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New insights into the role of mTOR signaling in the cardiovascular system

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Abstract

The mechanistic target of rapamycin (mTOR) is a master regulator of several crucial cellular processes, including protein synthesis, cellular growth, proliferation, autophagy, lysosomal function and cell metabolism. mTOR interacts with specific adaptor proteins to form two multiprotein complexes, called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). In the cardiovascular system, the mTOR pathway regulates both physiological and pathological processes in the heart. It is needed for embryonic cardiovascular development and for maintaining cardiac homeostasis in post-natal life. Studies involving mTOR loss-of-function models revealed that mTORC1 activation is indispensable for the development of adaptive cardiac hypertrophy in response to mechanical overload. mTORC2 is also required for normal cardiac physiology and ensures cardiomyocyte survival in response to pressure overload. However, partial genetic or pharmacological inhibition of mTORC1 reduces cardiac remodeling and heart failure in response to pressure overload and chronic myocardial infarction. In addition, mTORC1 blockade reduces cardiac derangements induced by genetic and metabolic disorders and has been reported to extend lifespan in mice. These studies suggest that pharmacological targeting of mTOR may represent a therapeutic strategy to confer cardioprotection, although clinical evidence in support of this notion is still scarce.

This review summarizes and discusses the new evidence regarding the pathophysiological role of mTOR signaling in the cardiovascular system.

Keywords

mTOR signaling; mTORC1; mTORC2; hypertrophy; ischemia/reperfusion; heart; cardiovascular diseases

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1. Introduction

The mechanistic (previously called mammalian) target of rapamycin (mTOR) is an atypical serine/threonine kinase, belonging to the phosphoinositide kinase-related kinase (PIKK) family [1-6]. It is an evolutionarily conserved protein that plays a central role in the regulation of cellular physiology, metabolism and stress responses[1-6].

mTOR interacts with specific adaptor proteins and forms two distinct macromolecular complexes, named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)[6-10]. The previous paradigm in mTOR biology included involvement of mTOR in the regulation of protein synthesis, cellular growth and ribosomal biogenesis, and sensing and integrating different upstream inputs, such as growth factors, nutrients, amino acids, starvation and hypoxia[1-6]. However, it is now known that the mTOR pathway also controls other important cellular processes, including cell survival, mitochondrial biogenesis and function, lipid synthesis and autophagy[6]. The signaling network of mTORC2 is less characterized than that of mTORC1. Some studies have suggested that the activities of mTORC1 and mTORC2 are strongly interconnected, with mTORC2 being less sensitive to acute rapamycin treatment than mTORC1[11]. Previous work also indicated that mTORC2 is involved in the regulation of cell survival, growth and proliferation, and controls cell architecture and polarity [7-10, 12].

Given its numerous functions, deregulation of mTOR signaling can lead to the development of several pathologies, such as cancer, metabolic syndrome and cardiovascular diseases[5, 12, 13].

Previous work has indicated that mTOR plays both adaptive and maladaptive functions in the heart. Studies conducted on mouse models with either systemic or cardiomyocyte-specific disruption of mTORC1 and mTORC2 demonstrated that mTOR signaling plays a central role in regulating the development of the cardiovascular system in embryo and in preserving cardiovascular integrity and function under unstressed conditions in the post-natal life [14-17]. In addition, complete genetic disruption of mTORC1 or mTORC2 in the heart impairs the development of compensatory cardiac hypertrophy in response to stress and abrogates the ability of the heart to adapt to mechanical and ischemic injury[15, 16, 18-20]. In contrast, partial and selective inhibition of mTORC1 confers cardioprotection in multiple cardiac pathological conditions, indicating that pharmacological inhibition of mTORC1 may represent a potential therapeutic intervention for the treatment of cardiovascular diseases. Either genetic or pharmacological partial inhibition of mTORC1 activity reduces pathological hypertrophy in response to pressure overload and chronic myocardial infarction, thereby improving ventricular function. mTORC1 inhibition also reverses cardiac aging, as well as genetic and metabolic cardiomyopathies[20-25].

This review article reports new insights into the biology of mTOR in the cardiovascular system, with a particular focus on the involvement of the mTOR complexes in different cardiac pathophysiological conditions.

2. Overview of the mTOR signaling pathway

mTOR is encoded by a single gene in mammals and represents the catalytic subunit of mTORC1 and mTORC2, which are the functional homologs of the yeast TORC1 and TORC2, respectively. mTOR was discovered as a direct target of rapamycin, which binds to the FK506-binding protein of 12 kDa–rapamycin complex (FKBP12), thereby leading to mTORC1 inhibition [1-3, 6]. The scaffold proteins of mTORC1 are the regulatory-associated protein of mTOR (Raptor), the mammalian lethal with SEC13 protein 8 (mLST8), the proline-rich Akt substrate of 40 kDa (PRAS40), the DEP domain containing mTOR interacting protein (DEPTOR) and Tel two interacting protein 1 (Tel2) [26-29]. Raptor and mLST8, together with mTOR, represent the core components of mTORC1. Raptor is needed for substrate recruitment and for subcellular localization of mTORC1, whereas mLST8, which is associated with the mTOR catalytic domain, is required for the kinase activity [30, 31]. DEPTOR and PRAS40 represent the inhibitory subunits of the complex (Figure 1)[32, 33].

mTORC2 also contains mTOR, mLST8, DEPTOR, and Tel2, but it contains the rapamycin insensitive companion of mTOR (rictor) in place of Raptor[34, 35]. In addition, the regulatory subunits mSin1 and protein observed with rictor (Protor1/2) have also been identified as possible mTORC2 partners (Figure 1). Moreover, several activators and inhibitors have been shown to act upstream and modulate mTOR complex activity, such as Akt (also known as protein kinase B) and adenosine monophosphate activated protein kinase (AMPK). Once modulated, mTOR transduces these signals to different downstream effectors, thereby controlling numerous cellular processes (Figures 2 and 3).

2.1 mTORC1 and protein synthesis

mTORC1 is the main regulator of cellular growth in response to different environmental and intracellular conditions. Therefore, it promotes anabolic processes such as protein, nucleotide and lipid synthesis, whereas it inhibits catabolic pathways, such as autophagy [6, 7]. The first step in protein synthesis in eukaryotic cells usually occurs through the cap-dependent translation process that involves interaction between a series of proteins with the 5′ end of an mRNA molecule. The first phase of translation involves the eukaryotic initiation factor (eIF) 4F, a complex formed by eIF4E, eIF4A and eIF4G. Briefly, eIF4F interacts with the 5′ end of the mRNA and the poly(A) tail, which recruits the 40S ribosomal subunit. eIF4B association with eIF4A is also required for binding of the mRNA to ribosomes[36].

The main substrates of mTORC1 involved in protein synthesis include the S6 kinase-1 (S6K1) and the eukaryotic translation initiation factor 4E (eIF4E)-binding protein-1 (4E-BP1)[7-10, 12]. mTORC1 activates S6K1 by direct phosphorylation at Thr389. Once phosphorylated, S6K1 promotes the initiation of translation through a number of different mechanisms. It activates eIF4B, a positive regulator of the 5′ cap-binding eIF4F complex, while at the same time it inhibits programmed cell death 4 (PDCD4)[37], a negative regulator of translation that prevents the incorporation of eIF4B into the translation initiation complex. Moreover, S6K1 also promotes translation through S6K1 Aly/REF-like substrate (SKAR), a component of exon-junction complexes[38]. In contrast, 4E-BP1 acts as a negative regulator of

translation, interacting with eIF4E and inhibiting eIF4F complex assembly. Phosphorylation of 4E-BP1 at multiple residues by mTORC1 prevents 4E-BP1 interaction with eIF4E, allowing eIF4E interaction with eIF4G and, consequently, cap-dependent translation[7-10, 12]. In cardiomyocytes, mTORC1 is also required for protein synthesis, in which it acts through the inhibition of 4E-BP1[15-17, 39]. mTOR gene deletion in these cells leads to a dramatic reduction of protein synthesis due to 4E-BP1 accumulation.

2.2 mTORC1 and metabolism

mTORC1 regulates the de novo synthesis of cellular membrane lipids through the activation of sterol responsive element binding protein 1/2 (SREBP 1/2) transcription factors [4], which trigger the expression of genes involved in fatty acid metabolism and cholesterol biosynthesis. SREBP 1/2 activation is also required for the oxidative pentose phosphate pathway, a process that generates metabolic intermediates needed for cellular growth [40]. mTORC1 can activate SREBP1/2 directly by phosphorylation[40] or indirectly, either through the S6K1 pathway or by mTORC1-mediated phosphorylation of Lipin-1, a negative regulator of SREBP 1/2[41]. Recently, glutamylprolyl-tRNA synthetase (EPRS) has emerged as a new downstream target of the mTORC1/S6K1 pathway in the regulation of lipid metabolism. When EPRS is phosphorylated at Ser999 by S6K1, it interacts with the fatty acid transporter protein 1 (FATP1). FATP1 then translocates to lipid membranes and mediates long fatty acid uptake. However, the mechanism by which EPRS activates FATP1 requires further investigation[42].

mTORC1 also enhances the expression of genes involved in mitochondrial biogenesis through activation of the transcription factor yin-yang 1 (YY1)/peroxisome proliferator–activated receptor γ coactivator-1α (PGC-1α) transcriptional complex[43]. In addition, mTORC1 favors cellular growth by promoting nucleotide synthesis through activation of the activating transcription factor 4 (ATF4)[44]. Lastly, mTORC1 promotes a metabolic shift from fatty acid oxidation toward glycolysis by enhancing the expression of hypoxia-inducible factor-1α (HIF- α), which regulates the expression of genes involved in glycolysis [40]. Hearts of mice with cardiac raptor gene ablation display an increase in carbohydrate metabolism together with a decrease in the expression of fatty acid regulatory genes[16].

2.3 Autophagy and lysosomal function

mTORC1 is an important negative regulator of autophagy (Figure 4). Autophagy is an evolutionarily conserved intracellular self-digestion mechanism that removes misfolded proteins and dysfunctional organelles[45, 46]. These damaged cargos are sequestered by double-membrane vesicles called autophagosomes, which ultimately fuse with lysosomes to form autophagolysosomes, in which the damaged cargoes are digested[47-49]. Once digested, the resulting molecular components (amino acids, lipids and carbohydrates) are recycled and reintroduced into the cellular metabolic processes. In this way, autophagy contributes to cellular homeostasis, especially during stress and nutrient deprivation. Autophagy is activated in response to cellular stress and mTORC1 inhibition contributes to autophagy activation under these conditions. mTORC1 inhibits autophagy at both the transcriptional and post-translational levels[9, 12, 45, 46], impairing both autophagosome and autolysosome formation[50]. mTORC1 phosphorylates unc-51-like kinase (ULK) 1 and ATG13, thereby

inhibiting the activity of the ULK1-ATG13-FIP200 multiprotein complex, which is crucial for autophagosome formation[51]. In the presence of nutrients, phosphorylation of ULK1 by mTORC1 also impairs its binding to AMPK, which phosphorylates ULK1 and activates autophagy[52, 53]. mTOR gene deletion in cardiomyocytes was found to be associated with autophagy activation[15]. The involvement of ULK1 in mediating mTOR-dependent inhibition of autophagy has been demonstrated in the hearts of cardiac raptor knockout mice and in animal models of genetic cardiomyopathy as well[16, 54]. In response to amino acid starvation, mTORC1 negatively regulates autophagy through inhibition of PIK3C3/VPS34 kinase, which forms a complex with ATG14 and promotes autophagosome formation. mTORC1 inhibits the ATG14-containing PIK3C3/VPS34 complex through multiple phosphorylation of ATG14 [55]. Previous work from Cecconi's group also showed that mTORC1 suppresses autophagy by directly phosphorylating AMBRA, a ubiquitin ligase protein of the Beclin1 complex needed for ULK1 stabilization through Lys-63-linked ubiquitination[56].

mTORC1 also transcriptionally regulates autophagy by directly regulating p73 and the transcription factor EB (TFEB). The latter regulates autophagy by enhancing the expression of autophagic proteins such as ATG7, which is fundamental for the initiation of autophagy[45, 57, 58]. However, mTORC1 activation in cardiomyocytes dramatically reduces ATG7 expression levels[54, 59]. Specifically, mTORC1 phosphorylates TFEB at Ser211, impairing its dissociation from the cytosolic chaperone 14-3-3 and consequent translocation into the nucleus, where it enhances the expression of lysosomal genes[60-62]. mTORC1 may also negatively regulate lysosomal biogenesis through regulation of the TFE3 and ZKSCAN3 transcription factors[63, 64]. In addition, mTORC1 directly impairs the activity of lysosomal proteins required for lysosomal lumen acidification, such as the proton pump v-ATPase[65], thereby affecting lysosomal function and the process of autophagosome-lysosome fusion, namely autophagic flux[66].

2.4 mTORC2 function: cell survival and polarity

Although mTORC2 signaling is less characterized than the mTORC1 pathway, recent evidence clearly shows its involvement in the regulation of cellular architecture and survival (Figure 2) [35, 67]. The best characterized mTORC2 substrates are those belonging to the AGC protein kinase family, including Akt, serum- and glucocorticoid-induced protein kinase 1 (SGK1), and protein kinase C-α (PKC-α). Akt is phosphorylated by mTORC2 at Ser473, which enhances Thr308 phosphorylation by phosphoinositide-dependent protein kinase-1 (PDK1) and ultimately leads to Akt activation and promotion of cell survival [68, 69]. mTORC2 also regulates cell survival through phosphorylation and activation of SGK1, which is known to promote cardiomyocyte survival and inhibit hypertrophy[70]. Cells with genetic mTORC2 disruption display an absence of SGK1 phosphorylation [71] and increased cell death[69].

We recently demonstrated the existence of crosstalk between mTORC2 and mammalian sterile 20 like kinase 1 (MST1) that is involved in the regulation of cardiomyocyte survival. MST1 is a serine/threonine kinase and the main component of the Hippo pathway. The Hippo pathway is a cellular transduction cascade that negatively regulates cellular survival and growth, partially through inhibition of YAP, a transcription co-factor that promotes

survival and proliferation. We showed that mTORC2 inhibits MST1 through phosphorylation at Ser438, thereby limiting its pro-death effects[19]. Finally, mTORC2 also controls cell polarity and cytoskeletal architecture. Cells with genetic deletion of rictor exhibit defects in cell polarity, resulting in actin-F accumulation and inhibition of cell spreading. PKC-α and Ras homolog gene family member (Rho)-A GTPase seem to be involved in cytoskeleton organization by mTORC2[34, 35].

3. Mechanisms of regulation of mTOR signaling and downstream targets

To date, several inputs are known to modulate mTOR activity. Generally, under unstressed conditions and in the presence of nutrients and growth factors, mTORC1 is activated. In contrast, mTORC1 activity is decreased in response to stress conditions such as nutrient starvation, hypoxia or DNA damage (Figure 5).

3.1 Growth factors

The Akt and AMPK pathways are the major upstream regulators of mTORC1. Akt is activated by nutrient-rich conditions and growth factors, and once activated Akt phosphorylates and inhibits PRAS40 and the tuberous sclerosis protein (TSC) 1/2, which are endogenous mTORC1 inhibitors[7-10, 72, 73]. TSC1/2 inhibit the small GTP-binding protein Ras homolog enriched in brain (Rheb), a positive regulator of mTORC1[74-77]. TSC1/2 and Rheb regulate mTORC1 on the lysosomal membrane, where mTORC1 seems to be primarily localized[74-77]. Excessive lysosomal accumulation of TSC2 is also responsible for mTOR inhibition, as shown in the heart in the presence of Pompe disease[78]. On the other hand, upon phosphorylation and inhibition by Akt, TSC1/2 dissociate from the lysosomal membrane, preventing it from interacting with and hydrolyzing Rheb- GTP[74]. Extracellular signal regulated kinase 1/2 (ERK 1/2) and inhibitor of nuclear factor κB (NF-kB) kinase β (IKKβ) were also reported to mediate TSC1/2 inhibition in response to growth factors[79, 80].

3.2 Energy, hypoxia and amino acids

During energy stress, nutrient deprivation and hypoxia, mTORC1 activity is reduced, leading to a shutdown of anabolic processes. AMPK was shown to inhibit mTORC1 in response to stress either directly, through the phosphorylation of Raptor with a subsequent impairment of complex assembly, or indirectly, through the activation of TSC1/2[77, 81-83]. During energy stress, mTORC1 is inactivated by glycogen synthase kinase (GSK) 3β, which also activates TSC1/2[81]. Activation of AMPK and GSK-3β and inhibition of Rheb also lead to mTORC1 inhibition in cardiomyocytes in response to energy deprivation[47, 59, 84]. p38-regulated/activated kinase also contributes to Rheb inhibition during energy deprivation, independently of TSC2. Similarly, a stress response protein named regulated in DNA damage and development 1 (REDD1) activates TSC1/2 during hypoxia independently of AMPK[85]. In addition, Miyamoto's group recently demonstrated that hexokinase-II (HK-II), a protein involved in the first step of glycolysis, increases autophagy and diminishes cell death in cardiomyocytes and non-cardiomyocyte cells during glucose deprivation through direct interaction with and inhibition of mTORC1[86]. We recently demonstrated that mTOR activity is also regulated through a redox mechanism. mTOR is oxidized at Cys1483 in response to oxidative stress in cardiomyocytes, and this modification reduces its activity.

Thioredoxin-1 binds to mTOR and reduces its oxidation, thereby preserving its activity, which is crucial for promoting cardiomyocyte survival under pro-oxidative conditions[87].

Interestingly, accumulating lines of evidence have recently demonstrated that amino acids are crucial regulators of mTORC1 activity through the control of Rag GTPases. Specific cytoplasmic and lysosomal amino acids maintain Rag in an active conformation, allowing it to positively interact with Raptor, leading to translocation of mTORC1 to lysosomal membranes and its subsequent activation by Rheb[32, 88]. This evidence indicates that simultaneous Rag and Rheb activation is required for mTORC1 activation. It was also demonstrated that the lysosomal amino acid transporter SLC38A9 interacts with Rag and acts as a sensor of arginine levels, together with the lysosomal v-ATPase, to control mTORC1 activity[89-91]. In contrast, Sestrin2, a leucine sensor, has emerged as an mTORC1 inhibitor during amino acid deprivation. In fact, in the absence of leucine, Sestrin2 deactivates GAP activity towards Rags (GATOR) 2, a positive regulator of mTORC1[92, 93]. Once inhibited, GATOR2 fails to bind GATOR1, which in turn deactivates mTORC1 through Rag inhibition. In fact, GATOR1 acts as the GAP protein of Rag GTPases. Similarly, the cellular arginine sensor for mTORC1 (CASTOR1) inhibits mTORC1 through the GATOR1/2 pathway in response to low arginine levels[94, 95]. A recent work also demonstrated that Rag A/B play an important role in the regulation of lysosomal function in cardiomyocytes[96].

3.3 Regulators of mTORC2

mTORC2 activity is also modulated by multiple upstream stimuli, including insulin/phosphoinositide 3 kinase (PI3K) signaling and growth factors[97]. In contrast, it appears to be insensitive to nutrient deprivation[8, 12]. However, the regulatory subunit mSin1 inhibits mTORC2 catalytic activity in the absence of insulin[98] and TSC1/2 complex may also inhibit mTORC2[99]. Interestingly, mTORC1 acts as a negative regulator of mTORC2, through inhibition of the insulin/phosphoinositide 3 kinase (PI3K) pathway[68, 69, 100]. This action is mediated by the activation of growth factor receptor-bound protein 10 (Grb10), a negative regulator of the insulin/IGF-1 pathway[101, 102], and by S6K1, which phosphorylates and promotes the degradation of insulin receptor substrate 1 (IRS1)[103, 104]. Recently, an interaction between mTORC2 and an atypical variant of the calcineurin Aβ gene was shown in mouse embryonic stem cells, which led to activation of the AKT/GSK3β/β-catenin pathway and cellular differentiation towards the mesodermal lineage[105].

4. mTOR in the heart

In recent years, the mTOR complexes have emerged as crucial regulators of cardiovascular embryonic development, homeostasis and adaptation to stress. In general, mTORC1 and mTORC2 are essential for the preservation of cardiac structure, growth and vascular integrity in both prenatal and postnatal stages. They are also required for cardiac adaptation to mechanical stress, contributing to the development of compensatory hypertrophy and limiting cardiomyocyte death (Table 1). However, mTORC1 activation in the heart during chronic stress has also been shown to have multiple maladaptive effects, such as the promotion of pathological hypertrophy, misfolded protein accumulation and energy stress. In

fact, partial inhibition of mTORC1 activity was shown to reduce cardiovascular damage in response to pressure overload, acute and chronic ischemic injury and aging, and in both genetic and metabolic cardiomyopathies (Table 1).

The most recent evidence regarding the role of mTOR complexes in the cardiovascular system will be discussed in the following paragraphs.

4.1 mTORC1 and the heart under unstressed conditions

mTOR regulates cardiac development and function both during embryogenesis and post-natal life[14, 15, 69]. Mice with systemic deletion of mTORC1 components die in utero and display multiple cardiac and vascular derangements [5]. Similarly, live born mice with constitutive cardiac-specific mTOR gene disruption suffer from cardiac dilatation, heart failure and metabolic derangements[14]. Similar results were observed in adult mice with inducible cardiomyocyte-restricted deletion of mTOR and Raptor genes, in which cardiac dilatation, dysfunction, heart failure and early mortality were observed soon after induction, together with sarcomere disarray, apoptosis, autophagy and mitochondrial dysfunction[15]. Cardiac-specific deletion of the Rheb gene was also associated with mTORC1 inhibition and rapid development of dilated cardiomyopathy, resulting in early mortality within 10 days after birth, although cardiomyocyte apoptosis was not observed in this model. Mechanistically, the detrimental effects of cardiac mTORC1 disruption in the postnatal stage appear to be partially mediated by 4E-BP1 accumulation[15], with consequent impairment of protein translation and a lack of fundamental protein production within cardiomyocytes. In support of this notion, simultaneous genetic deletion of 4E-BP1 partially reversed the dramatic phenotype observed in mTOR and Rheb knockout mice[15-17]. In a recent study, mice with early postnatal cardiac deletion of the mTOR gene displayed heart dilatation, cardiac fibrosis, apoptosis and heart failure, and died after three weeks. Apoptosis was associated with the accumulation of p53 and ankyrin repeat domain 1. These mice also displayed JNK activation, HIF-1α downregulation and a reduction of cardiac myoglobin content. These data suggest that mTOR in the early postnatal myocardium may be required for cardiomyocyte growth and oxygen supply maintenance[39]. On the other hand, no differences were observed in the phosphorylation status of the co-transcription factor YAP1; the activities of other components of the Hippo pathway, such as MST1/2 and LATS1/2, were not evaluated.

While these data indicate that complete disruption of the mTOR pathway is catastrophic for the heart, accumulating lines of evidence have demonstrated that partial inhibition of mTORC1 activity is beneficial for the heart during the aging process, likely due to reductions in energy expenditure and misfolded protein accumulation and to the activation of autophagy. Both partial genetic and pharmacological mTORC1 inhibition were shown to extend lifespan in lower organisms and in mammals[21, 22, 24]. Pharmacological inhibition of mTOR with rapamycin reduced age-related heart inflammation and fibrosis, and improved energy metabolism[22]. Similarly, caloric restriction was shown to improve diastolic function, reduce mTORC1 activity and activate autophagy in the aged rat[106]. Moreover, GSK-3α knockout mice displayed premature death and age-related cardiac abnormalities, such as hypertrophy and sarcomere disruption, as a result of mTOR activation and autophagy

inhibition[107]. Interestingly, short-term caloric restriction and rapamycin reversed cardiac hypertrophy and diastolic dysfunction in aged hearts. A global proteomic analysis after these treatments revealed a marked attenuation in age-dependent protein oxidation and ubiquitination, accompanied by an upregulation of mitochondrial proteins, such as those involved in the electron transport chain, citric acid cycle and fatty acid metabolism[108].

4.2 mTORC1 and cardiac hypertrophy

mTORC1 is activated in response to hypertrophic signals, such as pressure overload, β-adrenergic stimulation, angiotensin II and IGF-1, and plays both adaptive and maladaptive roles under these conditions. On the one hand, mTORC1 is required for the development of compensatory cardiac hypertrophy and maintenance of cardiac function in response to pressure overload. Mice with inducible cardiac-specific mTOR and raptor gene deletion develop cardiac dysfunction and heart failure without compensatory hypertrophy in response to transverse aortic constriction[15, 16]. However, it should be pointed out that whether a compensatory form of hypertrophy that favors cardiac adaptation to mechanical stress actually exists is currently under question [109].

On the other hand, deregulated mTORC1 activation in the heart leads to the development of pathological hypertrophy. In fact, it has been repeatedly demonstrated that partial genetic or pharmacological inhibition of mTORC1 is beneficial in various pro-hypertrophic conditions [110]. However, it should be noted that mTORC1 activation alone does not appear to be sufficient to induce cardiac hypertrophy. In fact, previous work showed that mTOR overexpression does not induce significant changes in ventricular mass [111, 112]. This evidence suggests that mTOR activation contributes to the development of cardiac hypertrophy together with other signaling pathways only in the presence of hypertrophic stimuli.

In a murine model of transverse aortic constriction (TAC)-induced cardiac hypertrophy, rapamycin reduced hypertrophy and improved cardiac function[113]. Similarly, pharmacological mTORC1 inhibition was found to reduce eccentric hypertrophy during volume overload induced by arteriovenous fistula in the abdominal aorta[114]. Mice with heterozygous cardiac deletion of Rheb showed reduced cardiac hypertrophy and fibrosis[23]. Cardiac overexpression of PRAS40 was also reported to reduce hypertrophy and fibrosis, improving cardiac function in response to pressure overload[115]. Moreover, mice with systemic TSC1 overexpression driven by the ubiquitin C promoter displayed decreased mTORC1 activity in the heart, preserved cardiac function and reduced hypertrophy in response to isoproterenol *in vivo*[116].

Multiple mechanisms contribute to the regulation of mTORC1 activation in the heart in response to hypertrophic stimuli and control the development of pathological hypertrophy.

Cardiac-specific deletion of Folliculin (FLCN), a tumor suppressor and negative regulator of mTORC1 activity, caused cardiac hypertrophy and dysfunction, which were reversed by rapamycin treatment[117]. Cardiac deletion of TSC2 also resulted in cardiac dysfunction and cardiomyocyte hypertrophy, associated with an impairment of autophagic flux, which were reversed by pharmacological autophagy reactivation[118].

Epigenetics also play a role in the regulation of mTOR during hypertrophy. Both genetic and pharmacological inhibition of class I histone deacetylases (HDACs) suppress pathological cardiac hypertrophy in mice subjected to pressure overload through TSC2 activation[119]. Interestingly, the heart-enriched long non-coding (lnc)RNA named 'Cardiac hypertrophy-associated epigenetic regulator' (Chaer) has recently emerged as a positive epigenetic regulator during cardiac hypertrophy development under the control of mTORC1. Cardiac disruption of Chaer reduced pathological cardiac hypertrophy and improved heart function in response to pressure overload. Chaer interacts with and inhibits the histone methyltransferase Polycomb Repressor Complex 2 (PRC2) in an mTORC1-dependent manner, reducing the level of histone methylation of pro-hypertrophic genes, thereby inducing their transcription[120].

MicroRNAs also appear to regulate cardiac hypertrophy by modulating mTOR activity. Mice with cardiac-specific overexpression of miR-211 showed cardiac dysfunction and heart failure, associated with reduced autophagy and increased mTOR activity. miR-211 downregulates the cyclin-dependent kinase (CDK) inhibitor p27, which in turn activates mTOR through CDK2-dependent mechanisms, thereby leading to heart failure[121]. Cardiac-specific miR-199a transgenic mice also developed spontaneous cardiac hypertrophy associated with a reduction of autophagy, through downregulation of GSK-3β and subsequent mTORC1 activation[122]. In contrast, mTOR is a direct target of miR-99a, which was shown to attenuate cardiac hypertrophy and reduce the mTOR/P70/S6K pathway in response to pressure overload[123]. Of note, in most of these studies the direct contribution of mTOR modulation to the effects of different epigenetic modifications was not clarified.

The valosin-containing protein (VCP), belonging to the type II AAA (ATPases associated with various cellular activities) ATPase family, modulates mTORC1 during pressure overload. VCP-overexpressing mice show a reduction of left ventricular hypertrophy and mTORC1 inhibition[124]. The DNA-damage-inducible transcript 4-like (DDiT4L) regulates mTOR activity in cardiomyocytes and is highly expressed in the heart of a murine model of pathological hypertrophy compared to a model of physiological hypertrophy. Overexpression of DDiT4L in the heart inhibits mTORC1, activates mTORC2, and stimulates autophagy, and these effects are associated with a subtle reduction of systolic function[125]. The specific contribution of mTOR complexes to the effects induced by DDiT4L was not investigated, but the authors found that heterozygous Beclin-1 gene deletion reversed the phenotype of the transgenic mice, suggesting that autophagy activation may contribute to the detrimental cardiac effects induced by DDiT4L overexpression. Recently, two novel obscurins (Obsc), Obsc40 and Obsc80, which are localized at the intercalated disc, have emerged as negative modulators of cardiomyocyte cell size and adhesion through inhibition of the mTOR pathway[126]. Additionally, p38γ and p38δ, two MAPKs involved in the regulation of stress response, modulate both physiological and pathological hypertrophy by enhancing mTOR activation through phosphorylation and consequent proteasome-mediated degradation of DEPTOR[127].

4.3 mTORC1 and ischemic damage

An increasing body of evidence suggests that mTORC1 also regulates cardiac adaptation to energy deprivation and ischemia. We previously demonstrated that mTORC1 activity is reduced in cardiomyocytes under these conditions through inhibition of Rheb[59]. Reactivation of Rheb/mTORC1 inhibits autophagy through ATG7 downregulation and promotes cardiomyocyte death[59]. In line with this evidence, we also found that inhibition of cardiac GSK-3β during prolonged myocardial ischemia attenuates ischemia-induced mTORC1 inhibition, inhibits autophagy and increases ischemic injury[84]. This detrimental effect of GSK-3β inhibition was reversed by rapamycin treatment. Overall, these results suggest that inhibition of the mTORC1 pathway is an adaptive response in cardiomyocytes, where it promotes cell survival-promoting mechanisms, such as autophagy, energy preservation, and reduction of misfolded protein accumulation [59].

mTORC1 activation also contributes to pathological cardiac remodeling in response to chronic ischemic injury [20, 25]. Attenuation of mTORC1 activity with RAD01 treatment activates autophagy and reduce chronic post-infarction cardiac remodeling[25]. Administration of rapamycin and S6K inhibitors during myocardial infarction also reduces cardiac ischemic damage and apoptosis through a PDK1-Akt-dependent pathway [128]. Interestingly, mTORC1 inhibition through overexpression of PRAS40 reduces post-infarction cardiac remodeling and improves cardiac function through activation of the mTORC2-Akt pathway[20]. Accordingly, cardiac-specific knockdown of rictor exacerbated cardiac remodeling and dysfunction after myocardial infarction[20]. These data demonstrate that pharmacological inhibition of mTORC1 attenuates chronic ischemic remodeling and highlight the importance of a proper balance between mTORC1 and mTORC2 activities in the regulation of the cardiac adaptation to stress.

In contrast, the role of mTORC1 in response to reperfusion injury is still debated. Although previous work demonstrated that pharmacological inhibition of mTORC1 before the ischemic phase reduces final ischemia/reperfusion damage[129], other studies showed that mTORC1 inhibition is ineffective when administered at the time of reperfusion injury[84]. Prolonged rapamycin treatment also decreases cardiac function and increases myocardial necrosis in healthy pigs, although these effects may be mediated by mTORC2 downregulation since long-term rapamycin treatment also impairs mTORC2 activity[130]. Rapamycin abrogates the beneficial effects of ischemic preconditioning[131], despite the fact that mTORC1 is already inhibited in response to ischemic preconditioning through G9a-mediated hypermethylation of the H3K9 site[132]. Overall, these data may suggest that mTORC1 activation is adaptive in response to reperfusion injury. In support of this notion, we previously found that heterozygous deletion of the GSK-3β gene reduces reperfusion injury through mTORC1 activation[84]. Cardiac-specific overexpression of Creb binding protein/p300–interacting transactivator with ED-rich carboxy-terminal domain (CITED)-4 also reduces cardiac remodeling after ischemia/reperfusion injury through activation of the mTORC1 pathway[133]. In addition, mice with mTOR overexpression in cardiomyocytes displayed reduced necrosis, inflammation and cardiac dysfunction after ischemia/reperfusion[134]. These results are in accordance with previous compelling data from Kirshembaum's group demonstrating that mTOR overexpression reduces cardiomyocyte

death in hypoxic conditions through the activation of NF-kB and downregulation of BCL2/adenovirus E1B 19 kDa protein–interacting protein 3 (Bnip3), a mitochondrial death gene[135]. However, the specific contributions of mTORC1 and mTORC2 in the beneficial effects exerted by mTOR overexpression were not evaluated in these studies. mTORC1 activation during reperfusion injury may be beneficial through several mechanisms. It may reduce maladaptive autophagy[47], it may limitmPTP opening [84] and it may also promote mitochondrial biogenesis and function[43], which is important for mitochondrial turnover, new energy production and functional cardiac recovery.

4.4 mTORC1 in genetic cardiomyopathies

Dysregulation of the mTOR pathway was also found to be associated with several genetic forms of cardiomyopathy. mTORC1 is hyperactivated in the heart of a mouse model of LEOPARD disease, which is characterized by hypertrophic cardiomyopathy, fibrosis and cardiac dysfunction. Rapamycin administration reduced hypertrophic cardiomyopathy and myocardial disarray[136]. Consistent with this result, mTOR activity was found to be increased in a variety of models of hypertrophic cardiomyopathies, including those caused by mutations in the TRIM63 gene, encoding for Muscle RING finger protein 1 (MuRF1), and in the Laminin A/C gene[54, 137, 138]. In the latter model, pharmacological inhibition of mTORC1 activated autophagy, reduced cardiac remodeling and improved cardiac function[54, 138]. In addition, *de novo* S75Y mutation of RagC GTP-binding protein is associated with the development of syndromic fetal dilated cardiomyopathy. This mutation leads to a reduction of RagC activity and its overexpression increases mTORC1 activity, making cells insensitive to amino acid deprivation *in vitro*[139]. This evidence corroborates previous evidence demonstrating that cardiac disruption of RagA/B proteins leads to a massive hypertrophic cardiomyopathy mimicking lysosomal storage disease. Finally, high levels of mTORC1 activation in human myocardial biopsies are associated with increased myocardial fibrosis and a worse prognosis in patients with non-ischemic cardiomyopathy, indicating that the impact of mTORC1 activation on the development and progression of genetic cardiomyopathies is clinically relevant[140].

4.5 mTORC1 in metabolic cardiomyopathy

Cardiac activation of mTORC1 also contributes to the development and progression of cardiac abnormalities induced by metabolic derangements, such as diabetes, obesity and metabolic syndrome. In obese mice, mTOR activation impairs autophagosome formation, causing a reduction of cardiac functionality, which is rescued by rapamycin administration [141]. Similarly, deregulated activation of the Akt2/mTORC1 pathway contributes to the development of cardiac dysfunction and autophagic flux abnormalities in response to high fat diet consumption [142]. Cardiac activation of the mTOR pathway is also responsible for the suppression of autophagosome formation in the hearts of *ob/ob* mice, independently of IGF1/Akt signaling[143]. mTOR activation is observed in the heart of a swine model of metabolic syndrome and is correlated with a reduction of autophagy, with increased apoptosis and with the development of cardiac dysfunction [144]. In type II diabetic mice, mTOR inhibition by chronic administration of rapamycin improves cardiac function, with a significant reduction of plasma glucose, insulin and triglyceride levels, and an attenuation of oxidative stress [145]. In addition, mTORC1 inhibition by PRAS40 treatment prevents the

development of high fat diet-induced diabetic cardiomyopathy, by improving metabolic function and insulin receptor signaling [146]. Metabolic syndrome and diabetes are often associated with the development of renal insufficiency, and mTOR inhibition attenuates cardiac fibrosis in uremic cardiomyopathy[147].

mTORC1 activation is also responsible for the reduction of myocardial tolerance to stress induced by metabolic defects. We previously found that ischemia-induced activation of autophagy is inhibited in the hearts of mice with high fat diet-induced metabolic syndrome through a dysregulation of Rheb/mTORC1 signaling. Either pharmacological or genetic mTORC1 inhibition rescues autophagy and decreases infarct size after ischemia in these animals[59]. Rapamycin also protects against ischemia/reperfusion injury through activation of the STAT3 signaling pathway in diabetic hearts[148].

Interestingly, increased circulating levels of branched-chain amino acids (BCAA) can usually be observed in patients with metabolic abnormalities[149]. Increased BCAA levels may contribute to the activation of myocardial mTORC1 and to the increased myocardial susceptibility to ischemic injury. In this regard, oral BCAA administration exacerbates cardiac hypertrophy and remodeling in response to chronic myocardial infarction, and these detrimental effects are alleviated by rapamycin administration[150].

4.6 The direct role of mTOR in cardiac fibroblasts

As described in the previous paragraphs, deregulated mTOR activation in cardiomyocytes often leads to the development of myocardial fibrosis. However, the direct role of mTOR in cardiac fibroblasts has been poorly investigated. A number of different profibrotic stimuli lead to mTOR activation in cardiac fibroblasts, and mTOR upregulation in this context is associated with an increase in collagen synthesis, fibroblast proliferation and transformation of fibroblasts into myofibroblasts[151]. mTOR was also found to mediate the cardiac profibrotic effects induced by the epidermal growth factor-like growth factor (HB-EGF). In the hearts of transgenic mice overexpressing HB-EGF, the Akt and mTOR signaling pathways were upregulated in cardiac fibroblasts, together with S6K. Furthermore, in cultured cardiac fibroblasts treated with HB-EGF, Akt-mTOR pathway activation correlated with collagen synthesis and fibroblast proliferation[152]. Similarly, in rat cardiac fibroblasts, angiotensin II induced the transformation of cardiac fibroblasts into myofibroblasts, which was associated with mTOR activation and AMPK downregulation[153]. In addition, a previous study demonstrated that knockdown of Sestrin 1 in cardiac fibroblasts leads to mTOR activation, promotes fibroblast proliferation, enhances collagen synthesis and stimulates connective tissue growth factor (CTGF) production, both at baseline and in response to angiotensin II treatment[154]. It should be pointed out that the direct contribution of mTOR signaling to fibroblast activation and collagen synthesis was not completely demonstrated in these studies.

4.7 The role of mTORC2 in cardiac homeostasis and stress

mTORC2 appears to be essential for normal cardiac development and for the maintenance of postnatal cardiac structure and function. Global deletion of rictor is embryonically lethal, although rictor-/- embryos die significantly later than raptor-/- ones[69], suggesting that

mTORC2 is important in a later stage of embryonic development with respect to mTORC1. Nonetheless, rictor-/- embryos show marked cardiovascular abnormalities[34]. Mice with constitutive cardiac-specific rictor gene deletion are born normally but develop signs of cardiac dysfunction at 6 months of age[19], in keeping with previous evidence that heterozygous rictor gene deletion reduces life span[155]. On the other hand, cardiac deletion of the rictor gene in adulthood does not significantly affect baseline cardiac function[18].

mTORC2 disruption also impairs the ability of the heart to adapt to stress. We previously found that mice with α-MHC-Cre-mediated cardiac deletion of the rictor gene develop cardiac dilation, fibrosis and apoptosis in response to 4 weeks of pressure overload. These mice also display an attenuated left ventricular hypertrophic response. We found that mTORC2 interacts with and negatively regulates MST1 by phosphorylating it at Ser438 in the SARAH domain, which is important for MST1 dimerization and activation. As a result, the activity of MST1 was significantly increased in the hearts of rictor knockout mice compared to in controls, and inhibition of this molecule in vivo, by the overexpression of a dominant negative form of MST1, rescued their phenotype[19]. Mice with tamoxifen-induced cardiomyocyte-specific rictor gene deletion develop cardiac dysfunction and dilation in response to 1 week of pressure overload, confirming that mTORC2 plays a pivotal role in cardiac adaptation to mechanical stress. Interestingly, these mice do not show significant differences in total ventricular weight with respect to controls, suggesting a similar hypertrophic response[18]. However, this result might also be interpreted as a defect in the development of compensatory hypertrophy, since ventricular weight should be higher in rictor knockout mice as a consequence of the presence of cardiac dysfunction and dilation[18]. In support of this latter hypothesis, a recent work demonstrated that stromal interaction molecule 1 (STIM1) is required for the development of compensatory hypertrophy and preservation of cardiac function in response to pressure overload through direct activation of the mTORC2/Akt pathway and inhibition of GSK-3β, a regulator of cardiomyocyte growth[156].

mTORC2 also appears to limit ischemic injury. Volkers et al. previously demonstrated that cardiac rictor gene knockdown accelerates cardiac dysfunction and remodeling in response to chronic myocardial infarction, suggesting that mTORC2 exerts important anti-remodeling effects in response to myocardial infarction[20]. The cardioprotective effects of ischemic preconditioning appear to be mediated by activation of the mTORC2/Akt pathway. In fact, dual mTORC inhibitors abrogated the beneficial effects of ischemic preconditioning but rapamycin did not [157]. Likewise, mTORC2 has been shown to mediate the cardioprotective effects of hydrogen sulphide in response to ischemia/reperfusion in rats[158].

5. Perspectives

Several aspects of the pathophysiological functions of mTOR in the cardiovascular system have been characterized, but still many others need to be understood.

mTOR complexes exert both adaptive and maladaptive functions and future studies are encouraged to understand the mechanisms underlying these dual functions, which may depend upon the condition and the cell type in which the activity of mTOR complexes is

modulated. The level of activation and inhibition may also determine whether the function of mTOR is physiological or pathological. However, it is also likely that the cellular effects of mTOR signaling may depend upon its regulators and upstream signals or may be determined by the specific downstream targets that are modulated given the specific condition and timepoint. For this reason, it is important to identify other regulators and effectors of mTOR, as well as adaptor proteins. In addition, the subcellular localization of mTOR under different conditions needs to be determined. This information may help identify new therapeutic targets that would selectively stimulate the beneficial effects of the mTOR pathway while inhibiting the detrimental ones.

In particular, the molecular mechanisms through which mTORC1 inhibition exerts beneficial effects during cardiac stress need to be clearly dissected. Autophagy activation, a reduction of misfolded protein accumulation, energy-saving mechanisms or a combination of all these mechanisms may be involved in these beneficial effects. It is also possible that the mechanisms underlying the beneficial effects of mTORC1 inhibition during cardiac stress are condition-dependent. Future studies are warranted to address these issues.

Additional studies are encouraged to better characterize the cardiac phenotypes of mice with loss of function of different components of the mTOR complexes in response to specific stress conditions. These results are fundamental to understanding the specific effects of mTOR complexes in various contexts. In fact, numerous studies have used pharmacological compounds to modify the activity of mTORC1 and mTORC2 or studied the effects of modulation of their upstream regulators. However, these approaches may not be specific, generating some confusion in the interpretation of the results. In particular, characterization of the phenotypes of mTORC1 and mTORC2 knockout models in response to myocardial ischemia/reperfusion may help to clarify the role of mTOR complexes in this condition.

In addition, several pharmacological modulators of mTOR have been developed in the last years, particularly mTORC1 inhibitors. However, most of them also have multiple non-specific targets and display side effects. Therefore, it is important to find highly selective and safe modulators that may be suitable for the treatment of cardiovascular diseases. To date, mTOR inhibitors have already been tested in several clinical settings. Rapalogs, such as everolimus and temsirolimus, have shown efficacy against certain types of cancer, such as renal cell carcinoma and mantle cell lymphoma[159, 160]. A new class of dual PI3K/mTOR inhibitors, called ATP competitive inhibitors, have also been tested in phase I and phase II clinical trials in cancer patients, but their toxicity has emerged as a major problem related to their use[161]. On the other hand, more selective mTOR inhibitors, such as Torin1, did not show significant toxicity in phase I clinical trials[161].

mTOR inhibitors have also been largely tested in patients with coronary artery disease, as pharmacological components of drug-eluting stents, which are used for the treatment of coronary artery narrowing due to atherosclerotic plaques. The release of mTOR inhibitors by these devices inhibits vascular smooth muscle cell proliferation and reduces the rate of stent restenosis after implantation. Of note, a clinical trial is currently recruiting participants with the objective of evaluating the effect of oral administration of everolimus on the infarct size after an acute myocardial infarction (NCT01529554). In addition, several trials tested the

effects of mTOR inhibitors after cardiac transplantation[162, 163]. Additional trials investigating the effects of mTOR inhibitors for the treatment of heart failure, cardiac remodeling and genetic or metabolic cardiomyopathy may also be organized in the future. However, dual mTOR inhibitors may not be completely beneficial for the treatment of cardiac diseases. In fact, preclinical studies demonstrated that mTORC2 exerts beneficial effects in the heart during stress and that abrogation of these functions by dual mTOR inhibitors may result in a lack of efficacy or even harmful effects. Very few selective modulators of mTORC2 are available. Pharmacological mTORC2 activation may also represent a potential therapeutic approach for the treatment of cardiovascular diseases although this hypothesis needs to be fully tested in preclinical studies.

The direct role of the mTOR pathway in cardiac fibroblasts and vascular cells also requires further investigation.

Finally, the activity of mTOR complexes should be evaluated in human specimens from patients with cardiovascular diseases in order to evaluate its impact on cardiovascular outcomes.

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Abbreviation List

4EB-P1	eukaryotic translation initiation factor 4E (eIF4E)-binding protein-1
Akt	protein kinase B
AMBRA1	activating molecule in Beclin1-regulated autophagy
AMPK	adenosine monophosphate activated protein kinase
ATG	autophagy-related gene
CASTOR 1/2	cellular arginine sensor for mTORC1 1/2
DEPTOR	DEP domain-containing mTOR-interacting protein
eIF4	eukaryotic translation initiation factor 4
FIP200	focal adhesion kinase family interacting protein of 200 kD
FKBP12	FK506-binding protein of 12 kDa–rapamycin complex
FoxO1/3	Forkhead box O1/3
GATOR 1/2	GAP activity towards Rags 1/2
GSK-3β	glycogen synthase kinase-3β
LC3	Light chain 3
mLST8	mammalian lethal with sec-13 protein 8
mTOR	mammalian or mechanistic target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2

PGC-1α	peroxisome proliferator–activated receptor γ coactivator-1α
PI3K	phosphoinositide 3 kinase
PKC-α	protein kinase C-α
PRAS40	proline-rich Akt substrate 40
Protor 1/2	protein observed with rictor 1/2
Raptor	regulatory-associated protein of mammalian target of rapamycin (mTOR)
Rheb	Ras homolog enriched in brain
Rictor	rapamycin insensitive companion of mTOR
S6K1	S6 kinase-1
SGK1	serum- and glucocorticoid-induced protein kinase-1
SREBP 1/2	sterol regulatory element-binding protein 1/2
Tel1/2	Tel two interacting protein 1
TFEB	transcription factor EB
TSC1/2	tuberous sclerosis protein 1/2
ULK1/2	unc-51-like kinase 1/2

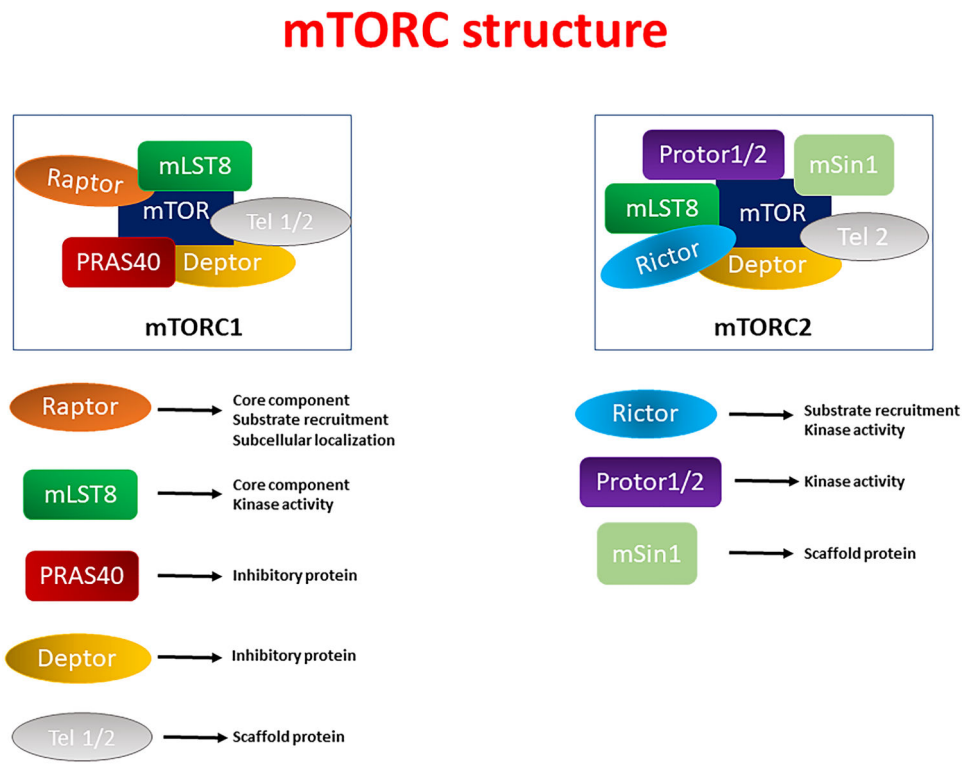

Figure 1. Structure of mTOR complex 1 (mTORC1) and mTORC2

Schematic representation of the different subunits of mTORC1 and mTORC2, together with a description of their functions.

Legend: DEPTOR, DEP domain-containing mTOR-interacting protein; mLST8, mammalian lethal with sec-13 protein 8; PRAS40, proline-rich Akt substrate 40; Protor 1/2, protein observed with rictor 1/2; Raptor, regulatory-associated protein of mammalian target of rapamycin (mTOR); rictor, rapamycin insensitive companion of mTOR; Tel1/2, Tel two interacting protein 1.

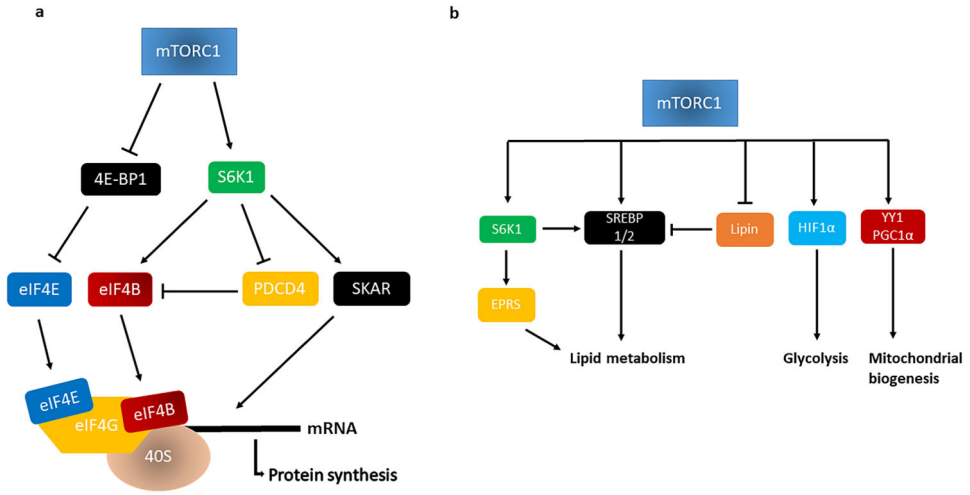

Figure 2. mTORC1 regulation of protein synthesis and cell metabolism

Schema representing the molecular mechanisms through which mTORC1 controls protein synthesis (a) and modulates cell metabolism (b).

Legend: 40S, eukaryotic small ribosomal subunit; 4EB-P1, eukaryotic translation initiation factor 4E (eIF4E)-binding protein-1; eIF4B, eukaryotic translation initiation factor 4B; eIF4G, eukaryotic translation initiation factor 4G; EPRS, glutamylprolyl-tRNAsynthetase; HIF-1, hypoxia-inducible factor-1α; PDCD4, programmed cell death 4; S6K1, S6 kinase-1; SKAR, S6K1 Aly/REF-like substrate; SREBP1/2, sterol regulatory element-binding protein 1/2; YY1/PGC-1α, transcription factor yin-yang 1/peroxisome proliferator–activated receptor γ coactivator-1α transcriptional complex.

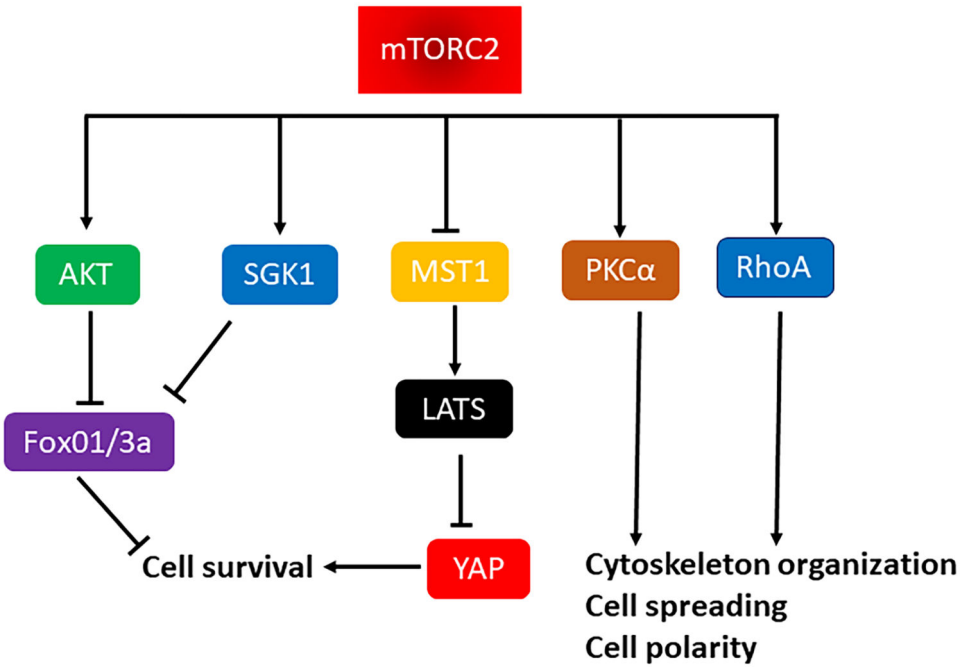

Figure 3. Main cellular functions and substrates of mTORC2

Schema representing the main cellular functions and substrates of mTORC2.

Legend: Akt, protein kinase B; FoxO1/3, Forkhead box O1/3; PKC-α, protein kinase C-α; LATS, large tumor suppressor kinase 1; MST1, mammalian sterile 20- like kinase 1; RhoA, Ras homolog gene family, member A; SGK1, serum- and glucocorticoid-induced protein kinase-1; YAP, yes associated protein.

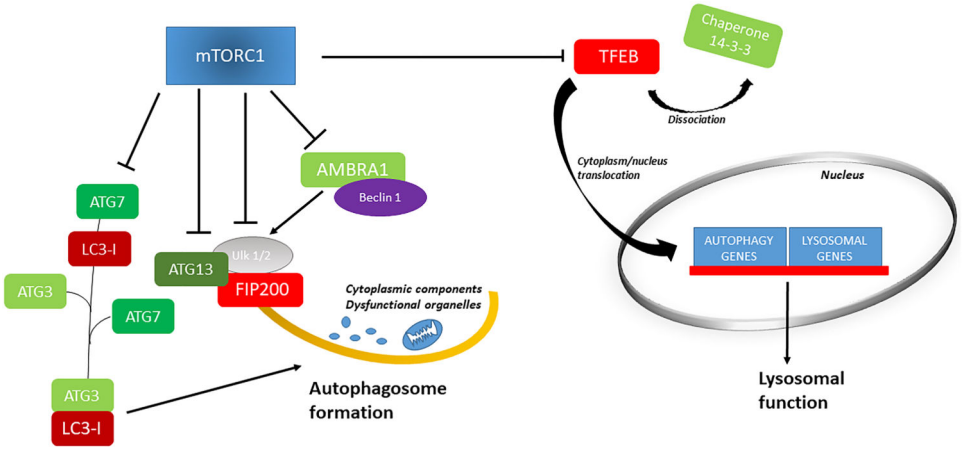

Figure 4. mTORC1 modulation of autophagy and lysosomal function

Schema representing the molecular mechanisms through which mTORC1 inhibits autophagy and lysosomal function. Legend: AMBRA1, (activating molecule in Beclin1-regulated autophagy; ATG, autophagy-related gene; FIP200, focal adhesion kinase family interacting protein of 200 kD; LC3, Light chain 3; ULK1/2, unc-51-like kinase 1/2; TFEB, transcription factor EB.

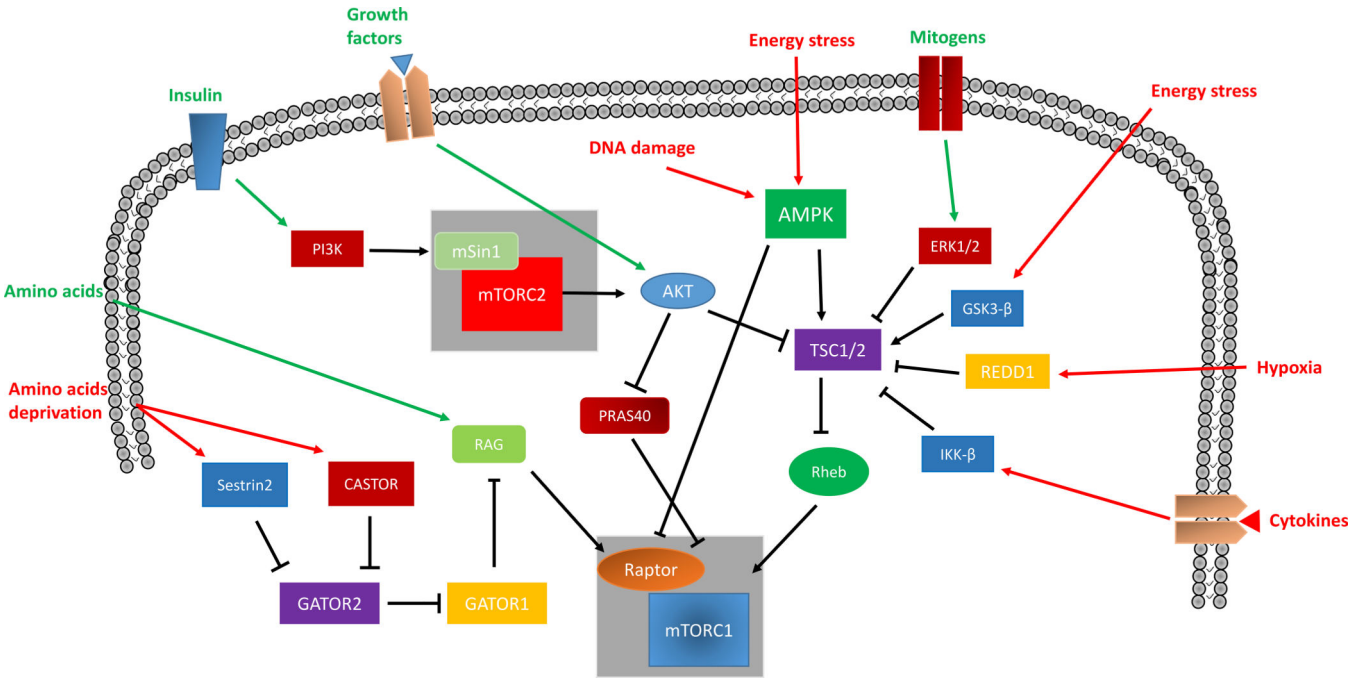

Figure 5. Schematic overview of the upstream signaling modulators of mTORC1 and mTORC2

Signaling network regulating mTORC1 and mTORC2 activity. Arrows indicate the effects of different conditions or cellular events on mTOR activity. Red arrows represent maladaptive input signals, whereas green arrows represent adaptive input signals.

Legend: AMPK, adenosine monophosphate activated protein kinase; Akt, protein kinase B; CASTOR 1/2, cellular arginine sensor for mTORC1 1/2; ERK1/2, extracellular signal regulated kinase 1/2; GATOR 1/2, GAP activity towards Rags 1/2; GSK-3β, glycogen synthase kinase-3β; IKKβ, inhibitor of NF-κB kinase-β; PI3K, phosphoinositide 3 kinase; PRAS40, proline-rich Akt substrate 40; Rag, Ras-related GTPase; Raptor, regulatory-associated protein of mTOR; REDD1, regulated in development and DNA damage response 1; Rheb, Ras homolog enriched in brain; TSC1/2, tuberous sclerosis protein 1/2.

Table 1

Cardiac effects of genetic and pharmacological mTOR modulation during development, physiology and stress.

Cardiac condition	mTOR modulation/ animal model	Cellular modifications and cardiac outcomes	Reference
Development/physiological conditions	Constitutive cardiac mTOR gene deletion	↑ Early mortality, heart failure, cardiac dilatation, cardiac dysfunction	14
	Constitutive cardiac rictor gene deletion	↓ Cardiac function over time	19
	Inducible cardiac mTOR gene deletion during adulthood	↑ Apoptosis, mitochondrial dysfunction, autophagy, sarcomere disarray ↓ Protein synthesis ↑ Early mortality, heart failure, cardiac dilatation, cardiac dysfunction	1516
	Constitutive cardiac mTOR gene deletion at early post-natal stage	↑ Apoptosis ↓ Protein synthesis ↑ Early mortality, hypoxia, cardiac dilatation	39
	Constitutive cardiac Rheb gene deletion	↓ Protein synthesis ↑ Early mortality, heart failure, cardiac growth, cardiac dysfunction	17
Aging	mTOR inhibition using rapamycin	↑ Energy metabolism ↓ Heart inflammation, cardiac fibrosis	22
	Systemic GSK-3α gene deletion	↓ Autophagy ↑ Sarcomere disarray ↑ Early mortality, cardiac hypertrophy, cardiac dysfunction during aging	107
Cardiac hypertrophy	Inducible cardiac mTOR gene deletion during adulthood and pressure overload	↓ Protein synthesis ↓ Compensatory hypertrophy ↑ Cardiac dysfunction, heart failure	1516
	mTOR inhibition using rapamycin and pressure overload	↑ Cardiac function ↓ Cardiac hypertrophy	110
	mTOR inhibition using rapamycin and TAC	↓ Cardiac hypertrophy, cardiac dysfunction	113
	mTOR inhibition using Akt inhibitor and volume overload	↓ Eccentric hypertrophy	114
	Constitutive heterozygous cardiac Rheb gene deletion and pressure overload	↓ Cardiac hypertrophy, cardiac fibrosis	23
	Cardiac PRAS40 overexpression and pressure overload	↓ Cardiac hypertrophy, cardiac fibrosis ↑ Cardiac function	115
	Systemic TSC1 overexpression and isoproterenol administration	↓ Cardiac hypertrophy ↑ Cardiac function	116
	Epigenetic mTOR inhibition and pressure overload	↓ Cardiac hypertrophy ↑ Cardiac function	119120
	Constitutive cardiac rictor gene deletion and pressure overload	↑ Apoptosis ↑ Cardiac dilatation ↓ Compensatory hypertrophy	19
	Inducible cardiac rictor gene deletion during adulthood and pressure overload	↑ Cardiac dysfunction, cardiac dilatation	18
Ischemic injury	Cardiac Rheb overexpression during prolonged ischemia	↓ Autophagy ↑ Apoptosis ↑ Infarct size	59
	Cardiac dominant negative GSK-3β overexpression and systemic	↓ Autophagy ↑ Ischemic injury	84

Cardiac condition	mTOR modulation/ animal model	Cellular modifications and cardiac outcomes	Reference
	heterozygous GSK-3β deletion during prolonged ischemia		
	Cardiac PRAS40 overexpression during chronic myocardial infarction	↓ Apoptosis ↓ Ischemic injury, cardiac remodeling ↑ Cardiac function	20
	mTOR inhibition using everolimus and chronic myocardial infarction	↑ Autophagy ↓ Cardiac remodeling, infarct size, cardiac dilatation ↑ Cardiac function	25
	mTOR inhibition using rapamycin and S6K inhibitor and myocardial infarction	↓ Apoptosis ↓ Ischemic injury	128
	Cardiac rictor gene deletion and myocardial infarction	↑ Cardiac remodeling, cardiac dysfunction	20
	Cardiac dominant negative GSK-3β overexpression and systemic heterozygous GSK-3β deletion and I/R	↓ Reperfusion injury	84
Genetic disorders	mTOR inhibition using rapamycin in a model of cardiomyopathy caused by mutation in Laminin A/C gene	↑ Autophagy ↓ Cardiac remodeling ↑ Cardiac function	54138
	mTOR inhibition using rapamycin in a mouse model of LEOPARD syndrome	↓ Cardiac hypertrophy, myocardial disarray	136
Metabolic disorders	mTOR inhibition using rapamycin in high-fat diet-induced obesity	↑ Autophagy ↑ Cardiac function	141
	Systemic Akt2 knockout in high-fatdiet-induced obesity	↑ Autophagy ↑ Cardiac function	142
	mTOR inhibition using rapamycin in type II diabetic mice	↓ Plasma glucose, triglyceride levels, oxidative stress ↑ Cardiac function	145
	Cardiac PRAS40 overexpression in diabetic mice	↑ Metabolic function, cardiac function ↓ Cardiac hypertrophy	146

Legend: Akt, protein kinase B; GSK, glycogen synthase kinase; I/R, ischemia/reperfusion; mTOR, mechanistic target of rapamycin; PRAS40, proline-rich Akt substrate 40; Rheb, Ras homolog enriched in brain; rictor, rapamycin insensitive companion of mTOR; S6K, S6 kinase; TAC, transverse aortic constriction; TSC1, tuberous sclerosis protein 1.