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Perspectives of targeting mTORC1–S6K1 in cardiovascular aging

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The global population aging is accelerating and age-associated diseases including cardiovascular diseases become more challenging. The underlying mechanisms of aging and age-associated cardiovascular dysfunction remain elusive. There are substantial evidences demonstrating a pivotal role of the mammalian target of rapamycin complex 1 (mTORC1) and its down-stream effector S6K1 signaling in mammalian lifespan regulation and age-related diseases such as type II diabetes mellitus and cancer. The role of mTORC1–S6K1 in age-related cardiovascular diseases is, however, largely unknown and the available experimental results are controversial. This review article primarily summarizes the most recent advances toward understanding the role of mTORC1–S6K1 in cardiovascular aging and discusses the future perspectives of targeting mTORC1–S6K1 signaling as a healthy lifespan extension modality in anti-aging and anti-cardiovascular aging.

Keywords: aging, endothelial senescence, eNOS, mTOR, S6K1, oxidative stress, rapamycin, resveratrol

INTRODUCTION

Global population aging is accelerating, which has been predicted to be a great challenge for our society in the twenty-first century (Christensen et al., 2009). This is due to the steady achievements of medicine and public healthcare over the past century, having resulted in an increased life expectancy, which is mainly attributed to improvement of infant and childhood survival and reduction of mortality in the elderly population (Christensen et al., 2008, 2009; Rau et al., 2008). It has been predicted that the global proportion above age 60 will increase from 10% of the total population in 2000 to 21.8% in 2050 and 32.2% in 2100 (Lutz et al., 2008). With the increased aging population, we are confronted with an increase in age-associated diseases including cardiovascular disease, cancer, diabetes, and neurodegenerative disease (Christensen et al., 2009). Although aging has been proven to be an prominent independent risk factor for cardiovascular disease (Najjar et al., 2005), the mechanisms of aging and age-associated cardiovascular dysfunction are still elusive. There are substantial evidences suggesting that oxidative stress plays a crucial role in cardiovascular aging (Ungvari et al., 2010). However, several prospective clinical trials with various combinations of antioxidants fail to show significant effects on the incidence of major adverse cardiovascular events (Vivekananthan et al., 2003; Sesso et al., 2008). The results suggest that more thorough research on the mechanisms of aging and age-associated cardiovascular diseases is required.

Research in the past years provides compelling evidences showing a potential role of the target of rapamycin (TOR) signaling pathway in lifespan regulation, which is remarkably conserved across various species. In model systems such as yeast, nematodes, fruit flies, and also recently in mice, inhibition of TOR signaling increases lifespan (Evans et al., 2011). A role of deregulated mammalian TOR (mTOR) signaling in mammalian aging and age-related diseases such as type II diabetes mellitus and cancer are demonstrated (Evans et al., 2011). The role of mTOR, especially its down-stream effector S6K1 in age-related cardiovascular diseases or cardiovascular aging is, however, largely unknown. The primary emphasis of this review article is to discuss emerging evidence and future perspectives for a role of mTOR and S6K1 signaling in mammalian aging and age-associated cardiovascular diseases.

BRIEF BIOCHEMISTRY OF mTOR SIGNALING

mTOR is a serine/threonine protein kinase which serves as intracellular sensor for energy, nutrients, and stress, regulating cellular and organism growth and metabolism. Therefore, dysfunctional mTOR signaling has been considered a central integral mechanism linking aging, metabolic disorders, and cancer (Zoncu et al., 2011). The detailed molecular signaling network and regulation of mTOR signaling have been reviewed comprehensively in several articles (Dann et al., 2007; Sengupta et al., 2010; Evans et al., 2011; Zoncu et al., 2011). A few important biochemical features of mTOR signaling network are summarized in **Figure 1**. mTOR, with other molecular components, forms two structurally and functionally distinct complexes namely mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). In these complexes, mTOR functions as the catalytic subunit and its enzymatic activity is regulated and distinguished by its unique accessory proteins Raptor and Rictor, respectively. Raptor and Rictor function as scaffold proteins for assembling mTORC1 and mTORC2 and also for binding substrates and regulators in the respective complex.

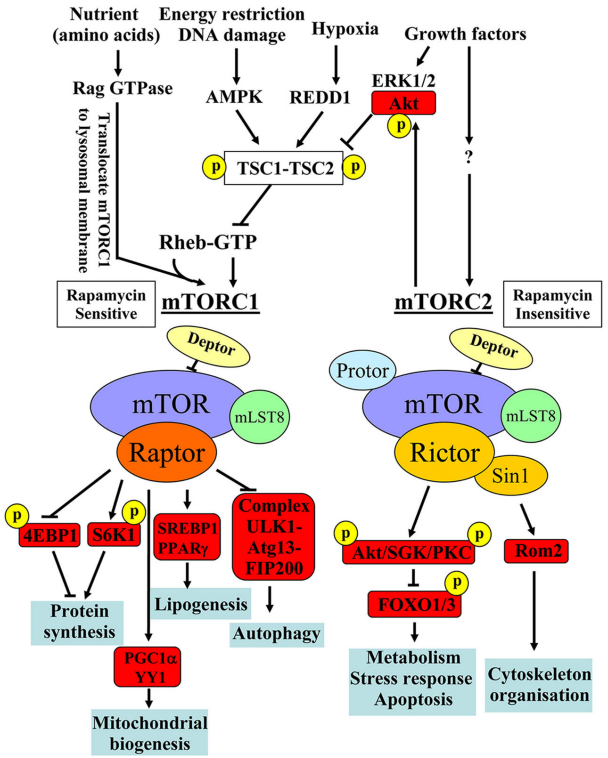

FIGURE 1 | Major distinct characteristics of the two known mTOR signaling complexes, mTORC1 and mTORC2, in composition, rapamycin sensitivity, upstream signals, substrates, and biological functions. Adapted from Foster and Finger (2010) and from Zoncu et al. (2011). 4E-BP1, eIF4E-binding protein 1; AMPK, AMP kinase; Atg, autophagy-related; FIP200, 200 kDa FAK family kinase-interacting protein; FOXO, forkhead box protein O; PPARγ, peroxisome proliferator-activated receptor-γ; REDD1, regulated in development and DNA damage response 1; Rom2, Rho1 GDP–GTP exchange protein-2; SGK, serum- and glucocorticoid-regulated kinase; SREBP, sterol regulatory element-binding protein; TSC, the tuberous sclerosis.

In addition to their different protein compositions, there are a few major important biochemical characteristics which distinguish the two complexes: (1) sensitivity to the immunosuppressant rapamycin: rapamycin inhibits mTORC1 but not mTORC2 activity, although prolonged treatment with rapamycin has been shown to be capable of inhibiting mTORC2 in certain cell types (Sarbassov et al., 2006); (2) the upstream signals they integrate: while mTORC2 seems to be regulated only by growth factors, mTORC1 is regulated by many stimuli including growth factors, energy status, stressors such as DNA damage, hypoxia, and nutrients. The small GTPase protein Rheb has been identified to be the key end point for mTORC1 activation by stimulating kinase activity of mTORC1. The TSC1–TSC2, a GTPase activating protein (GAP), negatively modulates Rheb by converting the active form Rheb-GTP into its inactive form Rheb-GDP through its GAP activity and thus reduces mTORC1 activity. Except nutrient amino acids, all of the above mentioned stimuli inputs regulate mTORC1 activity through modulation of TSC1–TSC2 activity. Growth factors inactivate TSC1–TSC2 via Akt/ERK1/2, leading to formation of Rheb-GTP and ultimately mTORC1 activation, whereas energy deficit, DNA damage, or hypoxia activate TSC1–TSC2 through activation of AMPK or REDD1 (regulated in development and DNA damage response 1), respectively, resulting in inactivation of Rheb and thus inhibition of mTORC1. Amino acids activate mTORC1 independently of TSC1–TSC2, but through action of Rag GTPases. In the presence of amino acids, Rag GTPases interact with mTORC1 and translocate the complex from cytoplasm to lysosomal membranes where it is activated by Rheb. For more detailed discussion about the regulatory mechanisms of mTORC1 activation by various inputs, please refer to the review article by Zoncu et al. (2011). In contrast to mTORC1, the regulatory mechanisms and functions of mTORC2 signaling are less well characterized; (3) the substrate they regulate and the biological processes they control: mTORC1 enhances protein synthesis through S6K1 and eIF4E-binding protein 1 (4E-BP1). Upon phosphorylation by mTORC1, 4E-BP1 dissociates from eIF4E, relieving its suppressing effect on mRNA translation, while S6K1, when phosphorylated by mTORC1, promotes mRNA translation. In addition, mTORC1 also induces lipogenesis in the liver through activating transcription factors SREBP1 and PPARγ, inhibits autophagy through phosphorylation of the ULK1–Atg13–FIP200 complex, and promotes mitochondrial biogenesis by activating PGC1α/YY1 (Zoncu et al., 2011). mTORC2 exerts its effects on metabolism, stress responses, apoptosis, and cytoskeleton organization through phosphorylation of many AGC kinases including Akt, serum-and glucocorticoid-induced protein kinase (SGK), protein kinase C-α (PKCα), and Rho1 GDP–GTP exchange protein-2 (Rom2; Sarbassov et al., 2005; Frost and Lang, 2011). Since mTORC2 activates Akt that in turn enhances mTORC1 activity through inactivation of TSC1–TSC2, mTORC2 is the upstream of mTORC1 upon stimulation by growth factors (see **Figure 1**).

EVIDENCE FOR A ROLE OF mTORC1–S6K1 IN REGULATION OF LONGEVITY

The role of mTORC1–S6K1 as a master determinant in longevity control stems from experiments in almost all model organisms including yeast, worms, flies, and mice. Genetic inactivation of TOR or core components of TOR signaling including S6K1 or pharmacological inhibition of the signaling pathway with rapamycin showed lifespan extension in yeast and invertebrates and also recently in mice (Chen et al., 2009; Harrison et al., 2009; Selman et al., 2009). These independent studies clearly verified the important role of mTORC1–S6K1 in mammalian aging. No data is available, yet, whether mTORC2 is also involved in regulation of longevity. The lifespan extension experiments with rapamycin may not fully exclude a possible role of mTORC2 in regulation of longevity, since long-term rapamycin treatment is able to inhibit mTORC2 in certain cell types (Sarbassov et al., 2006).

Furthermore, the effect of mTOR signaling inhibition on longevity can be mimicked by caloric restriction, i.e., reduction in calorie intake without malnutrition, across different species (Pan et al., 2007; Evans et al., 2011; Shinmura et al., 2011). A recent report demonstrating that caloric restriction also slows aging in rhesus monkeys (Colman et al., 2009) may suggest that caloric restriction could have anti-aging effects in humans (Kaeberlein and Kennedy, 2009). Indeed, it has been reported that the

Okinawan populations who consume 20% less calories compared to the average caloric consumption of the Japanese population live longer and display lower incidences of cancer and cardiovascular diseases in the elderly. The healthier aging status disappears in those who switched to Western diet (Kagawa, 1978; Willcox et al., 2007). A small caloric restriction study in adult humans over 6 years demonstrates less cardiovascular risk factors including lower body mass index, body fat deposition, blood pressure, fasting plasma levels of glucose and insulin, lower inflammation markers, i.e., tumor necrosis factor-α and C-reactive protein, and beneficial lipid profiles (Fontana et al., 2004) and shows better left ventricular diastolic function than healthy age-and sex-matched controls (Meyer et al., 2006). Also, caloric restriction improves cardiac function in patients with type 2 diabetes (Hammer et al., 2008). Whether the beneficial effects of caloric restriction in humans are due to healthier diet and could be translated to lifespan extension in healthy elderly subjects and improve survival in patients requires further investigation. The underlying mechanisms of caloric restriction on longevity may involve reduction of mTORC–S6K1 signaling. In yeast and flies, when TOR is inactivated, caloric restriction does not show further effects on lifespan extension (Kapahi and Zid, 2004; Powers et al., 2006). Whether this holds true also in humans needs to be confirmed.

Another piece of supporting evidence for mTORC1–S6K1 in regulation of longevity derives from experiments showing that down-regulation of insulin and insulin-like growth factor (IGF-1) signaling in mouse models such as haploinsufficiency of IGF-1 receptor and global deletion of insulin receptor substrate-1 (IRS-1) are associated with reduced mTORC1–S6K1 signaling and increased longevity in mice (Holzenberger et al., 2003; Selman et al., 2008) as in yeast, nematodes, and fruit flies (Stanfel et al., 2009). Moreover, caloric restriction prolongs lifespan in animal models, which is associated with reduced mTORC1–S6K1 mediated by IGF-1 signaling pathway (Estep et al., 2009; Fontana et al., 2010). These results further strengthen the role of mTORC1–S6K1 in lifespan regulation in mammals. The exact mechanisms of reduced signaling of mTORC1–S6K1 and IGF-1 under caloric restriction, however, remain elusive. It seems that AMPK and Sirt1, whose activities are augmented by caloric restriction, are involved in negative regulation of mTORC1–S6K1 signaling and in turn regulate aging process (Cohen et al., 2004; Canto et al., 2009; Shackelford and Shaw, 2009; **Figure 1**), although direct evidence for it in mammals is still lacking (Herranz and Serrano, 2010). It is also noteworthy that loss of *S6K1* in mice reciprocally results in activation of AMPK which has been proposed to mediate the lifespan extension (Selman et al., 2009). Intriguingly, persistent activation of AMPK seems to play an important role in vascular endothelial cell senescence (Zu et al., 2010). A working model of reciprocal regulatory effects of Sirt1 and AMPK on cellular senescence and aging is discussed by Wang et al. (2011). The molecular mechanisms of the reciprocal interplay between mTORC1–S6K1 and AMPK and Sirt1 are not clear. A mutual inhibition of mTORC1–S6K1 and AMPK has been reported (Lee et al., 2010).

Although the role of inhibition of mTORC1–S6K1 in lifespan extension is consistently demonstrated in animal models, very little information is available about the role of mTORC1–S6K1 in cardiovascular aging or age-associated cardiovascular diseases.

mTORC1–S6K1 SIGNALING IN CARDIOVASCULAR AGING

CARDIAC AGING

Age-associated cardiovascular diseases are accompanied by structural and functional changes in heart and blood vessels. These aging-associated changes in cardiovascular system are referred to as cardiovascular aging phenotypes. Cardiac aging is manifested by maladaptation to stress, cardiac dysfunction, and heart failure. Cardiac aging process involves cardiomyocytes and other cell types in the heart, such as interstitial fibroblasts and vascular cells. The morphological and functional changes of these cells with aging lead to cardiac hypertrophy and dilation, cardiac fibrosis due to chronic deposition, and remodeling of extracellular matrix produced mainly by fibroblasts. Moreover, a decreased regenerative capacity of cardiac stem cells, possibly due to impaired cell division and accelerated cell senescence and an increased cardiac myocyte death due to necrosis and apoptosis with aging, may also contribute to cardiac dysfunction in elderly. These pathological and clinical aspects of cardiac remodeling in aging have been recently reviewed in great details (Shih et al., 2011).

There are compelling evidences demonstrating that mTORC1–S6K1 signaling is involved in cardiac hypertrophy under various pathological conditions including diabetes and hypertension (Sadoshima and Izumo, 1995; Boluyt et al., 1997; Tu et al., 2002; McMullen et al., 2004; Soesanto et al., 2009; Kurdi and Booz, 2011; Sung et al., 2011). The underlying mechanisms have been proposed to be attributed to both an increase in protein synthesis upon stress stimulation and a decrease in protein degradation due to inhibition of autophagy by mTORC1 signaling (Hands et al., 2009). However, evidence for this hypothesis needs to be demonstrated. Inhibition of mTORC1–S6K1 pathway by rapamycin or activation of AMPK by 5-aminoimidazole-4-carboxamide riboside (AICAR) to negatively regulate mTORC1 signaling has been shown to attenuate pressure overload-induced cardiac hypertrophy *in vivo* (Li et al., 2007). Conversely, deficiency in AMPK enhances mTORC1–S6K1 signaling and exacerbates myocardial hypertrophy in response to pressure overload (Zhang et al., 2008).

Besides pressure overload, many hormonal factors, e.g., angiotensin-II, insulin, endothelin-1, catecholamine, etc., that are elevated in plasma and/or tissues under various cardiovascular pathologies and in aging, have been shown to stimulate mTORC1–S6K1 signaling in the cardiovascular system (Moschella et al., 2007; Muniyappa et al., 2007; Kim et al., 2012). Activation of mTORC1–S6K1 by the hormones, e.g., angiotensin-II and also by overnutrition (Glazer et al., 2009) participates in cardiac hypertrophy and vascular remodeling, and causes insulin resistance, an important cardiovascular risk (Reaven, 2011), through phosphorylation of IRS-1 at serine residues (Kim et al., 2012). Importantly, under the condition of cardiovascular insulin resistance, there is a selective inhibition of the metabolic pathway, i.e., Akt–eNOS in response to insulin, while the growth pathway, i.e., $p44/p42^{ERKs}$ remains active (Muniyappa et al., 2007). This selective insulin resistance may play an important role in decreased vascular relaxation due to impaired eNOS activation and enhanced cardiovascular remodeling in metabolic disorders such as type II diabetes (Muniyappa et al., 2007). mTORC1–S6K1 pathway is therefore

considered as an important molecular link between metabolic stress and cardiovascular abnormalities.

Despite the evidences for a role of mTORC1–S6K1 signaling in agonist or pressure-induced cardiac hypertrophy, only little information is available about the signaling of mTORC1–S6K1 in physiological process of aging heart. An early study using microarray analyses reported that gene expression pattern associated with mTOR is suppressed in aging heart of Fischer 344 rats (Linford et al., 2007), while a recent study shows no difference in mTORC1–S6K1 activity (measured by phosphorylated mTOR and S6K1 levels) in the heart between 8 and 30 month old rats of the same strain (Shinmura et al., 2011). There are two points that should be considered for interpretation of the inconsistent results. First, microarray has its limitation in elucidating signaling pathways, because signaling molecules are mainly enzymes whose activities could not be investigated with this experimental approach; second, aging-associated kinetics of mTORC1–S6K1 signaling may exist, meaning an enhanced signaling with time followed by a decreased signaling in old age. Indeed, a decreased mTOR signaling has been shown to play a role in sarcopenia in advanced age (Sakuma and Yamaguchi, 2010). Whether this also occurs in the heart requires investigation. Nevertheless, caloric restriction in the old rats showed improved diastolic function associated with reduced cell senescence and mTORC1–S6K1 signaling in the heart compared to the old animals fed *ad libitum* (Shinmura et al., 2011), suggesting that mTORC1–S6K1 is involved in caloric restriction-induced improvement of heart function in aging. It is also conceivable that the anti-cellular senescence effect and reduced mTORC1–S6K1 signaling contribute to the beneficial effects of caloric restriction on human heart function (Meyer et al., 2006; Hammer et al., 2008).

VASCULAR AGING

Endothelial dysfunction and inflammatory activation

In the vasculature, increased arterial wall thickening and generalized vascular stiffness occur with aging. This vascular aging phenotype is attributed to vascular calcification, increased collagen content and elastin breakdown, and elevated levels of advanced glycation end products. Vascular inflammation, oxidative stress, endothelial dysfunction, endothelial progenitor cell dysfunction, vascular cell apoptosis all intertwine with each other to affect vascular aging process, which accelerates coronary heart disease, heart failure, stroke, and dementia (Ungvari et al., 2010).

In the past decades, much attention has been devoted to endothelial dysfunction in aging. Evidence demonstrates that besides the enhanced production of endothelium-derived vasoconstrictor prostanoids, which is due to augmented expression and/or activity of cyclo-oxygenases in endothelial cells during aging (Vanhoutte et al., 2009), decreased endothelial nitric oxide (NO) bioavailability is a major characteristic of vascular aging, which is independent of other cardiovascular risk factors (Lakatta, 2001). Endothelial NO causes vascular relaxation, inhibits platelet aggregation, and leukocyte adhesion (Yang and Ming, 2006). Clinical studies provide evidence that endothelial dysfunction is not only highly associated with cardiovascular disease, it also predicts future cardiac events (Schachinger et al., 2000; Halcox et al., 2002; Bugiardini et al., 2004; Huang et al., 2007). eNOS dysfunction seems causally involved in cardiovascular aging, since eNOS−/− male mice have a significantly shorter lifespan than their wild type controls and exhibit accelerated cardiac dysfunction with age (Wei, 2004). Endothelial cells with dysfunctional eNOS in aging are also more vulnerable to apoptotic stimuli (Hoffmann et al., 2001; Csiszar et al., 2004). There is increasing evidence suggesting that the number and regenerative capacity of circulating endothelial progenitor cells which play a role in reendothelialization and repair after vascular injury are decreased in patients with cardiovascular diseases and risk factors including aging (Vasa et al., 2001; Hill et al., 2003; Rauscher et al., 2003; Werner et al., 2005). The underlying mechanisms of eNOS dysfunction in aging are multifactorial and have not been fully understood, yet. Decreased eNOS gene expression (Csiszar et al., 2002; Tanabe et al., 2003) or increased eNOS gene expression with impaired enzymatic activity due to oxidative stress are important mechanisms (Stockklauser-Farber et al., 2000; van der Loo et al., 2000; Ming et al., 2004; Desrois et al., 2010). The up-regulation of eNOS gene in aging and also in other vascular disease may represent a compensatory mechanism counteracting oxidative stress (Drummond et al., 2000; Stockklauser-Farber et al., 2000; Hink et al., 2001).

Oxidative stress has been proposed as the culminant mechanism impairing endothelial function via quenching of NO, i.e., inactivation of NO by increased production of O_2^-, which leads to formation of peroxynitrite, a strong oxidant that can further damage endothelial cells in aging (van der Loo et al., 2000; Brandes et al., 2005). Among other sources, eNOS itself produces significant amount of O_2^- in endothelial cells, when "eNOS uncoupling" occurs – that is, eNOS generates O_2^- instead of NO (Landmesser et al., 2003; Forstermann and Munzel, 2006; Rajapakse et al., 2011). It seems that normal function of eNOS requires homodimerization of the enzyme which is stabilized by the cofactor BH_4. The eNOS reductase domain generates electron flow from NADPH through FAD and FMN flavins, which are then transferred to the oxidase domain of other monomers in which L-arginine is metabolized to NO at the heme group in the active site (Forstermann and Munzel, 2006). In the absence of BH_4 due to oxidative inactivation, eNOS dimer/monomer ratio is decreased and the catalytic activity becomes uncoupled – that is – uncoupling of NADPH oxidation and NO synthesis, with oxygen instead of L-arginine as terminal electron acceptor, resulting in O_2^- generation (Forstermann and Munzel, 2006). In addition, limited specific pool of intracellular L-arginine bioavailability due to enhanced arginase activity (Csiszar et al., 2002; Berkowitz et al., 2003; Tanabe et al., 2003) or production of endogenous eNOS inhibitor asymmetric dimethylarginine (ADMA; Sydow and Munzel, 2003; Antoniades et al., 2009) have been also reported to contribute to endothelial dysfunction in aging. The finding that eNOS enzymatic dysfunction is the major mechanism for endothelial dysfunction in aging indicates that one should therapeutically focus on improving eNOS enzymatic function instead of increasing eNOS gene expression in blood vessels. Indeed, over-expression of eNOS under disease conditions for example in atherosclerosis prone $ApoE^{-/-}$ mice has been shown to accelerate atherosclerosis (Ozaki et al., 2002). Endothelial specific eNOS transgenic mice have enhanced O_2^- generation which can be inhibited by the eNOS inhibitor L-NAME or by endothelial-targeted GTP cyclohydrolase 1 over-expression to

increase BH_4 production (Bendall et al., 2005). The results further implicate that too much eNOS under pathological conditions where the enzyme is uncoupled, is detrimental. A recent study reveals that oxidative stress causes cysteine S-glutathionylation of the reductase domain of eNOS, resulting eNOS uncoupling (Chen et al., 2010). The eNOS S-glutathionylation level is increased in spontaneously hypertensive rats as compared to normotensive animals accompanied with impaired endothelium-dependent relaxations that can be reversed after the S-glutathionylation of eNOS is removed by thiol-specific reducing agents (Chen et al., 2010). Whether S-glutathionylation of eNOS also plays a role in endothelial dysfunction in aging requires further investigation.

Another important feature of endothelial aging is enhanced expression of inflammatory adhesion molecules such as ICAM-1 and VCAM-1 (Gorgoulis et al., 2003; Zhou et al., 2006; Ungvari et al., 2010), which leads to enhanced monocyte–endothelial interaction and accelerated atherogenesis. There are considerable evidences suggesting that oxidative stress is a major mechanism for promoting eNOS dysfunction as well as vascular inflammation which causes further endothelial dysfunction and aging in a positive-feedback manner (Herrera et al., 2010). However, evidence for a causal role of oxidative stress in vascular aging is still lacking, although there is no doubt that oxidative stress contributes to aging-associated vascular dysfunctions. Some authors suggest that mTOR signaling instead of oxidative stress might be the more powerful driving force for organismal aging (Blagosklonny, 2008).

Causal role of mTORC1–S6K1 signaling in endothelial aging

Although evidence for a role of mTOR–S6K1 signaling in regulation of organism lifespan has been well demonstrated (Evans et al., 2011; Zoncu et al., 2011), it is, however, not known, whether mTORC1–S6K1 signaling pathway participates in vascular aging. In contrast to the aging heart, an increased basal activity of mTORC1–S6K1 has been demonstrated in aortas of old Fischer 344xBrown Norway F1 hybrid rats (Rice et al., 2005), which implicates a potential role of mTORC1–S6K1 signaling in vascular aging. However, a study reported that inhibition of mTORC1–S6K1 by rapamycin and everolimus induces endothelial cellular senescence in culture (Ota et al., 2009), which does not support the role of mTORC1–S6K1 in endothelial aging. One has to take into account that the former study shows correlation between increased mTORC1–S6K1 signaling and vascular aging, while the latter used pharmacological inhibitors in young endothelial cells. The drugs may exert some non-specific effects which could interfere with the interpretation. Moreover, the function of mTORC1–S6K1 in young and old cells or organisms might be different. A regulated function of mTORC1–S6K1 signaling is necessary for organism development, while a persistent non-regulated mTORC1–S6K1 signaling is detrimental.

By pharmacological and genetic approaches, we demonstrate a hyperactive S6K1 activity in two aging model systems, i.e., in cultured senescent endothelial cells and in aortas of naturally aging rats as compared to young cells and young animals (Rajapakse et al., 2011). A persistent activation of mTORC1–S6K1 signaling is also shown to be associated with hematopoietic stem cells in old mice, which is responsible for decreased regenerative capacity of the stem cells in aging (Chen et al., 2009). Inhibition of mTORC1–S6K1 pathway either with rapamycin or with S6K1 silencing improves NO production and inhibits O_2^- production in senescent cells and old rat aortas. The enhanced O_2^- production in senescent cells and old rat aortas can be eliminated by the eNOS inhibitor L-NAME, demonstrating eNOS uncoupling in aging (Rajapakse et al., 2011). Conversely, over-expression of a S6K1 active mutant in young endothelial cells causes eNOS uncoupling and endothelial senescence, which provides the first evidence for a causative role of S6K1 in eNOS uncoupling and endothelial aging. How S6K1 drives endothelial senescence or aging and causes eNOS uncoupling, and whether S6K1 causes endothelial aging through eNOS uncoupling remain to be investigated.

Endothelial aging is also associated with increased expression of the coagulation factor tissue factor (TF) as well as adhesion molecules ICAM-1 and VCAM-1 (Csiszar et al., 2008), which may promote thrombosis and vascular inflammation in elderly subjects. Numerous studies including our own report the inhibitory effect of mTORC1 on TF expression based on the observation that rapamycin or silencing mTOR enhances TF expression in endothelial cells (Camici et al., 2010; Ming et al., 2010). However, silencing S6K1 reduces TF protein level in endothelial cells stimulated with thrombin or TNFα without affecting TF mRNA expression. Conversely, over-expression of a constitutively active S6K1 mutant enhances TF protein level even in the mTOR-silenced cells (Ming et al., 2010). The results reveal an unexpected opposing effect of mTOR and S6K1 on endothelial TF expression and are best explained by the mechanisms that the markedly enhanced TF mRNA expression under the condition of mTORC1 inhibition is translated by S6K1-independent pathways such as RhoA, NF-κB, and p38mapk (Ming et al., 2010), since blockade of RhoA, NF-κB, and p38mapk either pharmacologically or genetically is able to reduce the up-regulation of TF protein level (**Figure 2**). The uncoupling effect of mTOR and S6K1 has also been reported in skeletal muscle cells (Cunningham et al., 2007a). These findings may have potential clinical implications. TF is highly expressed in cells within atherosclerotic plaques (Hatakeyama et al., 1997). It has been reported that vascular injury after coronary intervention increases circulating TF activity in patients (Tutar et al., 2003), which favors thrombus formation after coronary intervention. With rapamycin (sirolimus)-eluting stents, TF expression in the vasculature and release from vascular cells including endothelial cells, smooth muscle cells, and macrophages might be exaggerated because of the stimulating effect of TF expression by the drug. This effect of rapamycin, may increase thrombotic risk, despite its property of preventing vascular restenosis in patients with coronary artery disease (Luscher et al., 2007), although inhibition of endothelial regeneration and in turn endothelialization of stent surface by drug-eluting stents may play a more prominent role in stent thrombosis (Inoue et al., 2011). It would be interesting to test whether inhibition of S6K1 alone, rather than mTORC1 as achieved by rapamycin, proves sufficient to prevent vascular restenosis and be superior in reducing thrombotic propensity by developing specific S6K1 inhibitors. The same consideration may be applied for anti-aging therapy. Moreover, silencing S6K1 in endothelial cells is able to prevent up-regulation of E-selectin induced by TNFα (Ming et al., 2009), implicating a possible role of

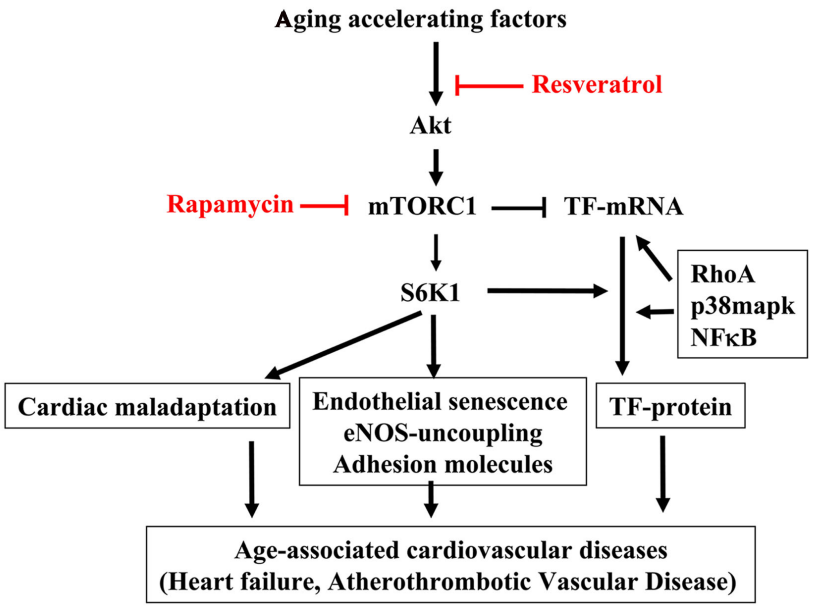

FIGURE 2 | mTORC1–S6K1 signaling in cardiovascular aging. Aging accelerating factors cause persistent activation of the Akt–mTORC1–S6K1 signaling. The hyperactive S6K1 has pleiotropic effects in cardiovascular system. In heart, it causes cardiomyocytes hypertrophy leading to cardiac maladaptation. In endothelium, it induces eNOS uncoupling, endothelial senescence, and adhesion molecule expression as well as TF protein expression. All of these effects contribute to age-associated cardiovascular diseases such as heart failure, atherosclerotic vascular disease. Thus inhibiting S6K1 with resveratrol or mTORC1 inhibitor rapamycin may have beneficial effect in treatment of age-associated cardiovascular diseases in clinic. It is important to recognize that there are some uncoupled biological function(s) of mTORC1 and S6K1 as reported for their opposing effect in regulation of TF expression. While mTOR suppresses endothelial TF mRNA expression, S6K1 is required for TF protein translation, which works in concert with other signaling pathways such as RhoA, p38mapk, and NF-κB. Given this finding, drugs more specifically inhibiting S6K1 rather than mTORC1 should be developed and the effects of targeting mTORC1 or S6K1 on aging-related cardiovascular diseases and cardiovascular aging and beyond should be investigated.

S6K1 in regulation of endothelial–leukocyte interaction. Whether S6K1 is involved in TF as well as adhesion molecule expression in aged endothelial cells and blood vessels warrants further investigation. The effects of mTORC1–S6K1 on cardiovascular functions are illustrated in **Figure 2**.

Resveratrol inhibits mTORC1–S6K1 and prevents endothelial aging

It is well demonstrated that resveratrol, a naturally occurring polyphenol, slows aging process and exerts protective effects on aging-associated pathologies including vascular diseases, type II diabetes in animal models (Baur et al., 2006; Lagouge et al., 2006; Barger et al., 2008; Pearson et al., 2008; Miller et al., 2011). Numerous studies including ours demonstrate that resveratrol inhibits ICAM-1 and VCAM-1 expression in endothelial cells in response to high glucose and TNFα (Csiszar et al., 2006; Park et al., 2009; Rajapakse et al., 2009). Interestingly, resveratrol is capable of recoupling eNOS, leading to inhibition of superoxide generation and increase in endothelial NO production in senescent endothelial cells and in aged rat aortas partly through inhibition of mTORC1–S6K1 signaling (Rajapakse et al., 2011), since NO production stimulated by acetylcholine is only partly restored by resveratrol and rapamycin in old rats. Additional defects in eNOS dysfunction as discussed must be present.

It also remains to be investigated whether resveratrol's inhibitory effect on mTORC1–S6K1–eNOS-uncoupling is mediated through activation of the NAD^+-dependent deacetylase Sirt1 (Lagouge et al., 2006; Zang et al., 2006; Baur, 2010), whose activation has been shown to exhibit protection against age-associated diseases including diabetes and atherosclerosis (Lagouge et al., 2006; Zang et al., 2006; Pearson et al., 2008), although current studies do not show life extending effect in mammals (Lagouge et al., 2006; Barger et al., 2008; Pearson et al., 2008; Miller et al., 2011). Recent studies provide evidence showing that resveratrol also exerts pleiotrophic effects independently of Sirt1 (Zhang, 2006; Pirola and Frojdo, 2008; Kitada et al., 2011). It has also been shown that resveratrol may indirectly activate Sirt1 through AMPK, which has been demonstrated to inhibit mTORC1–S6K1 pathway in different cell types (Ruderman et al., 2010). The underlying mechanisms by which resveratrol inhibits mTORC1–S6K1 in aging endothelial cells are not clear, yet. Since the hyperactive Akt, an upstream signaling of mTORC1–S6K1 pathway, is observed in senescent endothelial cells and is inhibited by resveratrol (Rajapakse et al., 2011), it seems that resveratrol negatively regulates mTORC1–S6K1 pathway through inhibition of mTORC2. The persistent activation of Akt–mTORC1–S6K1 signaling in endothelial aging observed in our study is consistent with the finding that a hyperactive Akt plays a role in endothelial cell senescence (Miyauchi et al., 2004). Further studies need to establish a role of mTORC2 in endothelial senescence and in regulation of longevity.

RAPAMYCIN AND RESVERATROL AS ANTI-AGING AND ANTI-CARDIOVASCULAR AGING DRUGS IN HUMANS?

Although caloric restriction seems the most promising approach to slow aging and onset of age-related diseases in humans with almost no recognizable adverse effects (Fontana et al., 2010), it is difficult to implement as a routine and long-term preventive or treatment modality in humans. The primary target of anti-aging strategy should focus on treatment of age-related diseases and not longevity, i.e., improvement of "healthy" lifespan. By treatment of age-related diseases, healthy lifespan extension, and improvement of life quality in elderly are expected.

In animal models, rapamycin is able to extend lifespan and prevents many age-related diseases such as cancer (Hudes et al., 2007), obesity (Um et al., 2004), cardiovascular diseases (Sadoshima and Izumo, 1995; Elloso et al., 2003; Waksman et al., 2003; McMullen et al., 2004; Pakala et al., 2005; Adelman, 2010). Moreover, a recent study reports that in cultured fibroblasts isolated from patients suffering Hutchinson–Gilford Progeria Syndrome (HGPS), a lethal genetic disease characterized by premature aging and death in adolescence or the teen years, rapamycin postpones cell senescence by activating autophagy, a process by which cells clear junk protein and trashed organelles and is inhibited by hyperactive mTORC1–S6K1 (Cao et al., 2011). These findings suggest an additional mechanism for the beneficial effects of rapamycin on aging.

Interestingly, many of the beneficial effects of rapamycin have been shared with resveratrol (Jang et al., 1997; Baur et al., 2006; Zang et al., 2006; Chan et al., 2008; Smoliga et al., 2011), although the lifespan extending effect could be demonstrated with rapamycin but not with resveratrol in mice (Pearson et al., 2008; Harrison et al., 2009; Miller et al., 2011). Both rapamycin and resveratrol inhibit hyperactive mTORC1–S6K1 signaling, improve endothelial function in senescent cells and aging rat aortas (Rajapakse et al., 2011), and also improve bone marrow-derived progenitor cell function and senescence (Chen et al., 2009; Huang et al., 2010). All the results suggest that rapamycin and resveratrol may be used as anti-aging therapeutics in humans. Rapamycin and analogs are indeed used in patients with organ transplantation and cancer and show clinical benefits (Kauffman et al., 2005; Law, 2005; Stallone et al., 2005; Zmonarski et al., 2005; Campistol et al., 2006). However, there is concern about some undesirable effects of rapamycin which may limit its systemic use as anti-aging or anti-cardiovascular aging drug. In contrast to the initial major concern about immunosuppressive effects, rapamycin has been shown to improve immune function and regenerative capacity of bone marrow stem cells in old mice (Chen et al., 2009). However, early studies in animal models treated with rapamycin reported multiple adverse effects including deregulated glucose homeostasis, hyperlipidemia (Cunningham et al., 2007b; Chang et al., 2009). In humans, rapamycin increases blood triglyceride and cholesterol resulting from lipolysis (Morrisett et al., 2002; Ribes et al., 2005), which may have negative impact on cardiovascular functions and metabolic homeostasis, although rapamycin has been shown to reduce atherosclerosis in mouse models (Basso et al., 2003; Elloso et al., 2003; Waksman et al., 2003; Pakala et al., 2005). The different effects of rapamycin in mice may be related to when the rapamycin therapy is initiated (early or late in life) or how long the therapy persists, or whether chronic persistent or pulsed therapy is instrumented. Finally, it may depend on whether young or old, healthy or diseased animals are treated with the drug. It is important to point out that rapamycin may be beneficial under the condition when mTORC1–S6K1 signaling is inappropriately consistently elevated, for example in aging, but detrimental if its activity under physiological conditions is abolished. Furthermore, taking into account that an opposing and uncoupling effect of mTORC1 and S6K1 has been demonstrated such as in regulation of endothelial TF expression (Ming et al., 2010), and that both rapamycin and resveratrol inhibit mTORC1 at the level and upstream of mTORC1, respectively (**Figure 2**), it would be worth to test whether targeting S6K1 directly would be a better approach than targeting mTORC1 as achieved by rapamycin or resveratrol. For this purpose, specific inhibitor of S6K1 should be developed. At the molecular level, since off-target effects of the drugs could not be excluded, more specific experimental approaches such as mutants (active or dominant negative), RNA interference, or targeted gene disruption of mTORC1–S6K1 signaling should be applied to evaluate their roles in cardiovascular systems.

So far, almost no undesirable effects of resveratrol have been reported, which might be due to the fact that this drug is newly investigated as compared to rapamycin. The first recently published human study with resveratrol confirmed metabolic beneficial effects which were previously observed in animals (Timmers et al., 2011). The study showed that treatment of healthy, obese men with 150 mg/day resveratrol for 30 days mimicked the effects of calorie restriction, significantly improved metabolic profiles on circulating glucose, triglycerides, liver lipid content, and decreased inflammation markers and systolic blood pressure (Timmers et al., 2011). The effects of resveratrol on cardiovascular diseases in patients remain to be demonstrated. Further, dosage dependent side effects of resveratrol should be investigated, especially for the newly developed derivatives which show much stronger effects than resveratrol on Sirt1 activity (Milne et al., 2007). The potency of a drug is usually associated with toxicity due to non-specific off-target effects.

CONCLUSION AND PERSPECTIVES

Emerging evidence demonstrates that targeting mTORC1–S6K1 signaling could be a promising therapeutic modality to slow aging process and treat cardiovascular disease in aging. Future work should further elucidate the mechanisms of persistent hyperactive mTORC–S6K1 and the mechanisms of cardiovascular aging driven by mTORC1–S6K1. Since mTORC1–S6K1 signaling is also essential for normal development and skeletal muscle mass growth, undesirable side effects of targeting mTOCR1–S6K1 may be avoided by short treatment instead long-term treatment. In addition, drugs more specifically inhibiting S6K1 rather than mTORC1 should be developed and the effects of targeting mTORC1 or S6K1 on aging-related cardiovascular diseases and cardiovascular aging and beyond should be investigated.

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