

Chapter 2

The mTOR Complexes in Cancer Cell Metabolism

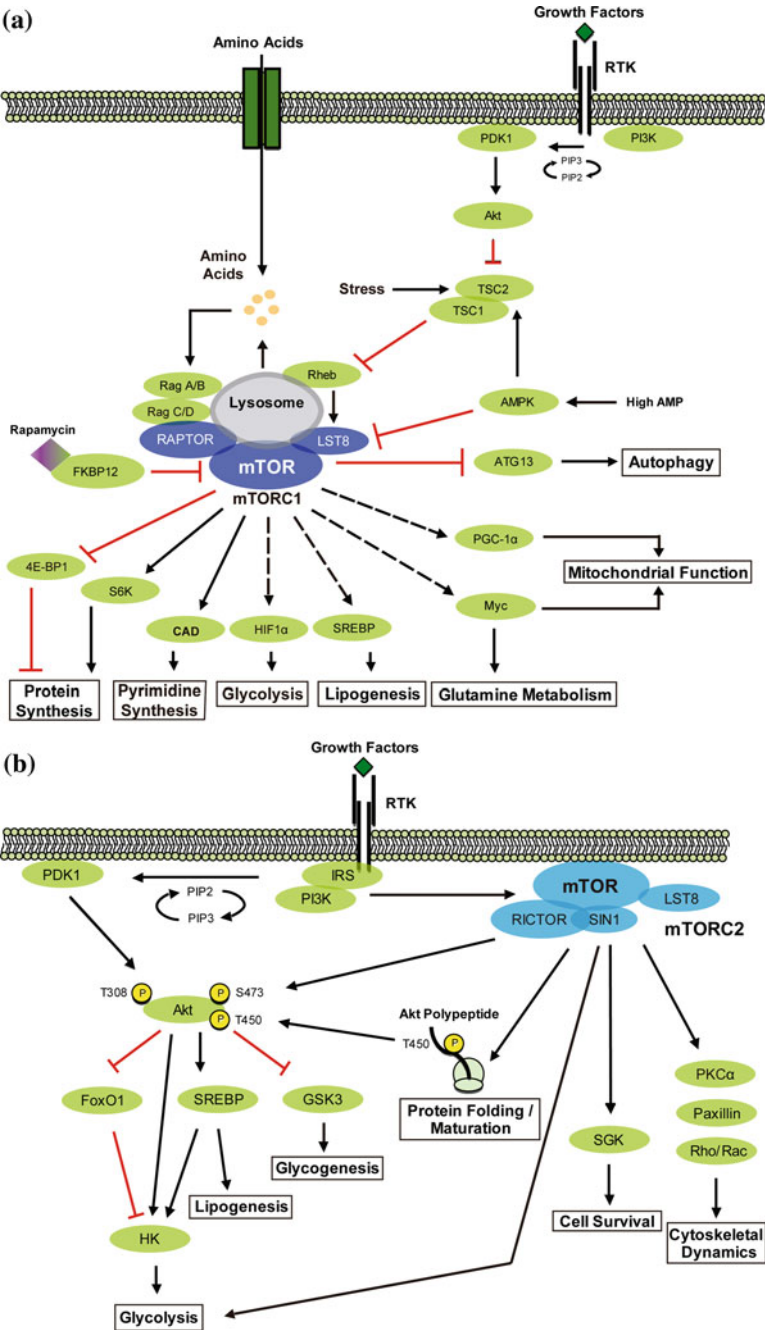
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Introduction

Our understanding of the intersection between cellular signaling pathways, nutrient sensing, and metabolic functions is rapidly growing, and at the center of this intersection is mTOR. mTOR is an atypical protein kinase, that is conserved from yeast to man, and is allosterically inhibited by a complex formed by the natural compound rapamycin and the prolyl isomerase FKBP12 [15, 61, 62, 85, 125]. Using rapamycin and genetic studies in yeast, it was shown that TOR promotes protein synthesis when nutrient conditions are favorable [3]. Its inhibition by rapamycin arrests cells in G1 phase of the cell cycle, eliciting a phenotype characteristic of starved cells [3, 15, 18, 59]. Moreover, rapamycin induces autophagy, a starvation response that degrades and recycles cellular components [14, 110]. In mammals, rapamycin blocks the activation of the translation regulator p70 S6K [22, 86, 118]. The major target of S6K is the 40S ribosomal protein S6, which is highly phosphorylated during G1 [148]. Around this time, the phosphorylation of S6 was discovered to be sensitive to amino acids, and that rapamycin and amino acids have opposite effects on autophagy and protein synthesis [14]. Another translation regulator, 4E-BP1 is also sensitive to rapamycin [9, 52]. Furthermore, phosphorylation of S6K diminishes upon amino acid withdrawal, resembling rapamycin treatment [58]. These observations, along with the early studies in yeast and in *Drosophila*, led to the notion that TOR/mTOR participates in nutrient sensing [3, 59, 114, 177].

Subsequent studies demonstrating that yeast TOR and mTOR play a role in the expression and trafficking of nutrient transporters, and that mTOR can sense levels of ATP, further reinforced the idea that mTOR is part of a nutrient signaling cascade [5, 34, 39, 133]. Moreover, studies on different model organisms have

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◀ **Fig. 2.1** mTOR forms two distinct protein complexes. **a** mTOR association with LST8 and RAPTOR characterizes the rapamycin-sensitive mTORC1. This complex responds to amino acids and its activity is enhanced by inputs from growth factor signaling via the PI3K/AKT pathway. mTORC1 is negatively regulated by TSC1/2 and AMPK, in response to nutrient/energy availability. Together, these opposing signaling pathways provide modulation for mTORC1 signaling to various downstream effectors that positively regulate anabolic metabolism and protein synthesis while negatively regulating catabolic processes including autophagy. **b**. mTOR association with LST8, RICTOR, and SIN1 characterizes mTORC2, the rapamycin-insensitive mTOR complex. mTORC2 is activated by growth factor/PI3K signaling and association with translating ribosomes. mTORC2 regulates protein maturation by a cotranslational phosphorylation mechanism, whereby nascent peptide stability is enhanced. AKT is stabilized in such a manner and is further activated by additional mTORC2 phosphorylation, leading to the upregulation of several metabolic pathways. mTORC2 activity regulates additional cellular processes through additional downstream effectors. Dashed lines refer to indirect regulation. Abbreviations used here include 4E-BP1: eukaryotic translation initiation factor 4E-binding protein 1, AMP: adenosine monophosphate, AMPK: AMP-activated protein kinase, ATG13: autophagy-related 13, CAD: carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase, FoxO1: forkhead box O1, FKBP12: 12-kDa FK506-binding protein, GSK3: glycogen synthase kinase 3, HIF1 α : hypoxia inducible factor 1 α subunit, HK: hexokinase, IRS: insulin receptor substrate, LKB1: liver kinase B1, LST8: lethal with SEC13 protein 8, mTOR: mammalian target of rapamycin, PDK1: phosphoinositide-dependent kinase-1, PGC-1 α : peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PI3K: phosphatidylinositol-3-kinase, PIP2: phosphatidylinositol-4,5-bisphosphate, PIP3: phosphatidylinositol-(3,4,5)-trisphosphate, PKC α : protein kinase C α , Rag: Ras-related GTP-binding protein, RAPTOR: Regulatory-associated protein of mTOR, RHEB: Ras-homolog enriched in brain, RICTOR: rapamycin-insensitive companion of mammalian target of rapamycin, RTK: receptor tyrosine kinase, S6K: ribosomal protein S6 kinase, SGK1: serum and glucocorticoid-regulated kinase 1, SIN1: stress-activated protein kinase-interacting 1, SREBP: sterol regulatory element-binding proteins, and TSC: tuberous sclerosis complex

unrevealed that TOR controls cell growth or increases in cell mass, as opposed to cell proliferation or cell cycle progression [132]. Genome-wide screening has further uncovered the effect of rapamycin on metabolic genes, revealing that TOR/mTOR mediates the expression of genes involved in nutrient metabolism [59, 114]. Together, these early studies have laid the groundwork that paved the way for understanding how nutrient signaling is linked to growth and metabolic signaling pathways via mTOR.

In multicellular organisms, cell growth is coordinated with tissue and organismal growth. Thus, in addition to nutrients, other extracellular inputs, such as growth factors and hormones, signal cell growth processes. The insulin and insulin-like growth factors play central roles in anabolic metabolism. The phosphatidylinositol-3 kinase (PI3K)/AKT signaling pathway couples signals from the insulin receptor to the control of gene expression and cellular growth responses [99]. In the presence of amino acids, insulin enhances S6K and 4E-BP1 phosphorylation, which can be inhibited by PI3K inhibitors or rapamycin, thus placing mTOR as a downstream effector of insulin signals [89]. The insulin/PI3K signals are wired to mTOR via the tuberous sclerosis protein complex (TSC1/TSC2) [67] (Fig. 2.1a). TSC1/2 are tumor suppressors and mutation of either one leads to hyperactivation of the mTOR/S6K1 branch. Numerous signals, including AKT, and stress signals impinge on TSC1/2,

modulating mTOR signaling. TSC1/2 negatively regulates mTOR (as part of mTOR complex 1/mTORC1, see below) via inactivation of Rheb. In its GTP-bound form, Rheb directly interacts and stimulates mTORC1. mTORC1 responds to amino acid signals via Rag GTPases [126, 127]. Amino acids promote GTP loading of RagA/B and enable the Rag heterodimers to interact with raptor and facilitate translocation of mTORC1 to the lysosomal surface [126]. Additionally, the presence of insulin or growth factors acutely dissociates TSC and thereby excludes this negative regulator of mTORC1 from this compartment [33, 103]. On the lysosomal surface, mTORC1 becomes activated possibly via Rheb, which is found throughout the endomembrane system. Thus, nutrient and growth factor signals converge to regulate the activation and subcellular localization of mTORC1.

In addition to compartmental regulation, mTOR is also highly regulated by its protein partners. Previous studies in yeast revealed a rapamycin-insensitive function of TOR [134, 179]. Unlike mammals wherein only one gene encodes mTOR, there are two in yeast, namely *TOR1* and *TOR2*. TOR2 performs a function involving actin cytoskeleton polarization that is insensitive to rapamycin treatment [134]. Biochemical purification has led to the identification of two structurally distinct TOR complexes in both yeast and mammals [46, 57, 73, 74, 81, 94, 128, 167]. The rapamycin-sensitive mTORC1 forms a complex with raptor and mLST8 (Fig. 2.1a), whereas the rapamycin-insensitive mTORC2 forms a complex with rictor (mAVO3), SIN1, and mLST8 (Fig. 2.1b). In addition to these conserved partners, the mTORCs also associate with other distinct, less well-conserved proteins that could regulate its activity and function [164]. The well-characterized function of mTOR in regulating the translation regulators S6K1 and 4E-BP1 is mediated by mTORC1. On the other hand, mTORC2, but not mTORC1, can phosphorylate AKT, and this phosphorylation which leads to optimal activation of AKT, is not acutely sensitive to rapamycin [64, 130]. Although AKT phosphorylation at its conserved hydrophobic motif site is used as a hallmark of mTORC2 activity, other direct substrates of mTORC2, such as SGK and PKC, are emerging from recent studies [164]. mTORC2 has also been linked to other cellular functions, such as actin cytoskeleton reorganization, translation, and protein maturation/folding [111]. The latter function entails cotranslational phosphorylation of AKT and PKC at a conserved turn motif that is critical for folding and stabilization of the kinase domain [43, 71, 112]. Consistent with this function of mTORC2, it was found to associate with ribosomes [112, 181]. Thus, both mTORCs function during translation, albeit by distinct mechanisms. Whereas mTORC1 promotes translation in response to amino acids, it remains unclear what activates mTORC2. The association with ribosomes enhances its activity toward AKT but precisely how mTORC2 gets activated remains elusive [181]. ATP depletion and glucose withdrawal can prevent optimal phosphorylation of AKT at the turn motif in vitro by mTORC2, suggesting that these signals could regulate mTORC2 [19].

AKT has long been studied as a central regulator of the response to insulin [99]. Among its many functions, it mediates the increase in glucose transporters on the plasma membrane upon insulin stimulation. Furthermore, it regulates numerous

enzymes in the glycolytic pathway, emphasizing its pivotal role in cell metabolism [122]. Thus, the finding that mTORC2 controls AKT and that both mTOR complexes feed back to regulate insulin/insulin receptor substrate (IRS) signals attest to a broader role of mTOR in the control of cellular metabolism [65, 82, 173]. However, much of the recent studies on the role of mTOR in cell metabolism highlight the involvement of mTORC1. This is partly due to the availability of rapamycin and TSC knockout models, which have expanded our knowledge on the functions of mTORC1. Availability of mTOR inhibitors (MTI) that block both mTORC1 and mTORC2, along with genetic models containing deficiencies in mTORC1 or mTORC2 components are now providing more insights on the role of these complexes in cancer cell metabolism.

A hallmark of cancer cells is increased aerobic glycolysis despite the presence of oxygen, also known as the Warburg effect [152]. The uncontrolled proliferation necessitates reprogramming of energy metabolism in order to sustain cell growth and division. Not only do cancer cells augment their uptake of nutrients, they also rewire signaling circuits in order to route nutrient catabolism toward macromolecular synthesis. The increased uptake of glucose enhances flux through glycolysis. When glycolytic flux is high, this leads not only to production of abundant ATP, but also produces intermediates that are required for biosynthetic pathways [31]. In this review, we will discuss evidence supporting the role of mTORC1 and mTORC2 in cell metabolism. We will discuss different metabolic pathways including glycolysis, mitochondrial, and biosynthetic pathways that become rewired in proliferating cancer cells and consider the role of the mTOR complexes in metabolic reprogramming.

Glucose Metabolism (Glycolysis)

In normal differentiated cells, glucose is metabolized to pyruvate via glycolysis. This process yields a net production of 2 mol of ATP/mol of glucose while reducing the cofactor NAD^+ to NADH. Cancer cells enhance their rate of glucose uptake and produce pyruvate at a higher rate than can be metabolized by the mitochondria. Under this condition, the excess pyruvate is diverted from being metabolized in the mitochondria and converted to lactate in the cytosol. This glycolytic switch can occur under aerobic conditions. Genetic mutations that lead to enhanced growth factor-PI3K/AKT/mTOR pathway signaling can drive and/or maintain this switch [152]. Indeed, multiple points along the glycolytic pathway are influenced by mTOR via regulation of two critical transcription factors HIF1 α and Myc (Fig. 2.2).

A crucial signaling protein that is involved in the glycolytic switch is HIF1 (hypoxia inducible factor 1). HIF1 is a heterodimer, consisting of an O_2 -regulated HIF1 α subunit and a constitutively expressed HIF1 β subunit [136]. Increased HIF1 α expression is sufficient to induce expression of genes whose products increase glycolytic flux [66]. While HIF1 α expression is normally elevated under

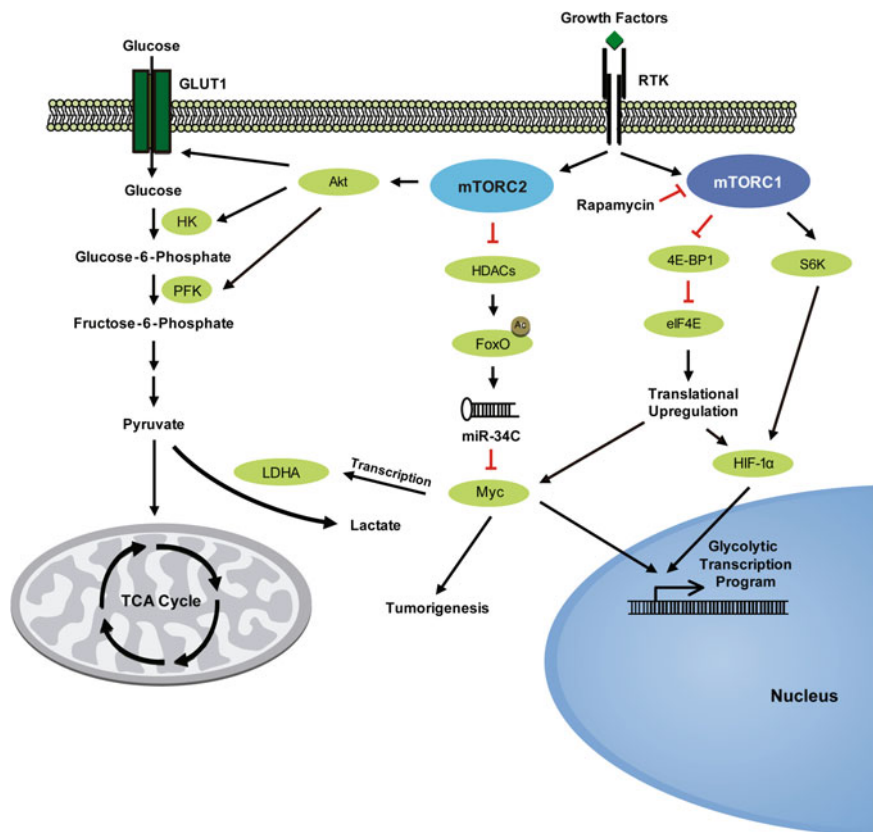


Fig. 2.2 mTORCs and glucose metabolism. mTORC1 contributes to the upregulation of the transcription factors HIF-1 α and Myc, both of which drive a pro-glycolytic transcriptional program. This activity can be inhibited by rapamycin. mTORC2, through regulation of AKT activity, can enhance the activity of various glycolytic enzymes, and through the stabilization of Myc, can additionally promote transcription of glycolytic enzymes. Myc has been associated with tumorigenesis. Additional abbreviations used here include GLUT1: glucose transporter 1, HDACs: histone deacetylases, HIF-1 α : hypoxia inducible factor 1 α , HK: hexokinase, LDHA: lactate dehydrogenase A, miR-34C: microRNA 34c, PFK: phosphofructokinase, RTK: receptor tyrosine kinase, S6K: ribosomal protein S6 kinase, and TCA Cycle: tricarboxylic acid cycle

hypoxia, deregulated mechanisms in cancer cells boost HIF1 α levels despite aerobic conditions. Its expression is upregulated in many primary and metastatic human tumors [136]. Early studies in prostate cancer cells have shown that inhibiting mTOR by rapamycin blocks the growth factor- and mitogen-induced HIF1 α expression [180]. Rapamycin also decreases HIF1 α stabilization and transcriptional activity under hypoxic conditions [69]. Elevated mTORC1 activation that occurs in TSC2^{-/-} cells also increases translation of HIF1 α mRNA [17] while rapamycin decreases its mRNA levels [90, 149]. The control of HIF1 α translation

involves the mTORC1 target, 4E-BP1 [38]. Thus, mTORC1 can regulate HIF1 α expression via translational and posttranslational mechanisms.

mTORC1 is also linked to regulation of genes that are controlled by HIF1 α . Transcriptional profiling of rapamycin-treated lymphocytes revealed altered glycolytic gene expression in these cells [53, 114]. In prostate epithelial cells of transgenic mice expressing active AKT, rapamycin diminishes the levels of glycolytic enzyme genes controlled by HIF1 α [98]. A combination of genomic, metabolomics, and bioinformatics approaches further confirmed the involvement of mTORC1 in inducing a HIF1 α -dependent transcriptional program to promote glycolysis [38]. Among the HIF1 α -regulated genes that are transcriptionally upregulated in an mTORC1-dependent manner include glycolytic enzymes and VEGF. The expression of the rate-limiting glycolytic enzyme pyruvate kinase M2 (PKM2), which is exclusively expressed in proliferating and tumor cells, is also regulated transcriptionally by mTORC1 via HIF1 α [145]. Hence, mTORC1 also regulates HIF1 α -target genes at the level of transcription.

Other genes that become upregulated during glycolysis are those encoding nutrient transporters. mTOR has been shown to play a role in regulating uptake of nutrients via control of gene expression or membrane trafficking of their transporters. Overexpression of kinase-inactive mTOR perturbs amino acid transporter trafficking while rapamycin diminishes glycolytic activity [39]. In differentiating T lymphocytes, rapamycin inhibits expression of HIF1 α and genes involved in glucose transport and metabolism [139]. Rapamycin treatment in vivo also reduces fluorodeoxyglucose (FDG) uptake in kidney cancers with loss of the tumor suppressor von Hippel Landau (VHL1), supporting a role for mTORC1 in glucose uptake [149]. The sensitivity to mTOR inhibition is attributed to a block in translation of mRNA encoding HIF1 α , a target of VHL. However, in another study using liver-specific *Tsc1* mutant mice, increased mTORC1 activation is accompanied by reduced glucose uptake [77]. This is likely due to the mTORC1/S6K1-mediated negative feedback loop that downregulates PI3K/AKT pathway, which plays a role in glucose transport [60]. The reduced glucose uptake under elevated mTORC1 activity is also in line with the findings that TSC-deficient cells are hypersensitive to glucose withdrawal [72]. Together, these findings suggest that under normal conditions, mTOR couples uptake of nutrients with the metabolic demands.

Another effector of mTORC1 that promotes glycolytic gene expression is the transcription factor Myc [49, 159]. Using a bioinformatics approach, Manning and coworkers have identified cis-regulatory elements among rapamycin-sensitive genes to be regulated by Myc [38]. In T cells, mTORC1 inhibition also diminishes the T cell receptor (TCR)-induced Myc expression, which is accompanied by reduction of glycolytic activity [155]. HIF1 and Myc have overlapping metabolic gene targets. One example of a common target gene is that encoding lactate dehydrogenase (LDH) [29, 135]. LDH is a tetrameric enzyme composed of a combination of the subunits LDHA and LDHB which converts pyruvate to lactate. Rapamycin treatment of prostate cancer cell lines downregulates LDHA expression among other metabolic effectors [151]. On the other hand, the expression of LDHB

is upregulated in an mTOR-dependent manner in murine embryonic fibroblasts that have deficiency in TSC1, TSC2, or PTEN and with activated AKT. The enhanced LDHB levels are critical for hyperactive mTOR-mediated tumorigenesis [176]. Another common target of HIF1 α and Myc is PKM2. Unlike transcriptional activation of the PKM2 gene by HIF1 α , Myc appears to regulate PKM2 expression in an mTORC1-dependent manner via the alternative splicing repressors, hnRNPs [145]. How mTORC1 contributes to the regulation of LDH and other common target genes of HIF1 α and Myc awaits further investigation. In addition, how mTORC1 can regulate Myc remains to be characterized.

Studies using raptor knockout models are also beginning to reveal more insights on specific roles of mTORC1 in cellular metabolism. More recently, Yang et al. showed that the metabolic programming that drives the exit of T cells from quiescence is dependent on mTORC1. TCR stimulation of raptor-deficient CD4⁺ T cells triggers reduced glycolytic activity compared to wild type cells [166]. This defect is accompanied by attenuated mRNA expression of glycolytic enzymes and protein expression of Myc. Furthermore, mTORC1 links glucose metabolism to cytokine receptor expression and responsiveness [166]. There is also evidence that downstream mTORC1 substrates mediate glycolytic metabolism. Knockdown of S6K1 in PTEN-deficient cells decreases HIF1 α expression and glycolysis [146]. In these studies, targeting the mTORC1 substrate S6K1 in PTEN-deficient mouse model of leukemia delays leukemogenesis. Additionally, pharmacological or genetic inhibition of another mTORC1 target, 4E-BP1, is also sufficient to block Myc-driven tumorigenesis [117]. Whether other substrates of mTORC1 in addition to S6K1 and 4E-BP1 mediate the function of mTORC1 in glycolysis will need to be addressed.

mTORC2 also plays a role in glycolytic metabolism. Early on, the mTORC2 substrate AKT was shown to couple growth factor signaling to glucose metabolism [122]. Using an experimental leukemia model, Elstrom et al. [41] demonstrated that AKT activation was sufficient in increasing the rate of glucose metabolism. They also found increased rates of aerobic glycolysis in glioblastoma cells harboring constitutive AKT activity. In prostate epithelial cells, activated AKT induces glycolytic genes via HIF1 α [98]. On the other hand, AKT deficiency is sufficient to suppress tumor development in PTEN \pm mice [20]. Thus, AKT plays a crucial role in enhanced aerobic glycolysis. The most compelling evidence demonstrating a role for mTORC2 in glycolytic metabolism was presented using liver-specific rictor knockout mice. In the liver of these mice, AKT phosphorylation is abrogated, glycolysis is impaired and the activity of glucokinase is reduced. Expression of a constitutively active AKT or glucokinase rescues glucose flux in these mice [55]. Thus, mTORC2 regulates glycolysis in the liver via AKT. Recently, mTORC2 was shown to control glycolytic metabolism in glioblastoma independently of AKT [101]. In this cancer model, mTORC2 controls the acetylation of FoxO through inhibition of Class IIa HDAC phosphorylation [101]. These findings support that mTORC2 has functions in glycolytic metabolism distinct from AKT.

Oxidative and Mitochondrial Metabolism

The maximum production of ATP under normal conditions requires metabolism of pyruvate in the mitochondria. Pyruvate enters the mitochondria and is converted to acetyl coenzyme A (acetyl CoA), which is further metabolized via the tricarboxylic acid (TCA) cycle. Whereas the TCA cycle serves to produce maximal ATP production in nonproliferating cells, it primarily generates intermediates that are utilized as biosynthetic precursors in highly proliferating cells [152]. mTOR has been linked to these processes occurring in the mitochondria (Fig. 2.3). Inhibition of mTOR by rapamycin reduces mitochondrial membrane potential, oxygen consumption, ATP

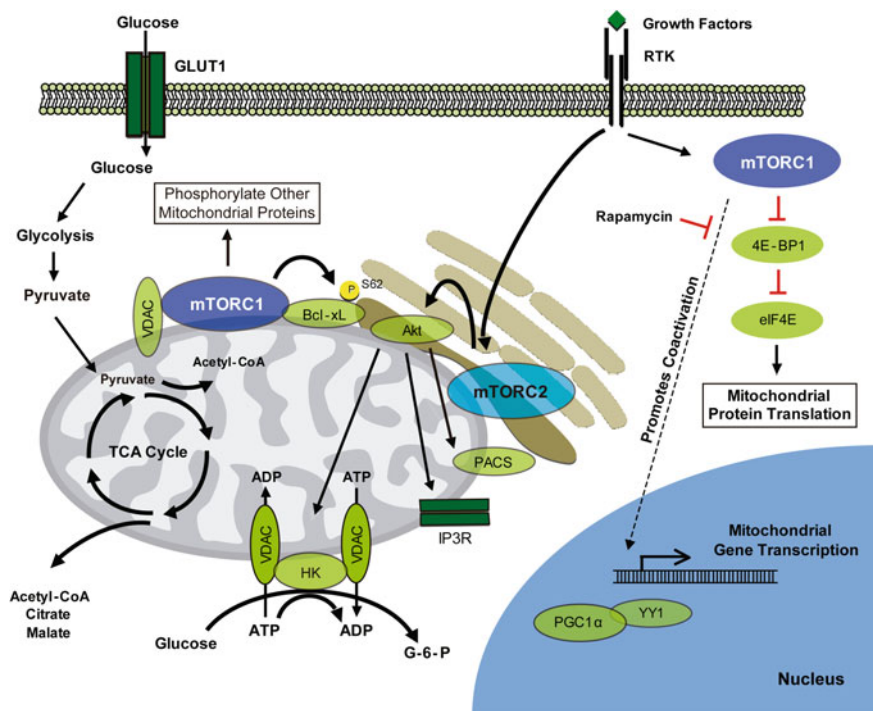


Fig. 2.3 mTORCs in oxidative and mitochondrial metabolism. Mitochondrial gene transcription is upregulated in an mTORC1-dependent manner, through the stimulation of PGC1 α /YY1 coactivation, and mitochondrial gene translation is upregulated through eIF4E activity. Additionally, mTORC1 promotes phosphorylation of mitochondrial proteins and associates with VDAC and Bcl-xL, regulating mitochondrial substrate availability and apoptosis, respectively. mTORC2 modulates mitochondrial metabolism and integrity through AKT activity. Additional abbreviations used here include ADP: adenosine diphosphate, ATP: adenosine triphosphate, Bcl-xL: B-cell lymphoma-extra large, G-6-P: glucose-6-phosphate, IP3R: inositol trisphosphate receptor, PACS: phosphofurin acidic cluster sorting protein, PGC-1 α : peroxisome proliferator-activated receptor gamma coactivator 1-alpha, VDAC: voltage-dependent anion channel, and YY1: yin yang 1

synthetic capacity and alters the mitochondrial phosphoproteome [131]. Interestingly, these effects of rapamycin do not seem to be mediated by S6K or 4E-BP1. In rapamycin-treated skeletal muscle tissue and cells, the expression of mitochondrial transcriptional regulators such as PGC-1 α is decreased along with mitochondrial gene transcription [27]. By bioinformatics analysis, the transcription factor YY1 was identified as a target of mTOR and PGC1- α in these cells. Knockdown of YY1 decreases mitochondrial gene expression and YY1 is required for the rapamycin-dependent repression of these genes. In TCR-stimulated raptor-deficient CD4+ T cells, reduced oxygen consumption rate and diminished expression of genes involved in oxidative phosphorylation are also observed [166]. Thus, mTORC1 could control mitochondrial metabolism via transcriptional mechanisms.

Other studies have shown a more direct control of mitochondrial function by mTOR [119]. mTOR can be found at the outer membrane of the mitochondria [35] and associates with the outer mitochondrial membrane proteins Bcl-xL and VDAC1, proteins that are involved in cellular apoptosis and substrate transport, respectively [119]. mTOR can phosphorylate Bcl-xL in vitro at Ser62, a site that regulates Bcl-xL activity. Although phosphorylation of VDAC has not been demonstrated, inhibition of VDAC2 in Jurkat cells generated some overlapping metabolic profile as rapamycin treatment including increased lactate, glycerol and upstream glycolytic intermediates [119]. Decreased levels of TCA cycle intermediates are also found. These findings suggest that mTORC1 inhibition could limit mitochondrial substrate availability and thus promotes diversion from mitochondrial respiration to aerobic glycolysis.

mTORC1 also controls genes involved in mitochondrial function at the level of translation. Raptor knockdown reduces mitochondrial respiration and the amounts of TCA cycle intermediates. mTORC1, but not mTORC2, induces the expression of nucleus-encoded mitochondrial proteins [105]. mTORC1 performs this function via inhibition of 4E-BP. S6K1 does not appear to affect mitochondrial respiration and glucose flux to pyruvate and lactate [105]. Knockdown of S6K1 also does not decrease expression of mitochondrial genes [27]. Thus, the mTORC1 effector 4E-BP plays a more significant role in mitochondrial metabolism.

The role of mTORC2 in oxidative and mitochondrial metabolism is poorly understood. Transformed cells that are mTORC2 addicted are highly dependent on mitochondrial functions [23]. Knockdown of rictor stimulates mitochondrial respiration while diminishing the amounts of pyruvate and lactate, suggesting a negative regulatory role in mitochondrial respiration [105]. mTORC2 localizes to mitochondria-associated endoplasmic reticulum (ER) membrane (MAM) upon growth factor stimulation [12]. In this compartment, mTORC2 controls growth factor-mediated MAM integrity, calcium flux, and mitochondrial membrane potential. This function of mTORC2 is mediated by AKT, which regulates hexokinase II, along with other proteins involved in MAM integrity. AKT can also regulate hexokinase-VDAC interaction at the outer mitochondrial membrane [50]. It was proposed that AKT increases coupling of glucose metabolism to oxidative

phosphorylation. mTORC2 at MAM is associated with ribosomes and while this suggests it is active in this compartment [12, 181], it remains unclear whether there are AKT-independent functions of mTORC2 in this compartment.

Glutamine Metabolism

Glutamine is the most abundant nonessential amino acid in the plasma and is avidly used by proliferating tumor cells. Glutamine is a versatile molecule, as it serves as a carbon source for energy production and its carbon and nitrogen are also used for biosynthetic reactions [30]. Glutamine is a precursor for α -ketoglutarate (α KG) and is used to replenish TCA intermediates (anaplerosis) in proliferating cells. Incorporation of α KG into the TCA is the major anaplerotic step in proliferating cells and important for production of oxaloacetate which reacts with acetyl CoA to produce citrate. α KG and glutamine are also precursors for nucleotides and other amino acids. Glutamine is also used for the production of UDP-GlcNAc, a metabolite produced by the hexosamine biosynthesis pathway, which, in turn is used for protein glycosylation. Glutamine is metabolized via glutaminolysis, which consists of two steps: the first is catalyzed by glutaminase (GLS) and converts glutamine to glutamate. The second is catalyzed by glutamate dehydrogenase (GDH) and converts glutamate to α KG. Oncogenic signals such as elevated levels of Myc increase glutamine uptake and metabolism through a transcriptional program that includes enhancement of expression of mitochondrial glutaminase [47, 161]. As with glucose metabolism, mTOR signaling impinges on multiple aspects of glutamine metabolism (Fig. 2.4).

mTORC1 is sensitive to glutamine levels. Glutamine, in combination with leucine activates mTORC1 by enhancing glutaminolysis and α KG production. Glutaminolysis correlates with increased mTORC1 activity and is necessary for GTP loading of RagB and activation of mTORC1 signaling. It also promotes cell growth and inhibits autophagy via regulation of mTORC1 [37]. The uptake of glutamine by the transporter SLC1A5 has also been suggested to be the rate-limiting step that activates mTOR. The heterodimeric glutamine antiporter SLC7A5/SLC3A2 (CD98) uses intracellular glutamine as an efflux substrate to regulate the uptake of leucine. This in turn leads to activation of mTORC1 [109]. On the other hand, glutamine depletion that can occur as an off target effect of using asparaginase, which has glutaminase activity, indirectly inhibits mTOR activity via decreased leucine uptake in AML [160]. Thus, although mTORC1 may not directly sense these amino acids, the above findings suggest an indirect mechanism whereby glutaminolysis activates mTORC1. Since glutaminase uses glutamine as the substrate and GDH is allosterically activated by leucine [137], the enzymes catalyzing glutaminolysis themselves instead sense glutamine and leucine directly [37].

mTORC1, via Myc, can also stimulate glutamine metabolism via regulation of transcription factors involved in expression of glutaminolysis-related genes. Deletion of Myc in T cells markedly inhibits T cell activation-induced

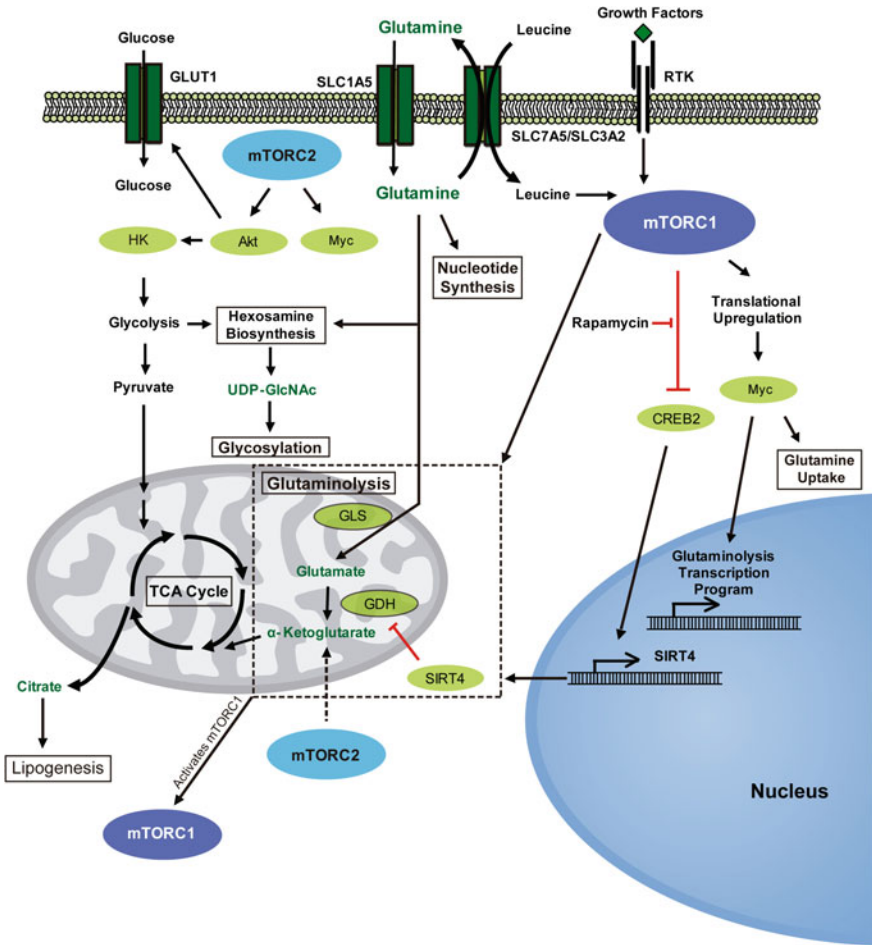


Fig. 2.4 mTORCs and glutamine metabolism. Glutamine serves as a precursor to generate molecules necessary for biosynthesis. mTORC1 itself is sensitive to levels of glutamine and leucine, both of which can drive glutaminolysis and α -ketoglutarate production, activating mTORC1. Through the translational upregulation of Myc, mTORC1 can drive the transcription of genes involved in glutaminolysis (indicated by dashed box). Further, mTORC1 directly inhibits CREB2, which drives the transcription of the GDH inhibitor, SIRT4. mTORC2 contributes to glycolytic and TCA cycle flux. The expression of Myc can be mTORC2-dependent but the mTORC2 role in glutaminolysis remains to be investigated. Additional abbreviations used here include CREB2: cAMP response element-binding protein 2, GDH: glutamine dehydrogenase, GLS: glutaminase, SIRT4: sirutin 4, SLC1A5: solute carrier family 1 member 5, SLC7A5/SLC3A2: solute carrier family 7 member 5/solute carrier family 3 member 2 (CD98), and UDP-GlcNAc: uridine diphosphate *N*-acetylglucosamine

glutaminolysis and decreases phosphorylation of mTORC1 substrates [155]. In the Myc-deleted T cells, the transcription and translation of glutaminase 2, along with other enzymes in the glutamine catabolic pathway are downregulated [155]. Since

mTORC1 mediates translational upregulation of Myc [159], these findings further highlight a regulatory loop wherein mTORC1 regulates Myc, which in turn promotes glutaminolysis that further activates mTORC1. Another transcription factor that is regulated by mTORC1 to promote glutaminolysis is CREB2. Using bioinformatics, Csibi et al. identified a CREB2 (cAMP-responsive element-binding 2) recognition motif in the promoter region of *SIRT4*. CREB2 is a transcription factor that plays a role in metabolic processes. mTORC1 promotes the proteasome-mediated degradation of CREB2 and represses *SIRT4* transcription [26]. *SIRT4*, which is localized in the mitochondria, is a member of the sirtuin family of nicotinamide adenine dinucleotide (NAD)-dependent enzymes that have been implicated in metabolism and longevity [56]. *SIRT4* negatively regulates GDH by ADP-ribosylation. Thus, repression of *SIRT4* by rapamycin treatment decreases GDH activity. These findings indicate that mTORC1 promotes glutamine metabolism via negative regulation of CREB2, which ultimately leads to activation of GDH.

Whether mTORC2 plays a role in glutamine metabolism is obscure. The expression of Myc in glioblastoma is mTORC2-dependent [101]. However, previous studies have shown that activation of PI3K and AKT is not required for glutaminolysis in Myc-expressing cells [161]. Inhibition of GDH or glutaminase does not affect phosphorylation of AKT, suggesting mTORC2 activity is intact under such conditions [37]. However, knockdown of rictor decreases levels of α KG that are likely derived from glutaminolysis, suggesting that mTORC2 could regulate this process as well [105].

Amino Acid Metabolism

Amino acids are the building blocks for protein synthesis and also serve as metabolic precursors. During nutrient-limiting conditions, protein synthesis is down-regulated, autophagy is induced, and amino acid biosynthesis is enhanced via mTOR-dependent mechanisms. Several recent reviews have discussed the role of mTOR in protein synthesis and autophagy in response to the presence of amino acids [76, 96]. Much work has been done characterizing the role that amino acids play in stimulating mTOR signaling, however, there has been comparatively less work done on describing the role mTOR plays in the regulation of amino acid synthesis. Here, we will discuss its implicated role in amino acid biosynthesis.

For humans, essential amino acids must be supplied through the diet; however, nonessential amino acids can be synthesized intracellularly. An abundant supply of amino acids, that are utilized for enhanced protein synthesis and as metabolic precursors, are required by cancer cells. Despite the apparent diversity of amino acids, there is a common source of precursor molecules for their synthesis. The importance of amino acid biosynthesis in metabolic reprogramming is underscored by the use of amino acid depleting enzymes such as asparaginase as an anticancer drug. Asparaginase has been used for the treatment of pediatric and adult acute

lymphocytic leukemia as well as pediatric AML [108]. Asparaginase catalyzes the hydrolysis of the nonessential amino acid asparagine into aspartic acid and ammonia, thereby depleting the serum of asparagine. Malignant cells that are auxotrophic for asparagine (due to lower asparagine synthetase activity than normal cells) have impaired protein synthesis due to limiting amounts of this amino acid. Rapamycin treatment of cells decreases asparagine levels and gene expression of asparagine synthetase, suggesting a role for mTOR in regulating amino acid biosynthesis [114, 119]. In contrast, argininosuccinate synthetase-1 (ASS1), the rate-limiting enzyme for arginine biosynthesis, is increased by rapamycin treatment [114]. The mechanism for this is not clear, but it is interesting to note that arginine-auxotrophic tumors, such as melanoma and hepatocellular carcinoma [87], develop resistance to therapy using arginine deiminase, which degrades extracellular arginine. This resistance to the deiminase is due to elevation of ASS and these tumors become particularly sensitive to PI3K/AKT inhibitors [95]. Future studies should reveal how the mTORCs can regulate amino acid synthetases.

The mTORCs may also regulate amino acid metabolism at the level of their transporters. Genomic studies identified neutral amino acid transporters to be decreased upon rapamycin treatment [114]. In contrast, metabolic profiling of rapamycin-treated cells revealed intracellular upregulation of specific amino acids due to increased uptake via transporters rather than anabolic processes [119]. Lastly, mTORCs are indirectly involved in amino acid biosynthesis based on their role in other metabolic pathways. Intermediates from glycolysis, the pentose phosphate pathway (PPP), and the citric acid cycle supply the necessary building blocks used for synthesizing the nonessential amino acids in human cells. Since mTOR is known to regulate various steps along these pathways [38, 55, 171], it can be deduced that mTOR regulates amino acid synthesis indirectly via different mechanisms.

Pentose Phosphate Pathway and Nucleotide Synthesis

Cells utilize the PPP for two critical functions; to generate reducing equivalents in the form of NADPH and ribose-5-phosphate for nucleic acid synthesis (Fig. 2.5). About 5–30 % of glucose is metabolized via the PPP. Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the rate-limiting reaction in the PPP [143]. PPP is divided into an oxidative and a nonoxidative branch, which are irreversible and reversible, respectively. In the oxidative branch glucose-6-phosphate (G6P) is oxidized by G6PD to produce NADPH. In contrast, the nonoxidative branch is a series of reversible reactions, converting glycolytic intermediates into ribose-5-phosphate. Both pathways ultimately generate phosphoribosyl pyrophosphate, the precursor for nucleotide synthesis. Rapidly dividing cells have increased PPP activity. In addition to the requirement for pentose phosphates in nucleotide production, NADPH is also used as a reducing agent in several synthetic steps of fatty acid, cholesterol, and steroid hormones, along with detoxification reactions.

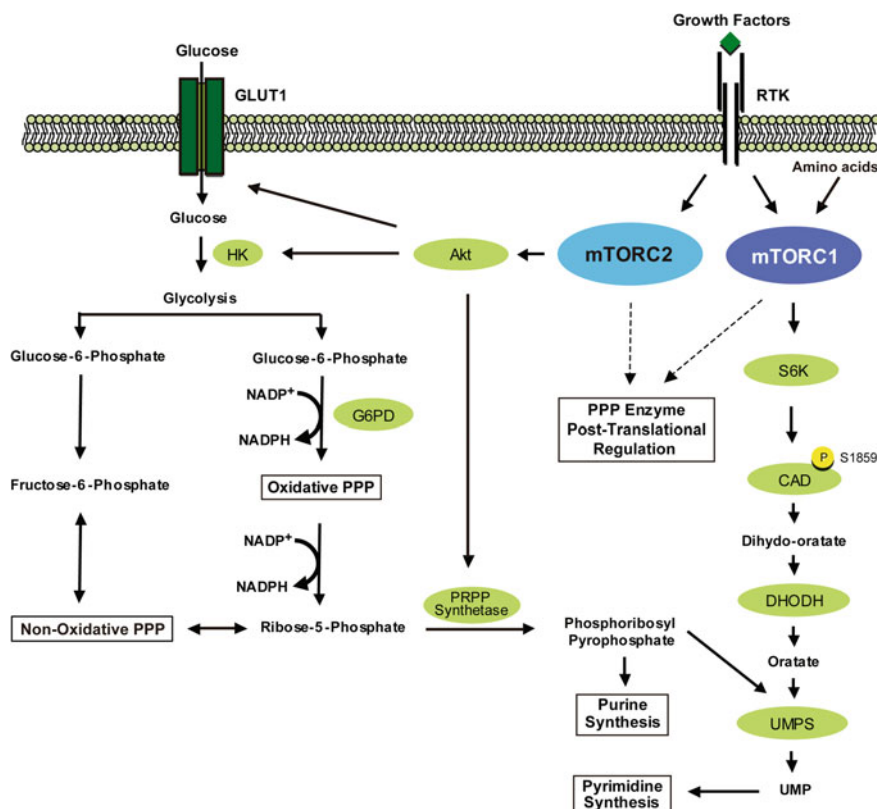


Fig. 2.5 mTORCs in the pentose phosphate pathway and nucleotide synthesis. Both mTORCs enhance transcription of enzymes involved in the PPP. mTORC2 has also been linked to posttranslational regulation of PPP enzymes. mTORC2, through AKT, promotes flux through the PPP and enhances levels of PRPP, the precursor for nucleotide synthesis. mTORC1, through S6K activity, can stimulate nucleotide synthesis through CAD, which catalyzes the initial steps of de novo synthesis. Additional abbreviations used here include: CAD: carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase, DHODH: dihydroorotase dehydrogenase, G6PD: glucose-6-phosphate dehydrogenase, PPP: pentose phosphate pathway, PRPP: phosphoribosyl pyrophosphate, UMP: uridine monophosphate, and UMPS: uridine monophosphate synthetase

mTORC1 can stimulate flux through the oxidative branch [38, 172]. mTORC1 is involved in this pathway via transcription of genes encoding enzymes that drive the PPP. By regulating expression of these genes, mTORC1 promotes production of ribose-5-phosphates, which are used in purine and pyrimidine nucleotide synthesis and production of NADPH.

Rapidly proliferating cells require an ample pool of nucleotides which are critical for cellular processes such as ribosome biogenesis. These pools are synthesized through two pathways: the salvage pathway, which generates nucleotides

from degradation intermediates and the de novo synthesis pathway, which assembles complex nucleotides from basic molecules. Utilizing phosphoproteomic and metabolomic profiling approaches, mTORC1 has been linked to production of pyrimidines via de novo pathways [7, 123]. mTORC1, via phosphorylation of S6K1, promotes activation of CAD (carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase). S6K1 directly phosphorylates CAD on Ser1859. In raptor-, but not rictor-depleted MEFs, CAD phosphorylation is abrogated, demonstrating that this phosphorylation is mTORC1-specific [123]. CAD catalyzes the initial steps of pyrimidine synthesis by utilizing glutamine, bicarbonate, and aspartic acid to generate pyrimidine rings. mTORC1 and S6K1-mediated phosphorylation of CAD enhances its oligomerization [123]. De novo pyrimidine synthesis is enhanced by mTORC1 and S6K in response to growth factor or amino acid stimulation, although they are not essential for de novo synthesis per se [7].

mTORC2 also plays a role in the PPP. Hyperactive mTORC2 can result in increased flux through the PPP. AKT phosphorylates hexokinase and drives its association with the mitochondria, where hexokinase can phosphorylate glucose, and thus elevate the levels of G6P, the substrate for the PPP [144]. Moreover, in an insulin-driven model of hepatocellular carcinoma cells, Evert et al. [42] have shown that AKT drives the upregulation of the PPP through several mechanisms, including via increase of phosphate dehydrogenase and ribose 5-phosphate isomerase A expression and activity, as well as through driving glycolysis. A significant role for mTORC2 in regulating the PPP was recently demonstrated using a chemical genetic screen. The yeast TORC2 specifically interacts with the PPP [83]. Proteins that play a role in the PPP physically associate with TORC2. Furthermore, metabolic intermediates such as 6-phospho-D-gluconate (6PG) and ribose-5-phosphate are strongly downregulated in response to TOR2 inhibition. Since the decrease in PPP metabolite levels is rapid, it was proposed that TORC2 likely plays a posttranslational role in the regulation of the PPP.

So far, the role of mTORC2 in nucleotide metabolism is likely via stabilization of AKT, which regulates purine synthesis [156]. The PI3K/AKT signaling axis regulates the early steps of the nonoxidative PPP at the level of phosphoribosylpyrophosphate (PRPP) synthesis and later steps by modulating the activity of aminoimidazole-carboxamide ribonucleotide transformylase IMP cyclohydrolase [156]. Whether mTORC2 has a more direct role in regulating the expression or activity of the enzymes involved in nucleotide synthesis remains to be elucidated.

Lipid Metabolism

Cancer cells undergo increased de novo lipid synthesis. Production of lipids and fatty acids are enhanced for biosynthesis of membranes and signaling molecules. Cell membrane lipids including phospholipids, sterols, sphingolipids, and lyso-phospholipids are derived in part from acetyl CoA. The enhanced glutamine

metabolism that occurs in cancer cells leads to elevated citrate production. Citrate, in turn, is exported from the mitochondria to the cytosol. Cytosolic citrate is processed by ATP citrate lyase (ACL) to generate cytosolic acetyl CoA, the building block for endogenous synthesis of acyl groups and sterols. Indeed, ACL expression is found upregulated in a number of cancers [6, 104, 157].

Early studies on the use of rapamycin in transplantation have revealed that an adverse side effect of mTOR inhibition is hyperlipidemia [162], underscoring the role of mTOR in lipid metabolism (Fig. 2.6). Although the *in vivo* studies imply a negative regulatory role for mTOR in lipid biosynthesis, cellular studies have revealed the opposite. Rapamycin reduces the expression of acetyl CoA carboxylase, fatty acid synthase and stearoyl CoA desaturase, which are lipogenic enzymes whose transcription is targeted by the transcription factor SREBP (sterol regulatory element-binding protein) [16, 102, 114]. Rapamycin also elevates levels of glycerol

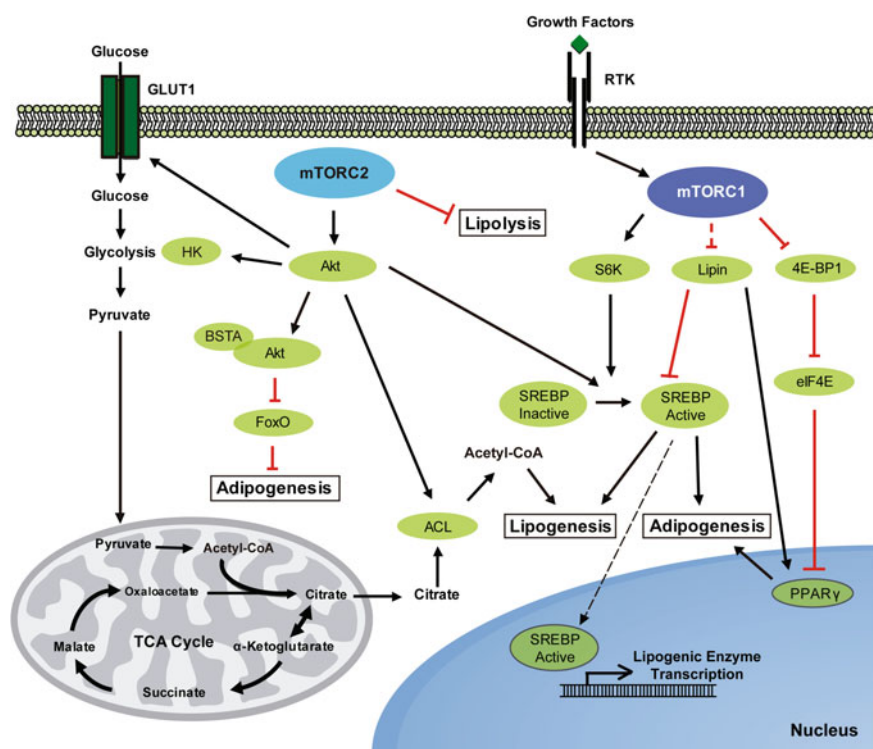


Fig. 2.6 mTORCs and lipid metabolism. mTORC1, through regulation of downstream effectors such as SREBP and PPAR γ , modulates lipogenesis and adipogenesis. mTORC2 regulates lipogenesis via an AKT-dependent and -independent manner. mTORC2, via AKT can stimulate lipogenesis by enhancing glycolytic and TCA flux and promotes adipogenesis by enhancing Acetyl CoA levels and FoxO inhibition. Additional abbreviations include: ACL: ATP citrate lyase, PDH: pyruvate dehydrogenase, PPAR γ : peroxisome proliferator-activated receptor γ , SREBP: sterol regulatory element-binding proteins

and promotes accumulation of acetyl CoA and malonyl CoA, which are substrates for lipid synthesis [119]. These findings suggest that the hyperlipidemia observed upon rapamycin treatment in vivo is probably not due to increased synthesis in the liver but is due to delayed peripheral clearance [16]. Indeed, numerous studies support a positive role for mTOR in lipid biosynthesis [88, 121].

Several studies have uncovered a role for mTORC1 in lipogenesis [38, 116, 166]. In cells expressing active AKT, the induction of lipid synthesis is dependent on mTORC1 [116]. In TSC2-deficient fibroblasts where mTORC1 activity is upregulated, there is a rapamycin-sensitive increase in de novo lipid biosynthesis [38]. A more direct analysis in liver-specific *raptor* knockout confirmed the requirement for mTORC1 in lipogenesis [154]. Similarly, raptor-deficient CD4⁺ T cells have defective de novo lipid synthesis and fails to induce genes involved in lipogenic pathways upon TCR stimulation [166]. In T regulatory cells (T_{reg}s), mTORC1 promotes cholesterol and lipid metabolism, with the mevalonate pathway being particularly important for T_{reg} proliferation and upregulation of mediators important for suppressive function [175]. Several studies support that the mTORC1 function in lipid metabolism is mediated via SREBP. An enrichment in DNA binding elements that recognize the transcription factor SREBP is found in rapamycin-sensitive genes identified in microarray studies using TSC-deficient cells [38]. There are three isoforms of SREBP in mammalian cells, SREBP1a, SREBP1c, and SREBP-2 that regulate distinct, yet overlapping transcriptional programs governing lipid synthesis. In raptor-deficient T cells, the protein expression of both SREBP1 and SREBP2 is attenuated [166]. In hepatocytes, the insulin-mediated increase in SREBP-1c mRNA is mTORC1-dependent but S6K-independent [113, 154]. Thus, mTORC1 can regulate SREBP at the level of transcription and protein expression to control lipogenesis.

SREBPs are synthesized as inactive precursors that reside in the ER and translocate to the nucleus after processing from the Golgi. This active processed form induces transcription of SRE-containing target genes. The processing step is thus sensitive to sterol levels and controlled by mTORC1 signaling. An enhancement of processed forms of SREBP1 has been observed in the TSC-deficient cells and the mTORC1 target S6K1 has been shown to regulate SREBP processing [38, 113]. The stability of its processed active form has also been linked to AKT regulation [116]. mTORC1 also regulates SREBP by controlling the nuclear localization of lipin 1, a phosphatidic acid phosphatase that represses SREBP activity [115]. Thus, mTORC1 signals promote accumulation of the active form of SREBP, serving as another mode of regulation of lipogenesis by mTORC1.

In addition to SREBP, mTORC1 has been linked to the regulation of peroxisome proliferator-activated receptor γ (PPAR γ). Upregulation of the mTORC1 pathway, such as by inactivation of TSC2, promotes adipocyte differentiation, in which PPAR γ plays a central role [178]. The mTORC1 effectors 4E-BP1 and S6K can in part mediate this function of mTORC1 in adipogenesis. mTORC1 can also increase the activity of lipin 1, which promotes triglyceride synthesis and enhances the PPAR γ adipogenic activity [70]. How this function of mTORC1 in adipogenesis becomes deregulated in cancer is not clear but reducing fatty acid availability to

cancer cells by diverting them to storage pathways or blocking their release from storage could be promising as therapeutic targets [28].

The role of mTORC2 in lipid metabolism is revealed in studies from knockout models using different organisms. In yeast, the TORC2 component, Avo3 (rictor orthologue) regulates ceramide and downstream sphingolipid synthesis [2]. Sphingolipids are a major component of cell membranes and are required for cell growth. In *C. elegans*, a high body fat phenotype occurs in Ric1 null worms, which is independent of AKT, but rather dependent on SGK1 [79, 142]. In mice, knockout of *ric1* specifically in the liver decreases lipogenesis [55, 174]. Hagiwara et al. have shown that in these mice, there is a loss of SREBP1c activity and in turn, loss of lipogenesis, suggesting that there is an mTORC2 specific role for the regulation of lipogenesis, as these mice have functional mTORC1. Further, expression of a constitutively active AKT is able to rescue de novo lipogenesis [55]. In glucose stimulated hematopoietic cells, knockdown of ACL impairs AKT-induced tumorigenesis [4]. ACL is itself phosphorylated by AKT [11]. The requirement for AKT in lipogenesis is strengthened by the findings that mTORC1 activation is not sufficient to stimulate lipogenesis under conditions wherein AKT signaling is downregulated [154, 172]. On the other hand, Yuan et al. [174] reported that active AKT is unable to drive hepatic lipogenesis in the absence of mTORC2. Thus, mTORC2 plays an additional role in lipogenesis that is independent of AKT.

mTORC2 also plays a role in adipogenesis. The BSD domain containing protein, BSTA, is targeted by mTORC2 and its interaction with AKT enhances its activation and suppression of FoxC2, driving adipogenesis [169]. Given the link between obesity and cancer, the impact of mTOR signaling in adipogenesis and cancer progression needs further investigation.

Acetylation

Protein modifications such as acetylation are dependent on the generation of metabolites. In addition to its use in lipogenesis, acetyl CoA is a key metabolite for acetylation and is also used to generate metabolites required for glycosylation [158]. Protein acetylation is a critical posttranslation modification in the regulation of various cellular processes. Analogous to phosphorylation, acetylation can modify protein function and interactions. Histone acetylation is the most common example of protein acetylation, in which the acetylation and deacetylation of lysine residues alter the interaction of histones with DNA, providing access for or inhibiting transcription of specific genes. Thus, levels of acetyl CoA could have profound effects on epigenetic regulation. As discussed above under lipid metabolism, the PI3K/AKT pathway plays a role in the production of acetyl CoA via regulation of ACL. Whether mTORC2 functions in regulating ACL remains unclear. Nevertheless, mTORC2 has a role in the indirect regulation of acetylation. Recent work has shown that mTORC2 regulates class IIa histone deacetylases [101]. Using a model of glioblastoma, this regulation of histone deacetylases occurs via

phosphorylation of histone deacetylases, which suppresses acetylation of FoxO, ultimately resulting in an increase in c-Myc expression and metabolic reprogramming [101]. Histone acetylation changes that precede tumor development are also stimulated by AKT and that this effect of AKT is mediated via ACL [91]. Since the mTORC2-mediated AKT phosphorylation at Ser473 correlated with histone acetylation marks in gliomas and prostate tumors, these findings also support a role for mTORC2 in acetylation. Interestingly, multiple sites of acetylation have been identified on the mTORC2 subunit Rictor [48]. Glidden et al. [48] identified a region along Rictor that is the target of acetylation and that the consequence of this acetylation is increased mTORC2 activity, as determined by stimulation of AKT activity. These findings reveal a regulatory loop wherein mTORC2 can control and is controlled by acetylation.

Clinical Relevance

mTOR sits within a key regulatory node, integrating extracellular cues such as growth and stress signals to control cell growth, metabolism and proliferation. This role makes mTOR a viable target for cancer therapeutic intervention (Table 2.1). Deregulated activation of the mTOR pathway is prevalent in many types of cancer. Mutations in mTOR itself that increase its activity have been found in solid tumors [51, 153]. Furthermore, mutations in upstream regulators of mTOR occur frequently in human tumors, including the oncogene *PIK3CA* (encoding PI3K) and the tumor suppressor *PTEN* [163]. There are also other genetic lesions that induce activation of the PI3K/mTOR pathway in cancer cells such as those encoding Ras, AKT, TSC1/2, Notch1, and receptor tyrosine kinases [10, 138]. Mutations and genomic alterations in metabolic enzymes and other key regulators of metabolic pathways of which mTOR has been linked are also common in cancers [54, 136]. Altogether, these mutations reprogram metabolism to favor biosynthetic processes crucial for the growth and proliferation of cancer cells. Hence, there is substantial effort to test MTI in the clinic and utilize existing drugs that target the pathways that become deregulated due to the above mutations (Table 2.1). More importantly, research efforts are geared toward improving cancer therapeutic strategies via better understanding of the growth and metabolic signaling network and identifying predictive biomarkers. This would allow more specific targeting using single agent therapies and/or more efficacious approach by combined therapeutic approaches. A more targeted approach holds promise particularly when specific mutations driving the malignancy are identified and the gene products are druggable. In support of this notion, a recent Phase I study wherein a urothelial cancer patient had an exceptional response to the mTOR inhibitor, everolimus, turned out to have activating mutations in mTOR [153]. In another study that analyzed gene expression patterns of patients with different types of gastric cancer, it was found that tumors of the mesenchymal subtype are particularly sensitive to MTI [92]. Whether common mutations in the mTOR/PI3K pathway in this cancer subtype occur

Table 2.1 Compounds currently being evaluated as anticancer agents targeting mTOR, as well as other aspects of cellular metabolism

Drug	Target	Mechanism of action	Clinical trial/status
<i>mTOR inhibitors</i>			
AZD8055	mTORC1/2	Inhibits mTOR signaling	NCT01316809, NCT00973076, NCT00999882, NCT00731263
Everolimus	mTORC1	Inhibits mTOR signaling	NCT00510068, NCT00912340, NCT00410124
MLN0128	mTORC1/2	Inhibits mTOR signaling	NCT01058707
OSI-027	mTORC1/2	Inhibits mTOR signaling	NCT00698243
Ridaforolimus	mTORC1	Inhibits mTOR signaling	NCT00770185, NCT00736970, NCT01234857, NCT01605396
Temsirolimus	mTORC1	Inhibits mTOR signaling	NCT01111825, NCT00909831
<i>Glucose metabolism (glycolysis)</i>			
2-deoxyglucose	Hexokinase	Inhibits glucose flux	NCT00096707, NCT00633087, NCT00247403
AZD-3965	MCT1	Inhibits lactate transport	NCT01791595
Dichloroacetate	PDK	Promotes oxidative metabolism	NCT00566410, NCT00703859, NCT00540176, NCT01163487
Metformin	AMPK	Impairs glucose metabolism	NCT02149459, NCT2145559, NCT02048384
PX-478	HIF1 α	Decrease HIF1 α levels	NCT00522652
TLN-232	PKM2	Inhibits anaerobic glycolysis	NCT00422786
<i>Oxidative and mitochondrial metabolism</i>			
AG-120	IDH1/2	Inhibits mutant IDH1/2	NCT02073994, NCT02074839
Dichloroacetate	PDK	Modulates mitochondrial metabolism	NCT00566410, NCT00703859, NCT00540176, NCT01163487
Metformin	Mitochondrial complex I	Inhibits oxidative metabolism	NCT02145559, NCT02048384
<i>Glutamine metabolism</i>			
L-asparaginase	Asparagine	Impairs glutamine uptake	Approved agent
<i>Amino acid metabolism</i>			
L-asparaginase	Asparagine	Depletes asparagine	Approved agent
Methotrexate	DHFR	Impairs folate metabolism	Approved agent
<i>Pentose phosphate pathway and nucleotide synthesis</i>			
5-fluorouracil			Approved agent

(continued)

Table 2.1 (continued)

Drug	Target	Mechanism of action	Clinical trial/status
	Thymidylate synthase	Irreversibly inhibits thymidine synthesis	
Gemcitabine	Nucleoside analog	Impedes nucleotide incorporation	Approved agent
Hydroxyurea	Ribonucleotide reductase	Impairs deoxyribonucleotide production	Approved agent

remains to be examined. Nevertheless, these studies support the concept that more effective therapy could be achieved as we gain better understanding of the molecular basis of tumor heterogeneity.

The clinical use of rapamycin is limited due to poor water solubility and stability. Thus, several pharmaceutical companies have developed rapamycin analogs (rapalogues) with improved pharmacokinetic properties. Rapalogues are already being used for the treatment of specific types of cancers and are also undergoing clinical trials for a number of different types of malignancies. We refer the reader to previous excellent reviews on rapamycin clinical trials and we focus our discussion here on more recent findings [8, 36]. Everolimus (Afinitor, RAD001; Novartis) has been efficacious for the treatment of renal cell carcinoma [106], subependymal giant cell astrocytoma and angiomyolipoma in tuberous sclerosis [13, 84]. Temsirolimus (Torisel, CCI779; Wyeth) is currently approved for advanced renal cell carcinoma and refractory mantle cell lymphoma [63]. Everolimus significantly prolonged progression-free survival (PFS) among patients with advanced pancreatic neuroendocrine tumors in a Phase III study [168], making everolimus the first effective treatment in prolonging the life of patients with this type of cancer. Ridaforolimus (A23573, deforolimus; Merck/Ariad) delayed tumor progression albeit modestly in patients with metastatic sarcoma [32]. It also has antitumor activity in advanced endometrial cancer patients in a Phase II clinical trial [24]. However, in most cancer types, rapalogues only stabilize the disease. Studies using cell lines have provided clues on possible molecular basis for this. Rapamycin and its analogs do not inhibit all the functions of mTORC1 and can inhibit mTORC2 indirectly only in certain cell types [21, 45, 129, 150]. Furthermore, inhibiting mTORC1 can also trigger a feedback loop that activates the PI3K/AKT pathway [60]. In addition, there are likely other bypass mechanisms or alternative pathways that tumor cells employ to acquire resistance against mTOR inhibition and evade cell death [68]. Thus, identifying such mechanisms would provide additional viable targets.

Indeed, although rapalogues as a single agent therapy have not lived up to expectations, combination therapy is yielding more promising clinical results. Everolimus in combination with the aromatase inhibitor, exemestane, is now approved for the treatment of ER-positive, HER2-negative advanced breast cancer resistant to nonsteroidal aromatase inhibitors based on extended median PFS of patients treated with everolimus + exemestane compared to placebo + exemestane

in the BOLERO-2 Phase III trial [170]. In the BOLERO-3 trial, use of everolimus in addition to trastuzumab and vinorelbine also significantly prolonged the PFS of patients with trastuzumab-resistant, taxane pretreated, HER2-positive advanced breast cancer [1]. Several clinical trials are ongoing to evaluate rapalogues in different breast cancer types either as combination or adjuvant/neoadjuvant therapy [75]. Temsirolimus in combination with the autophagy inhibitor hydroxychloroquine gave significant antitumor activity in melanoma patients in a Phase I clinical trial [120].

When combined with conventional chemotherapeutic agents (such as doxorubicin, camptothecin, paclitaxel, carboplatin, cisplatin, and vinorelbine), preclinical studies have revealed that rapamycin and rapalogues have enhanced antitumor activity. Thus, rapalogues in combination with chemotherapeutic agents are undergoing clinical trials for a number of different cancers. Phase I clinical trials have been conducted for use of everolimus with conventional chemotherapeutic agents for the treatment of advanced/metastatic pancreatic cancer [78], recurrent/metastatic squamous cell carcinoma of the head and neck [124], cholangiocarcinoma [25]. Phase II clinical trials of everolimus with carboplatin for the treatment of patients with triple negative breast cancer demonstrated efficacy [141].

While the use of rapalogs have mainly cytostatic effects, use of pan-mTOR kinase inhibitors either by itself or in combination with other targeted therapies and chemotherapeutic agents have been more successful in inducing cytotoxicity in preclinical studies. Unlike rapamycin, which allosterically inhibits mTOR and blocks some of mTORC1 functions, the ATP-competitive or active site inhibitors selectively target the ATP-binding pocket of mTOR and thus inhibit the catalytic activity of both mTORC1 and mTORC2 [45, 150]. The pan-mTOR inhibitors are currently undergoing a number of preclinical and early clinical trials. In U87-MG glioma xenografts, treatment with AZD8055 (AstraZeneca) led to a rapid decrease in uptake of labeled glucose, suggesting that this response can be used as an early biomarker for the metabolic changes that occur upon mTOR inhibition [80]. The mTOR kinase domain is structurally related to PI3K. Thus, some PI3K inhibitors can block both mTOR and PI3K activity. Dual mTOR/PI3K inhibitors are also undergoing early clinical trials. A clinical trial investigating the combination of mTOR and p100 α -specific PI3K inhibition in non-hematological cancers is ongoing (NCT01899053) based on preclinical studies demonstrating that mTORC1 inhibition was required for PI3K p100 α inhibitor sensitivity in breast cancer cells harboring *PIK3CA* mutations [40].

The activation of the mTOR pathway in cancers leads to altered expression and/or activity of a number of metabolic enzymes. This represents another attractive strategy particularly since antimetabolic agents have been in clinical use to effectively treat various cancers. For example, folate analogs were one of the first agents to cure liquid and solid tumors and remain in use as adjuvant therapy and for the management of several cancers [44, 93]. In preclinical studies, combined methotrexate (an antifolate) and MTI were synergistically effective for treatment of ALL in xenograft mouse models [147]. Gemcitabine, a nucleoside analog, is used in various cancers. Phase II clinical trials are ongoing that combine gemcitabine

with MTI based on promising results from a Phase I study on patients with advanced solid tumors and xenografts of sarcoma and leiomyosarcoma [100]. The antidiabetic drug, metformin, which acts by inhibiting the mitochondrial complex I and suppressing glucose production in the liver, has also been combined with MTI in several clinical studies. In a phase I clinical trial combining metformin and temsirolimus, one of 11 enrolled patients experienced partial response and five of the 11 patients experienced stable disease for 22 months [97]. Additional studies are ongoing investigating the combination of metformin and sirolimus in advanced solid tumors (NCT02145559), as well as metformin and rapamycin in pancreatic cancer (NCT02048384) (Table 2.1).

As we continue to gain insights from current preclinical and clinical studies about the role of mTOR in cancer, we can better design agents and combinatorial strategies to combat oncogenesis and tumor progression. Further understanding of the unbalanced metabolic processes that uniquely occur in different cancer subtypes and perhaps even at the individual level would pave the way for more rational therapeutic strategies with better treatment specificity and efficacy while having minimal toxicity.

Conclusions and Future Perspectives

The mTORC complexes control metabolic pathways at different levels, from transcription, translation, and posttranslational mechanisms. Most of the mTORC functions in cellular metabolism that have been uncovered point to the regulation of metabolic enzymes, transcription factors, and other effectors that ultimately modulate metabolite production and/or flux through a metabolic and biosynthetic pathways. While direct regulation of the mTORCs by nutrients remains elusive, most of the metabolic enzymes and effectors that are mTORC-dependent are allosterically regulated by nutrients or metabolites or utilize nutrients as substrates. Localization of mTORCs and association with specific regulators and metabolic enzymes in cellular compartments could serve to acutely modulate mTORC activity while triggering a cascade of events that ultimately induce gene expression of metabolic effectors.

An outstanding question is how the two mTOR complexes can overlap or diverge in the regulation of metabolic pathways. A number of studies using rapamycin, particularly in vivo models, have employed prolonged treatment of this drug, which can also inhibit mTORC2 under such conditions [129]. Furthermore, studies on mTORC2 and its function in metabolism, in comparison to mTORC1, are lagging behind. Thus, the function of mTORC2 on metabolism is so far underestimated. Knockout mouse models are shedding light on the distinct functions of these two complexes. Combined metabolomics and genomics using these mouse models along with cancer models would further enhance our understanding on the function of these two complexes. Development of specific inhibitors for each complex would also accelerate our analysis of their metabolic functions.

Phosphoproteomic studies have identified a number of mTOR targets, both directly and indirectly [65, 123, 173]. A number of these targets are involved in metabolic pathways and protein synthesis. Most of the mTORC metabolic targets that have been characterized and described herein appear to be indirectly regulated by mTOR. Although a number of transcriptional targets involved in metabolism have been pulled out, it remains unclear how mTORCs can regulate transcription in a more direct manner, i.e., whether its protein kinase activity is required. In some cases, it has been shown that the function of mTORCs is mediated by their canonical substrates such as AKT and S6K1. Given the predominant role of both mTORCs in control of protein synthesis, future studies should address precisely how metabolic enzymes and effectors could be regulated at the level of translation. Subcellular compartmentalization of mTORCs is emerging to play a key role in how mTOR integrate nutrient signals with growth and metabolic pathways. Thus, how mTORCs become recruited to membrane compartments and whether they control protein synthesis in such compartments would provide important clues on its regulation and functions in response to a specific nutrient. Further understanding of the structure of mTOR in complex with its partners would also shed light on its activation mechanisms and development of more specific MTI [165].

Lastly, we are just beginning to understand how oncogenic mutations can trigger metabolic reprogramming and the role mTOR plays in these metabolic processes. Future studies should further delineate how the mTORCs reprogram metabolic and biosynthetic pathways under specific oncogenic mutations. It has been recognized early on that although rapamycin by itself does not induce apoptosis, it augmented apoptosis and increased sensitivity to chemotherapeutic agents [140]. Nevertheless, tumors develop resistance to mTOR/PI3K inhibition due to induction of alternative pathways [107]. Identifying synthetic-lethal interactions and drug resistance mechanisms inherent to metabolic or growth signaling pathways upon mTOR inhibition will be important in order to develop more effective cancer therapy.

Acknowledgments The authors gratefully acknowledge funding from the NJ Commission on Cancer Research (TL), NIH (GM079176 and CA154674) and AACR/Stand Up to Cancer-Innovative Research Grant (IRG0311) (EJ). Stand Up to Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research.

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PI3K-mTOR in Cancer and Cancer Therapy

Dey, N.; De, P.; Leyland-Jones, B. (Eds.)

2016, XXIV, 294 p. 31 illus. in color., Hardcover

ISBN: 978-3-319-34209-2

A product of Humana Press