IGF-1 induced muscle hypertrophy could be blocked by Cyclosporin A (CsA), a calcineurin inhibitor, it was believed that the calcineurin pathway was responsible for muscle hypertrophy. However, calcineurin is also required for myocyte development; therefore, the calcineurin pathway may not be the sole mediator of skeletal muscle hypertrophy. Recent studies have implied that the AKT-TSC-mTOR pathway may also play a role in skeletal muscle hypertrophy.

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Treatment of mature myocytes with IGF-1 leads to the induction of the mTOR pathway, and conversely overexpression of AKT also leads to myocyte hypertrophy (71). To further distinguish whether IGF-1-associated skeletal muscle hypertrophy was due to calcineurin or AKT/mTOR, animals were treated with CsA or rapamycin. At concentrations sufficient to inhibit cardiac hypertrophy, CsA was unable to inhibit functional overload-induced skeletal muscle hypertrophy. However, treatment with rapamycin was able to inhibit the compensatory hypertrophy. Additionally, muscle recovery after induced atrophy was inhibited by rapamycin but not CsA (72). Further study of the roles of calcineurin and AKT-mTOR has shown that signaling through calcineurin predominately affects the types of muscle fibers generated, while it has little effect on the size of the muscle fibers. On the other hand, activation of AKT by transfection of myr-AKT or innervation and electrical stimulation of regenerating muscles has little effect on the specification of muscle fiber type; however, it leads to increases in muscle fiber size, which can be inhibited with rapamycin. This suggests that the hypertrophic effect on muscle fibers by AKT predominately signals through mTOR (73).

Clinically, the effects of mTOR activation can be seen in differences in exercise training. Endurance training generally promotes mitochondrial biogenesis and muscle fiber switch from fast twitch to slow twitch fiber types, whereas resistance training has little effect on fiber-type selection; however, there is a stimulation of protein synthesis. The effects of exercise training can be mimicked by electrical stimulation ex vivo. Low-frequency electrical stimulation (LFS) for long periods of time has been shown to promote muscle changes similar to endurance training, whereas short, intermittent, higher-frequency electrical stimulation (HFS) has been shown to cause muscle changes similar to resistance training. It was shown that HFS induces the activation of the mTOR pathway as seen by phosphorylation on AKT, TSC2, mTOR, S6K1, and 4EBP-1; however, LFS caused no change in the phosphorylation of AKT, mTOR, and S6K, and perhaps also a modest decrease in the phosphorylation of TSC2 and 4EBP-1. Therefore, it is possible that resistance training may cause differential activation of the mTOR pathway to generate the physiological consequences of muscle hypertrophy (74).

Similar to MEFs derived from knockout mice, S6K1$^{-/-}$ myocytes also show decreases in cell size, whereas S6K2$^{-/-}$ myocytes have sizes similar to that of wild-type cells, and double knockouts of S6K1 and S6K2 yield myocytes of similar size to S6K1$^{-/-}$ cells. Despite decreases in cell size, the cell number per muscle fiber remains unchanged in the S6K1$^{-/-}$ cells. Also similar to the manipulation of AKT, knockout of either S6K1 or S6K2 had little effect on the type of muscle fiber specified, only the cell size (75).

Clinically, it has been suggested that age-related muscle loss may be due to decreased activation of S6K1. When comparing different age groups, younger (mean age = 25) individuals have high protein synthesis in muscles compared with older (mean age = 72) individuals when the muscles were stimulated by infusion of insulin and amino acids. Moreover, insulin and amino acid stimulation lead to phosphorylation of the AKT, mTOR, 4EBP-1, and S6K1 in younger individuals; whereas in older individuals the response was similar except S6K1 was not phosphorylated (76).

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Although this data suggests an attractive model for age-related muscle atrophy, the correlation is still rather tenuous. Further study is needed to firmly establish that failure to activate S6K1 is responsible for age-related muscle atrophy.

Cardiac Muscles

Similar to the effects seen in skeletal muscle, rapamycin may also have an effect on cell size in cardiac myocytes. In response to increased load and demand on the heart, the cardiac myocytes often increase in size and lead to hypertrophy of the heart. Although these compensatory measures help the heart gain physiological function in the short term, cardiac hypertrophy leads to increased morbidity and mortality; therefore, treatments such as beta-blockers, which decrease the effort exerted by the heart, have been the standard of care to prevent further exacerbation of heart disease. Like skeletal myocytes, cardiac myocytes treated with rapamycin also show decreased cell size in response to growth factors. More important perhaps is that cardiac hypertrophy owing to increased load on the heart may also be inhibited by rapamycin. In mice, cardiac hypertrophy owing to increased load can be induced by ligation of the aorta. Ligation of the aorta leads to increased S6 phosphorylation, which returns to basal levels after a week postoperation. This upregulation of S6 phosphorylation is also coupled with cardiac hypertrophy as measured by heart weight; however, treatment with rapamycin prior to ligation is able to decrease cardiac hypertrophy in the ligated animals (77). Additionally, in mice with preexisting cardiac hypertrophy owing to aortic ligation, rapamycin was also able to significantly decrease the heart weight to body weight ratio. Although rapamycin significantly reduced the size of both compensated hypertrophy and decompensated hypertrophy, the reduction was more dramatic in the case of compensated hypertrophy (40% versus 70% reduction, respectively). In decompensated hypertrophy, the mice are showing signs of heart failure. However, neither the etiology of compensated versus decompensated hypertrophy nor the mechanism for differences in rapamycin response are well understood. Additionally, in mice with decompensated hypertrophy, rapamycin treatment helped regain heart function as seen by decreased left ventricular end-systolic dimensions, increased fractional shortening, and increased ejection fraction (78).

Even though the mTOR pathway seems to be implicated in cardiac hypertrophy, it would be unfair to neglect mentioning that perhaps the pathway most well characterized for its role in cardiac hypertrophy is the MAPK pathway. Activation of the MEK/ERK MAPK pathway seems to be critical for cardiac hypertrophy as a response to activation by phenylepherine (PE). S6K2, which is activated by insulin signaling, is also activated by PE. It has also been shown that rapamycin treatment is capable of significantly reducing protein synthesis induced by PE, but the inhibition is not complete, which implies that PE may stimulate protein synthesis by both mTOR-dependent and mTOR-independent pathways (79). Additionally, the activation of protein synthesis by PE occurs in a PI3K/AKT-independent manner (80). Because the MAPK-activated Kinase RSK1 was shown to phosphorylate TSC2 and thereby inhibit its activity (81), it was proposed that inactivation of TSC2 by RSK1

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was the mechanism by which PE promotes mTOR-dependent protein synthesis (82). However, it is also possible that PE promotes mTOR-dependent protein synthesis via ERK, as phosphorylation by ERK also inactivates TSC2 (83). Taken together, this cross-talk between the MAPK pathway and the mTOR pathway may be important for the induction of cardiac hypertrophy.

Smooth Muscles

Angiotensin II (Ang II) has been shown to be important for the induction of smooth muscle hypertrophy and proliferation. However, the effects of Ang II are not generalizable across all smooth muscle vessels. The effect of Ang II on blood vessels and airways seem to be dependent on species and vessel of origin. Consequently, this complexity of regulation has added difficulty in the study of smooth muscle proliferation, but conversely this variability in response may one day prove to be useful for targeted therapies. In human coronary arteries and the saphenous vein, it has been shown that Ang II is capable of inducing smooth muscle hypertrophy independent of cell proliferation. However, in rats, Ang II stimulated smooth muscle hypertrophy in the aorta but also induced cell proliferation in arterioles. To date, the etiology of these differences is not clearly understood. However, it has been suggested that the differences between the growth versus proliferation response may be due to relative activation of mTOR versus ERK pathways in response on Ang II. Treatment of human saphenous vein cultures with Ang II leads to hypertrophy without increases in proliferation, which was coupled with poor activation of ERK. However, PDGF, which strongly activates ERK in saphenous vein cultures, induced cell proliferation. In rat aortic smooth muscle cells, treatment with Ang II led to increased protein synthesis and phosphorylation of S6K1, both of which could be decreased by similar levels when using equal amounts of rapamycin (84). Similarly, in human coronary artery smooth muscle cells, Ang II leads to increased protein synthesis, which is indicative of cellular hypertrophy, which may be a cause of vascular wall thickening. This increase in protein synthesis was associated with activation of the AKT-mTOR signaling pathway, which thereby showed inhibition by both rapamycin and PI3K inhibitors (85).

It is therefore attractive to postulate that inhibition of protein synthesis is the mechanism by which rapamycin-eluding stents are capable of preventing restenosis. In the European arm of a double-blind study, it was shown that stenting of small coronary arteries with rapamycin-eluding stents as compared with bare wire stents led to patients with larger minimum lumen sizes at 8 months (2.22 mm versus 1.33 mm), less major cardiac events at 9 months (8% versus 22.6%), and lower need for revascularization (4% versus 20.9%) (86). Published concurrently, the American arm of the study showed similar efficacy of rapamycin-eluding stents (87). However, critics of the study have suggested that the endpoint used for efficacy, major cardiac events, is based on the need for revascularization, which skews the endpoint toward the measurement of lumen size as opposed to clinical efficacy (88). The two-year follow up of the study demonstrated the effects on restenosis were maintained after two years; however, despite preventing the need for revascularization, rapamycin-eluding stents

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had no effect on mortality or incidence of myocardial infarction (89). From this study it is not apparent whether this is due to lack of statistical power or lack of clinical efficacy, so larger studies must be done to clarify these findings.

Asthma is a disease of bronchoconstriction and inflammation, which can be further characterized into two subtypes. Type I asthma shows smooth muscle hyperplasia around the central bronchi. Type II asthma shows only mild hyperplasia around the central bronchi and involves smooth muscle hypertrophy throughout the bronchioles (90). Treatment with a rapamycin analogue has shown some efficacy in the treatment of severe asthma; however, this has mostly been attributed to the antiinflammatory effect of rapamycin (91). More recently, it has been suggested that effectiveness of rapamycin analogues on asthma may involve more than rapamycin's immunosuppressive role. When S6K1 is activated in the absence of serum, smooth muscle cells increase in cell size and levels of smooth muscle myosin heavy chain (smMHC) also increase. Conversely, inhibition by rapamycin or a PI3K inhibitor leads to decreases in development of long contractile smooth muscles.

The role of the mTOR pathway has been of particular interest in regard to smooth muscle proliferation because the clinical presentation of lymphangioleimyomatosis (LAM) in tuberous sclerosis (TSC) patients. In addition to TSC, mutations of TSC2 have also been connected to lymphangioleimyomatosis. This rare lung disease results in the invasion and proliferation of LAM nodules in the lungs. These nodules contain a mixture of both smooth muscle and melanocytes, and consequently, this overgrowth of cells leads to severe dyspnea and decreased pulmonary function. In primary cultures of LAM nodules, either adding back of wild-type TSC2 or treatment with rapamycin can decrease aberrant phosphorylation of mTOR targets and decrease the mutation-associated increases in DNA synthesis (92, 93). It is attractive to speculate whether rapamycin may also have a beneficial role in the treatment of smooth muscle hypertrophy associated with LAM.

Beyond Hypertrophy

Rapamycin analogues. In addition to the traditional use of rapamycin as an agent for immunosuppression, recently, many clinical studies have also been conducted on the use of rapamycin and its analogues as an antineoplastic agent. Perhaps the best characterized rapamycin analogues include CCI-779 (Wyeth Ayerst), RAD001 (Novartis Pharma), and AP23573 (Ariad Pharma). Although all of these compounds are effective at inhibiting mTOR, the new analogues that have been developed have favorable pharmacologic properties that may prove to be useful for therapy. Interestingly, intermittent administration of these analogues did not result in immunosuppression. However, most clinical trials involving rapamycin and its analogues are still in either Phase I or Phase II trials. The studies that have already reached Phase III include the following studies: On Aug 18, 2005, AP23573 was approved for the treatment of soft-tissue and bone sarcomas by the FDA. Additionally, a Phase III clinical trial on the concurrent use of CCI-779 with interferon therapy on advanced renal carcinomas has just been completed; however, the results have not yet been published. A Phase III study of CCI-779 in addition to letrozole as first-line hormone therapy

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for metastatic breast cancer was terminated before completion. Currently, patients for Phase III clinical studies are being recruited to study the effects of CCI-779 on mantle cell lymphoma and also to see the secondary effects on skin cancer in kidney transplant recipients who received rapamycin as therapy. It has been suggested that tumors with mutated PTEN may show increased sensitivity to rapamycin (94). However, this has not been shown conclusively yet.

Diabetes and cell size. As a downstream target of insulin signaling and as a regulator of cell size, the PI3K-mTOR-S6K1 pathway is important in metabolic disorders such as obesity, insulin resistance, and diabetes. In addition to reduced body size, S6K1-deficient mice show hypoinsulinemia and glucose intolerance, which is not due to loss of glucose-sensing mechanisms or insulin production capability. Instead, this change is due to reductions in pancreatic endocrine mass, which is accounted for by a selective decrease in β-cell mass and size. Intriguingly, this phenotype is only observed in β-cells and not in other endocrine cells such as α-cells and adrenal cells. These observations clearly demonstrate that S6K1 activity is essential for maintenance of β-cell growth and insulin secretion (95). Consistent with this observation, β-cell-specific knockouts of PDK1, which is an activator of S6K1 and AKT, also show decreases in β-cell mass (96). Despite the reduction of circulating insulin levels in S6K1-deficient mice, these mice are resistant to the development of obesity by enhanced β-oxidation and to the development of insulin resistance in both fat and muscle tissue by inhibition of negative feedback on IRS-1 (47).

In addition to regulation of β-cell mass, the mTOR pathway may also play a role in the regulation of kidney hypertrophy. Kidney hypertrophy is a compensatory measure for loss of kidney function. This can be seen in hypertrophy of the remaining kidney in the event of a unilateral nephrectomy. This hypertrophy of the kidney can be prevented in mice by treatment with rapamycin (97). Similarly, in mouse models of early diabetic nephropathy induced by streptozotocin, renal hypertrophy can also be seen. This early hypertrophy is mainly due to hypertrophy of the proximal tubules, and is associated with increased S6K1 phosphorylation. Treatment of the mice with rapamycin decreased both kidney hypertrophy owing to diabetes and S6K1 phosphorylation. Cultures of tubular cells also showed that overexpression of active S6K1 increased cell size, whereas overexpression of dominant negative S6K1 decreased the size (98). Another report suggests that rapamycin can reduce glomerular hypertrophy and prevent further progression to kidney disease. Glomerular hypertrophy is believed to be one of the hallmarks for progression to diabetic nephropathy. Moreover, in streptozotocin-induced diabetic nephropathy, rapamycin treatment attenuated albuminuria, a marker of diminishing renal function in early nephropathy (99, 100). Although rapamycin might interfere with the development and growth of pancreatic β-cells, the above studies suggest that rapamycin could be a potential therapeutic agent for diabetic complications.

Regulation of autophagy in Huntington's disease. As seen in myocyte, high levels of amino acids are capable of stimulating myocyte hypertrophy in an mTOR-dependent manner. Conversely, low levels of amino acids are able to inhibit mTOR

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activity (31). During amino acid starvation, cells undergo autophagy, which breaks down cytoplasmic organelles and proteins. Recently, it has been shown that in *Drosophila* inactivation of dTOR by overexpression of TSC1/2, loss of dTOR, or rapamycin treatment stimulated the formation of autophagic vesicles that were not dependent on S6K (101, 102). It is attractive to speculate that this mechanism is also involved in atrophy, but this has yet to be shown clearly. However, the stimulation of autophagy by rapamycin may have interesting clinical consequences. Huntington's disease is an autosomal dominant disease associated with the expansion of the trinucleotide-repeat CAG. This leads to the accumulation of aggregates with expanded polyglutamine tracts within neurons. Although the role of these neuronal aggregates is still unclear, it has been suggested that these aggregates may have toxic effects on the cells, and that they are subjected to clearance by the autophagic system. Additionally, mTOR seems to be sequestered by these aggregates, which leads to decreases in mTOR activity. This may be a compensatory mechanism, as activation of mTOR activity by overexpression of Rheb increases the toxicity of Huntington repeats in *Drosophila* models (103). Furthermore, treatment with rapamycin decreased the neuronal death associated with Huntington's repeats in *Drosophila* models and improved symptoms of Huntington's disease in mouse models (103, 104). Therefore, it has been suggested that rapamycin can be useful in the treatment of Huntington's disease by increasing autophagy of protein aggregates. Although Huntington's aggregates decrease upon rapamycin treatment, it is not clear whether this is due to the inhibitory effect on protein synthesis or the stimulatory effect on autophagy by rapamycin. Furthermore, it is also not clear whether the rapamycin effects are due to changes of the protein aggregates because in certain conditions rapamycin protects cells from apoptotic insults. One possible mechanism for this protection is stimulation of the autophagic processing of mitochondria, which would prevent subsequent cytochrome C release (105). However, the relationship between apoptosis and the mTOR pathway is very complicated, and it has yet to be shown what is the dominant mechanism mediating the antiapoptotic effects of rapamycin.

CONCLUSION

In recent years, the complexity of mTOR signaling has exploded, and it is clear that it touches many different pathways both upstream and downstream. With two different TOR complexes and the feedback inhibition of TORC2 by TORC1 activation, the simplification to linear pathway regulation is impossible. Additionally, as the biochemical regulation of mTOR has increased in complexity, the relevance to clinical processes has also followed. In addition to roles in cell size regulation, the mTOR pathway has also been implicated in tumorigenesis and cell survival. With the myriad of functions and multitude of potential targets in pathogenesis, clear clinical relevance for these targets still remains to be shown. Although it is likely that inhibition of mTOR may be clinically useful for treatment of diseases such as TSC, which directly involve misregulation of mTOR, more needs to be done to better understand the importance of mTOR signaling in a more complicated physiological context as seen by various disease states.

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