HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2019 September 26.

Published in final edited form as:

Biochim Biophys Acta. 2016 May ; 1862(5): 992–1007. doi:10.1016/j.bbadis.2015.11.010.

Vascular mTOR-dependent mechanisms linking the control of aging to Alzheimer's disease☆

Veronica Galvan[a,*], **Matthew J. Hart**[b]

[a]Department of Physiology and the Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio

[b]Department of Biochemistry, University of Texas Health Science Center at San Antonio

Abstract

Aging is the strongest known risk factor for Alzheimer's disease (AD). With the discovery of the mechanistic target of rapamycin (mTOR) as a critical pathway controlling the rate of aging in mice, molecules at the interface between the regulation of aging and the mechanisms of specific age-associated diseases can be identified. We will review emerging evidence that mTOR-dependent brain vascular dysfunction, a universal feature of aging, may be one of the mechanisms linking the regulation of the rate of aging to the pathogenesis of Alzheimer's disease. This article is part of a Special Issue entitled: Vascular Contributions to Cognitive Impairment and Dementia edited by M. Paul Murphy, Roderick A. Corriveau and Donna M. Wilcock.

Keywords

Alzheimer's; Neurovascular aging; Target of rapamycin; MTOR; Aging; Geroscience

1. Introduction

Alzheimer's disease (AD) is characterized by a progressive loss of memory followed by the disintegration of other cognitive functions. In its early stages, deterioration is specific to the ability to learn new information; motor and sensory functions are for the most part spared. Over time, neurodegeneration expands to other domains and leads to overt dementia and death approximately a decade after disease onset [1]. The progressive disability associated with AD entails significant suffering for patients and caregivers, and imposes a major financial toll on individuals and families affected and society at large. The incidence of AD has been steadily rising mostly as a consequence of increased life expectancy in most populations. The number of AD cases, which is currently estimated at 36 million worldwide, is expected to triple by 2050. The very high costs associated with long-term care for AD patients are expected to create a potentially overwhelming burden on healthcare systems.

*Corresponding author at: University of Texas Health Science Center at San Antonio, 15355 Lambda Drive, San Antonio, TX 78245, USA., galvanv@uthscsa.edu (V. Galvan).

☆This article is part of a Special Issue entitled: Vascular Contributions to Cognitive Impairment and Dementia edited by M. Paul Murphy, Roderick A. Corriveau and Donna M. Wilcock.

Transparency Document

The Transparency document associated with this article can be found, in online version.

Aging is, by far, the strongest known risk factor for AD [2]. However, very little is known about the molecular mechanisms that link the regulation of brain aging to diseases like Alzheimer's. Vascular dysfunction, a universal feature of aging [3,4], is one of the earliest events in AD [3–7]. A hallmark of vascular aging is impaired endothelium-dependent vasodilation [8–10], which leads to chronically decreased cerebral blood flow (CBF) [11]. Decreased CBF is not necessarily an indication of a disease state since reduced CBF is present in otherwise healthy subjects when compared to young adults [11]. Chronic reduction in CBF, however, has been linked with decreased neuronal plasticity and impairment of cognitive function. Prominent vascular pathologic conditions are present in AD and other age-related brain diseases, including Parkinson's disease, dementia with Lewy bodies, and vascular dementia [5–7,12,13]. Cardiovascular risk factors increase the risk for AD; conversely, controlling cardiovascular risk factors decreases the risk for AD [14]. Furthermore, cerebrovascular damage and aberrant protein aggregation and deposition, are commonly linked in a number of dementias [15]. Thus, age-associated brain vascular changes may represent an early and universal event of aging that underlies the increased susceptibility of aged brains to specific neurological diseases of aging, including AD.

Neurons are damaged or lost in AD [1]. Neurons depend critically on blood vessels for the continuous delivery of oxygen and nutrients and the removal of products of metabolism and of potentially toxic brain metabolites [4]. Because 90% of the energy in brain is provided through aerobic metabolism, cerebral blood flow (CBF) is tightly regulated to meet the brain's metabolic demands. The brain consumes 25% of the body's oxygen and receives ~ 20% of cardiac output. The dependence of neuronal activity on delivery of nutrients and oxygen through increased blood flow, a process called neurovascular coupling, is so tight that local increases in blood flow and blood oxygenation are used to directly infer changes in neural activity in functional MRI studies [16].

The target of rapamycin (TOR), also referred to as mechanistic TOR (mTOR) [17–19], is a central regulator of cell growth and survival that is a signaling hub for cellular pathways sensing nutrients, insulin, and growth factor availability. TOR has a clear role in the control of the rate of aging in invertebrates [20–24], and systemic attenuation of the mammalian TOR/mTOR increases lifespan in mice [25–28]. The tissue- and cell-specific mechanisms by which mTOR controls mammalian aging are, however, still unknown. mTOR controls protein homeostasis by promoting cap-dependent translation and inhibiting autophagy [29], and it regulates key aspects of cellular metabolism [18,30]. mTOR functions as a central switch between anabolic *versus* catabolic processes in response to nutrients, growth cues, and cellular energy status by integrating multiple inputs that result in its activation or inhibition. In addition to these critical activities, emerging evidence discussed in this review suggests a key role of mTOR in the regulation of vascular function by specific mechanisms active in vascular endothelial and smooth muscle cells [31–38]. mTOR has been shown to regulate vascular function acutely [33] as well as chronically [34,39] and our recent studies suggest that mTOR has a role in vascular contributions to the pathogenesis of AD [40]. Evidence for acute and chronic mTOR-dependent effects on vascular function are discussed in this review. The mechanisms by which mTOR regulates mammalian aging and those by which it regulates brain vascular function acutely and chronically, however, are largely unexplored.

Because aging is the major risk factor for AD and other dementias, it is imperative that the mechanistic interface between the regulation of aging and the specific pathogenesis of age-associated neurological diseases be defined. This review will discuss current evidence for TOR-centered brain vascular dysfunction as a critical mechanistic link between aging and the pathogenesis of AD, and will highlight specific therapeutic opportunities.

2. Alzheimer's, Aβ, and synaptic function

Synapses are a major target in the pathogenesis of AD [1], and the release of the amyloid-beta (Aβ) peptide at synaptic sites, triggered by neuronal activity, has a critical role in this process. The Aβ peptide is a product of proteolytic processing of a large transmembrane precursor protein, the amyloid precursor protein (APP), for which ligands have been identified [41–44]. Generation of Aβ depends on specific proteases that catalyze the cleavage of APP intramembranously and at different sites of its extracellular domain [45–47]. Aβ monomers spontaneously form a wide range of soluble oligomeric species including dimers, trimers, tetramers, dodecamers (5–25 nm in diameter) and higher-order oligomers and protofibrils (over 40 nm in lenght) [48] as well as mature fibrils with high β-sheet content [49,50] which can assemble and desposit in the extracellular space to seed the formation of microscopically visible plaques and cerebral amyloid angiopathy (CAA) lesions in brain parenchyma and vasculature respectively [51–53] (Fig. 1).

Numerous studies have shown that before plaques form, soluble oligomeric forms of Aβ [54] may exert toxicity at synapses by various mechanisms, prominently by impairing glutamatergic synaptic transmission strenght and plasticity [55,56] and causing synaptic loss [56]. Aβ secretion at synaptic sites is activity-dependent [57,58] and the available evidence supports the notion that APP and Aβ are part of a feedback loop that regulates neuronal excitability, in which secreted Aβ acts as a neuromodulator that suppresses excitatory synaptic activity postsynaptically [57,58]. A recent study, however, suggested that small increases in Aβ secreted from wild-type neurons stimulate synaptic activity at the presynaptic level in a manner that is inversely dependent on the neuron's firing rate [59]. These observations are consistent with prior studies that showed dose-dependent effects of Aβ on synaptic transmission, with picomolar and low nanomolar concentrations of Aβ having positive and negative effects on synaptic transmission respectively, through mechanisms involving activation of the α7-nicotinic acetylcholine receptor [60–62]. Higher levels of Aβ associated with pathology, however, impair long-term potentiation (LTP), a persistent strenghtening of synapses that is one of the mechanisms underlying synaptic plasticity [56], and induce long-term depression (LTD), a process functionally opposite to LTP that reduces the efficacy of synapses [63]. Glutamate receptors of the NMDA (NMDARs) and AMPA (AMPARs) type regulate the changes in neuronal excitability associated with LTP and LTD. Aβ induces endocytosis of AMPARs postsynaptically, thus attenuating glutamatergic excitatory neurotransmission [57,58,64]. The mechanisms of Aβ-induced LTP block [56] may also involve a partial block of NMDA-dependent signaling by desensitization, ultimately leading to synaptic depression and potentially the induction of LTD [57,58,65], spine shrinkage, and synaptic loss [57,58,65].

The generation of Aβ by proteolytic processing of APP and its release into brain interstitial fluid (ISF) is continuous throughout life and does not trigger disease when in its normal, soluble form [66]. Pathologically high levels of Aβ and Aβ oligomers, however, will disrupt synaptic plasticity and ultimately lead to neuronal loss. Dyshomeostasis of Aβ levels in ISF can thus have significant consequences for brain function and is hypothesized to be a critical causative event in the pathogenesis of AD.

3. Mechanisms of Aβ removal from brain

The cerebrovascular system, comprising endothelial cells, vascular mural cells (smooth muscle cells or pericytes) astrocytes and neurons, critically contributes to brain function [4,6]. In the central nervous system (CNS), endothelial cells are connected by specialized structures, ‘tight junctions’, to form a practically impermeable barrier [67,68]. Because paracellular movement of water and blood-borne substances is effectively abolished, the interchange of substances happens only transcellularly and depends on passive or active transport through endothelial cells. This highly selective permeability interface, the blood–brain barrier, allows the passage of water, some gases, and lipid-soluble molecules (by passive diffusion). The blood–brain barrier thus regulates the selective transport of molecules such as glucose and amino acids, crucial to neural function, and limits or impedes entry of potential neurotoxins such as amino acids, which are kept in brain at 10% of their concentration in blood, by systems involving active transport. A prominent example is the sodium-dependent excitatory acidic amino acid cotransporter (*e.g.* glutamate and aspartate; EAAT) transporter [69] that promotes the removal of glutamate and prevents the entry of glutamate from blood into the brain to maintain low glutamate concentrations in the interstitial fluid. Whereas the blood–brain barrier is localized at the tight junctions between brain endothelial cells, astrocytes, pericytes and neurons are also integral to blood–brain barrier structure and function [67,68]. Pericytes encircle capillaries through numerous cytoplasmic projections, make tight junctions and adherens junctions with endothelial cells, and regulate microvascular stability by secretion of extracellular matrix and permeability by secretion of specific growth factors [70]. Pericytes belong to the vascular smooth muscle cell (VSMC) lineage [71]. Recent studies have suggested that pericytes, like VSMC are contractile [72], and contribute to regulating brain capillary blood flow, although this has not been fully established yet [73]. In addition to having a key role in the regulation of synaptic activity, astrocytes are a prominently associated with brain vascular endothelial cells, pericytes and neurons through ‘foot processes’. Astrocytes interact with brain endothelial cells to regulate water and electrolite levels in brain [74] and have a major role, together with VSMC, in the synchronization of metabolic demand arising from neuronal activation with increases in local CBF, a process known as neurovascular coupling [75]. Another type of glial cells, microglia, are resident macrophages in brain that have phagocytic and antigen-presenting capacities. A subset of microglial cells associated with neurovasculature known as ‘perivascular microglial cells’ is bone-marrow derived and can signal to circulating immune cells [76] (Fig. 1).

After its generation by neurons and release at synaptic sites, Aβ in ISF can be cleared by various mechanisms, including uptake and degradation by microglia and astrocytes [77–80], proteolytic degradation [81–83], transport to the CSF with subsequent reabsorption into the

venous blood [84], and direct transport across the blood–brain barrier [67,85](Fig. 1c). Impaired Aβ clearance from brain has been documented for AD [86] and slower clearance kinetics resulting in increased Aβ half-life was strongly associated with increasing age [87]. Cellular and direct proteolytic degradation, involving enzymes like neprilysin and insulin-degrading enzymes in the extracellular space [81], contribute to clearance of Aβ from brain. A significant fraction of sub-arachnoid CSF cycles through the brain interstitial space, entering the parenchyma along the paravascular space that surrounds penetrating arteries and cleared along paravenous drainage pathways. Paravascular flow contributes to clearing ISF solutes, including Aβ [84]. However, most Aβ is removed from the brain through the blood–brain barrier, extruded into the circulation, and degraded largely by the liver and kidney [88].

The low-density lipoprotein receptor-related protein 1 (LRP1), a member of the low-density lipoprotein receptor (LDLR) family, is a large multi-functional cell surface receptor protein that regulates endocytosis of different ligands and associates with other cell membrane receptors to regulate intracellular signaling pathways [89,90]. LRP1 is widely expressed, with its highest levels in the liver, brain, and lung [89]. In the brain, LRP1 is expressed in neurons, glia, and cells of the cerebrovasculature. Neurons both produce Aβ and clear it from the synaptic space through reuptake followed by lysosomal degradation [91,92]. Endocytosis is critical for the generation of Aβ [93,94] and its retrieval from the cell surface [95]. LRP1-dependent clearance of Aβ [96] by neurons through endocytosis was demonstrated in studies in which LRP1 was knocked out exclusively in forebrain neurons; the result was a significantly increased Aβ half-life in ISF and exacerbated Aβ plaque deposition [97].

The autophagy-lysosomal pathway also plays a central role in the regulation of intracellular and extracellular levels of Aβ [98–101](Fig. 1). In addition to being a major Aβ degradative pathway, autophagy may contribute to Aβ secretion [102]. Significant neuronal loss, as seen in AD, is rare in mutant APP transgenic mice that robustly deposit Aβ, but it is usually extensive in APP/Aβ models that accumulate Aβ intraneuronally [103]. If the accumulation of Aβ intracellularly is more toxic than its release in the ISF, it has been suggested that the expected beneficial effects brought on by degradation of intracellular Aβ through autophagy may supersede the negative impact associated with release of Aβ in ISF [104].

LRP1 also has a critical role in clearing Aβ from brain through the cerebrovasculature [85,97,105–107]. LRP1 is highly expressed in cells of the brain vasculature, including endothelial cells, where it is the major Aβ receptor for clearance of the peptide across the blood–brain barrier [105] (Figs. 1 and 2). LRP1-mediated transport can be saturated at high concentrations of Aβ (70 nM-100 nM [105]). High levels of interstitial Aβ, such as those observed in transgenic mouse models, may thus trigger further increases in ISF Aβ levels by saturation of LRP1-mediated transport, and promote Aβ aggregation. During its transport across the blood–brain barrier, neuronally-generated Aβ in ISF is bound by LRP1 expressed on brain endothelium, at the abluminal side of the blood– brain barrier [107]. A wealth of evidence indicates that LRP1 binding by Aβ initiates the process of Aβ clearance from brain to blood [108–112]. The critical role of LRP1 in the export of Aβ out of the brain is substantiated by multiple *in vitro* and *in vivo* studies. When Aβ is present at high levels, its transport across the blood–brain barrier is almost completely abolished by LRP1 antagonists

such as anti-LRP1 antibodies or binding by the LRP-specific chaperone, receptor-associated protein (RAP). However, at lower peptide loads, neutralization of LRP1 by antibody or receptor-associated protein binding reduces (but does not abolish) Aβ clearance [105]. This effect suggested the existence of other transport mechanism(s) at the blood–brain barrier that operate to clear Aβ when the peptide is present at very low levels. The nature of this high-sensitivity pathway, however, is still unknown.

The phosphatidylinositol-binding clathrin assembly (PICALM) protein [113], which participates in endocytosis and internalization of cell surface receptors, was recently linked to AD in genome-wide association studies [114,115]. PICALM associates with LRP1 during Aβ transcytosis across endothelial monolayers [116]. Also, iPSC-derived endothelial cells carrying an AD-protective PICALM allele show significantly higher expression of PICALM and substantially increased Aβ clearance, compared to iPSC-derived endothelial cells expressing a non-protective PICALM allele [116]. These data suggest that PICALM regulates Aβ transcytosis and clearance and that polymorphisms in the PICALM locus enhance Aβ clearance and thereby reduce the risk of AD.

LRP1 is also expressed in vascular smooth muscle cells (VSMC), where it mediates lysosomal degradation of Aβ [117–119]. Uptake and degradation of Aβ by VSMC is inhibited by serum-response factor and myocardin, which reduce clearance of Aβ by downregulating LRP1 [119]. Early in AD, VSMC develop a hypercontractile phenotype [120] that contributes to deficits in cerebral blood flow. In addition, Aβ degradation by VSMC becomes impaired [120]. Accumulation of Aβ and Aβ deposition in pial and intracerebral arteries and arterioles thus leads to cerebral amyloid angiopathy (CAA; Fig. 1c). In agreement with a the critical role of LRP1 in VSMC-mediated Aβ clearance, conditional deletion of LRP1 in VSMC exacerbates Aβ plaque deposition and CAA in a mouse model of AD [117].

Microglia and astrocytes also have important roles in the removal of Aβ from ISF [78–80]. Both microglia [77,78,121,122] and astrocytes [79, 123,124] have a prominent role in clearing Aβ from ISF by internalization and degradation. In addition, pericytes may also be integral in Aβ clearance, since *Pdgfr*β haploinsufficiency –which leads to pericyte loss – dramatically decreases Aβ clearance and increases Aβ deposition early in progression of AD-like pathogenesis in a mouse model of AD [125]. Pericytes clear extracellular Aβ *via* LRP1 [125,126]. A critical role for pericytes in vascular dysfunction in aging [127,128] and in AD is also suggested by the fact that APOE4 carriers show accelerated pericyte degeneration correlated with the degree of blood–brain barrier deterioration and decreased levels of LRP1 [129].

Another multi-ligand receptor protein, the receptor for advanced glycated end-products (RAGE), has been implicated in the movement of Aβ back into the CNS [85,130]. Re-entry of Aβ into the brain can also occur *via* ApoJ-mediated blood-to-brain transport down its concentration gradient [89]. This system, however, is expected to be saturated under physiological conditions, favoring the net efflux of Aβ out of the brain (Fig. 1).

4. Vascular dysfunction in aging

Vascular dysfunction is a universal feature of aging. Regulation of blood flow by arteries and arterioles relies on the communication between endothelial cells and VSMC at the vascular wall [131]. Thus, a recognized central injury of vascular aging is impaired endothelial function [132,133]. Functional and structural changes in both endothelial cells and VSMC, and dysfunction in the mechanisms that mediate communication between them will induce changes in their structure that in turn augment endothelial dysfunction [133,134]. Major age-associated mechanisms that contribute to age-dependent dysfunction in the vascular wall are less bioavailability of nitric oxide due to decreased nitric oxide biosynthesis and its increased scavenging by free radicals associated with increased oxidative stress [135], increased activity or levels of vasoconstrictors, and increased inflammation [131,134,136]. A critical role of oxidative stress and inflammation in brain vascular aging [137] was indicated by studies that showed that caloric restriction, an intervention that robustly extends lifespan in many species [138], prevents impairment of angiogenesis, reduces oxidative stress and inflammation, and inhibits apoptosis in aged cerebromicrovascular endothelial cells [139]. Aging, in turn, exacerbates loss of pericyte coverage and subsequent cerebromicrovascular rarefaction and neurovascular uncoupling associated with increased inflammation in obese mice [127]. Endothelial senescence is also associated with altered function of endothelial nitric oxide synthase (eNOS), such that it produces superoxide instead of nitric oxide. This change in enzymatic activity is referred to as “eNOS uncoupling” and leads to reduced nitric oxide bioavailability and increased oxidative stress [140]. The main result of the synergistic interaction between these age-associated changes is less endothelium-dependent vasodilation, considered the central injury of vascular aging (Fig. 2) and a main mechanism by which aging increases the risk for CVD and atherosclerosis in humans [131,134]. The progressive impairment of endothelial function begins in middle age in humans and is associated with aging as an independent factor, even without other cardiovascular risk factors [141]. Yet the mechanisms that link the regulation of the rate of aging to age-associated vascular dysfunction are still unknown, although the activation of sirtuins and changes in telomere maintenance have been suggested [131].

5. The mechanistic target of rapamycin (mTOR) and the control of aging

The mechanistic/mammalian target of rapamycin (mTOR) is a serine/threonine kinase of the phosphatidylinositol-3-OH kinase (PI3K)-related family that functions as a major regulator of cellular growth and metabolism by integrating signaling cascades activated in response to nutrient and growth factor availability [18,19,142]. mTOR functions as hub for the switch between anabolic *versus* catabolic processes in response to nutrients, growth cues, and cellular energy status because of the multiple inputs that lead to its activation or inhibition, and its role in the regulation of critical cellular functions. mTOR kinase associates with specific companion proteins to form two complexes with distinct specific substrates: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [18,19] (Fig. 2).

Rapamycin, an antifungal macrolide compound, has been used in combination with other drugs for immunosuppression after transplant therapy. Various derivatives (everolimus, temsirolimus, umirolimus, ridaforolimus, and zotarolimus) are currently approved for use in

clinical conditions including elution from vascular stents to prevent restenosis following angioplasty, and as a treatment for some cancers [19,142].

Rapamycin inhibits mTORC1 through binding to the immunophilin FKBP12. The rapamycin-FKBP12 complex subsequently binds to the kinase in the context of the mTORC1 complex and potently inhibits its activity [143]. Although mTORC2 is not directly inhibited by the rapamycin-FKBP12 complex, prolonged exposure of cells to high levels of rapamycin can reduce the availability of mTOR kinase such that mTORC2 assembly is inhibited [144]. This effect of rapamycin is thought to be associated with glucose intolerance and hyperlipidemia, which are negative metabolic consequences of prolonged rapamycin treatment [145]. An important output of mTORC1 signaling is the positive regulation of lipid biosynthesis mainly through sterol-regulatory-element-binding protein (SREBP) transcription factors 1 and 2 by unclear mechanisms [142,146–148]. mTORC1 potently upregulates SREBP-2 [146,147], a transcriptional suppressor of the major Aß clearance receptor, LRP1 [149].

mTORC1 is activated by the insulin and insulin-like growth factor 1 pathways through PI3K and AKT signaling and amino acid availability, and is repressed by AMP-activated protein kinase (AMPK), a critical sensor of cellular energy status detected by the kinase as low AMP:ATP ratios [142]. In response to activating signals such as growth factor receptor binding or amino acid transport into the cell, mTORC1 promotes mRNA translation and protein synthesis through at least three of its substrates: ribosomal protein 6 kinases 1 and 2 (S6K1 and S6K2), and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1). Active repression of mTORC1 activity will not only suppress these energy-demanding cellular functions, but also relieve mTORC1-dependent inhibition of autophagy, effectively switching the cell from anabolic to catabolic state [18,29,142,150] (Fig. 2).

Autophagy initiation is regulated by two kinases, unc-51-like kinase (ULK1) and vacuolar protein sorting-34 (VPS34). mTORC1 potently represses ULK1 through direct phosphorylation and destabilization [151]. The mechanistic link between sensing of nutrient availability by mTORC1 and the regulation of autophagy is enabled by the localization of many signaling complexes and regulation of activation of mTORC1 at the lysosome, and depends on a class of small G proteins, the Rag GTPases [30]. Active RagA or RagB bound to GTP bind Raptor to translocate mTOR to the lysosomal surface. This is required for active Rheb to activate mTORC1 as a response to growth factor stimulation. Amino acids generated in lysosomes by catabolism are sensed by the vacuolar H^+-ATPase that signals to activate the Rag GTPases [30]. An overlapping system of control of metabolism by mTORC1 involves the mTORC1-dependent inhibition of transcription factors essential for lysosomal biogenesis, transcription factor EB (TFEB), and transcription factor binding to IGHM enhancer 3 (TFE3) [152]. Consistent with the concept that mTORC1 is critical in regulation of autophagy, hyperactivation of mTORC1 by loss of function of its upstream regulator tuberous sclerosis complex 1 (TSC1) potently inhibits autophagy [29,153]. Evidence for the involvement of mTORC2 in the regulation of autophagy is limited, although mTORC2 can indirectly repress autophagy by activation of AKT and the forkhead box O3 (FOXO)3A transcription factor. mTORC2, however, is also required for stability of

the actin cytoskeleton, which is in turn required for early autophagosome formation. Thus, mTORC2 may have a dual role in the regulation of autophagy [29].

Activation of mTORC1 has potent effects on the cell's metabolic state. mTORC1 regulates glucose metabolism through the hypoxic response transcription factor subunit HIF-1alpha by its phosphorylation and stabilization [154]. Stabilized HIF-1alpha associates with its companion subunit HIF-1beta activity to promote the transcription of a large set of genes, notably VEGF and erythropoietin, both involved in glucose and iron metabolism [155] (Fig. 2). To terminate mTORC1-driven responses, mTORC1 activation by the insulin and insulin-like factor 1 is regulated through a primary negative-feedback inhibitory pathway whereby high activation of the mTORC1/S6K1 pathway suppresses Akt activity [150]. This mechanism depends on the phosphorylation of IRS-1 by mTORC1-activated S6K1 [156]; this process induces IRS-1 inactivation and degradation, effectively blocking signaling through insulin and insulin-like and other growth factor receptors [156,157] (Fig. 2). This process is thought to be a major cause of insulin resistance in peripheral tissues [158]. In addition, mTOR directly phosphorylates the insulin receptor, triggering its internalization, thus in turn decreasing its own activation. mTOR hyperactivity is though to contribute to insulin resistance in diabetes mellitus. mTORC2 phosphorylates Akt on Serine 473 to activate it [159,160], which facilitates activation of eNOS by Akt [161,162]. Using a similar mechanism to ensure negative feedback inhibition, phosphorylation of Akt on Ser473 also targets Akt for ubiquitination and degradation by the proteasome [163].

Experiments in invertebrates [22,164–168] provided the first demonstration that TOR regulates aging. Chronic pharmacological reduction of mTOR signaling by rapamycin or genetic manipulation by deletion of its downstream target S6K1 also extends lifespan in mice by delaying aging [25–28]. The mechanisms by which TOR regulates aging in mammals, however, are still not understood. Dietary restriction – reduced nutrient intake in the absence of malnutrition – extends lifespan in many species [169,170]. To date, dietary restriction and TOR attenuation are the only two interventions that extend lifespan in yeast, worms, flies, and mice [142]. There is genetic evidence that mTOR is a critical effector of lifespan extension by caloric restriction in yeast and in *C. elegans* [20,166], but the interaction between mTOR activity and caloric restriction responses is complex. There is general consensus, however, that reduced mTOR signaling is important in dietary restriction-dependent lifespan extension [142,171].

Lifespan extension by mTOR attenuation in mice was first reported in 2009 in studies conducted by the National Institute on Aging's Interventions Testing Program (ITP) [172]. In these studies, chronic systemic rapamycin, fed in the chow, extended the lifespan of genetically heterogeneous mice arising from a 4-way cross at the three independent testing locations of the ITP [25–28]. Remarkably, attenuation of mTOR activity began at 600 days of age, approximately comparable to 60 years of age in humans [25]. That rapamycin effectively extended lifespan when administered late in life is highly relevant because all previous experimental manipulations that increased lifespan in mammals (mice or rats) were initiated early in life [173]. Subsequent studies showed that initiating rapamycin treatment at the same dose but earlier in life did not further extend the lifespan [27]. Rapamycin extended median lifespan by 18% and 10% in female and male mice respectively. These results were

confirmed in a genetic model of mTOR attenuation by S6K1 knockout, in which lifespan was also increased, but only in females [174]. Further studies showed that the frequency and form of causes of death in rapamycin-treated animals were not altered [27]. In addition to potential beneficial effects of rapamycin on neoplasias, many forms of age-dependent change – such as alterations in heart, liver, adrenal glands, endometrium, and tendons, as well as age-dependent decline in spontaneous activity – occur more slowly in rapamycin-treated mice, suggesting that mTOR attenuation retards multiple aspects of aging in mice [26]. Increasing the dose of rapamycin by threefold compared to the original studies by Harrison *et al.* extended median lifespan by 23% and 26% in males and female mice, respectively; maximal longevity was also increased in both sexes [28]. These studies further compared endocrine and metabolic changes and expression of hepatic genes involved in xenobiotic mechanisms in rapamycin-treated and caloric-restricted mice. Patterns of change from these interventions differed significantly, suggesting that these two interventions that extend lifespan differ in many respects. Various studies have reported changes in mTOR signaling with age, but the direction of change varied for different strains, sexes, tissues and ages compared [175–179]. An exhaustive analysis by Baar *et al.* [180] in which gender and fed state were variables, used C57BL6/Jnia animals up to 30 months of age to show that aging is not associated with increased mTOR activity in most tissues, suggesting that mTOR inhibition by rapamycin does not promote lifespan extension through reversal of an age-associated increase in mTOR [180]. This is in agreement with the notion that mTOR activity may be, in a manner comparable to the relationship of high levels of tosterone and risk for prostate cancer in late life, a pleiotropic trait selected for its beneficial effects in early life (*e.g.* by increasing fitness during reproductive age) that has negative effects post-reproductively in later life, when selective pressure wanes [181–183].

If mTOR attenuation slows the rate of aging in mice, then the age at onset and the progression of age-dependent diseases as modeled in this species should also be delayed in animals in which mTOR activity is attenuated. Thus, increasing lifespan by mTOR inhibition should also lengthen the period of life devoid of significant chronic disease or disability. Current evidence suggests that this is may be the case for dietary restriction, at least in rodents [169,184] and in rhesus monkeys [185,186]. In agreement with the hypothesis that mTOR attenuation slows aging, accumulating experimental evidence suggests that mTOR attenuation can delay or block the progression of neurological diseases or dysfunctions of aging such as Parkinson's [187–190], Alzheimer's [40,191–196], tauopathy [197–199], frontotemporal lobar dementia [200–203], and age-associated cognitive decline [204–207].

6. mTOR and vascular function

The senescent phenotype of aortic endothelial cells in C56BL/6 J mice made obese by consumption of high-fat diet was shown to be Akt/mTOR-dependent [36]. In this model, inhibition of the Akt/mTOR axis by rapamycin restored endothelial cell replicative life span, endothelial sprouting, eNOS activity, and endothelium-mediated vasorelaxation. *In vivo*, these phenotypes correlated with improved angiogenic response, blood flow recovery, decreased limb necrosis, and increased capillary density after hindlimb ischemia [36]. Augmented arginase activity in endothelial cells is causally implicated in the reduction of

eNOS activity through decreased availability of the eNOS substrate L-arginine [208]. Arginase-II upregulated the mTOR/S6K1 pathway in isolated mesenteric arteries, and thus may be implicated in a feedforward mechanism of mutual positive regulation that can be abolished by attenuating mTOR activity [37]. A recent study showed that persistently hyperactive S6K1 promoted endothelial senescence with eNOS uncoupling, increased superoxide generation, and decreased NO production *in vitro* in senescent primary HUVEC and *ex vivo* in aortas of aged rats [35].

Chronic *in vivo* mTOR inhibition by intravenously injected rapamycin enhances endothelial-dependent vasodilation in isolated rat aortic rings acutely [31,33,34] and mesenteric arterioles chronically treated with rapamycin [209] via endothelial-dependent nitric oxide release. Several *in vitro* studies using isolated rat aortic rings showed that acute *ex vivo* [31,33] and chronic *in vivo* [34] treatment with rapamycin has pronounced vasodilatory effects *via* an endothelium-dependent mechanism. Other studies have reported either no effect [38,210] or negative [32] effects of mTOR attenuation on vasodilation, but these effects were not specific to endothelium-dependent reponses [32]. While vascular responses elicited *ex vivo* consistently showed a vasodilatory effect of rapamycin-induced attenuation of mTOR activity, the systemic effects of mTOR attenuation with rapamycin have yielded conflicting results. This is likely a result of different study designs, drug doses, and durations of treatment duration [31–34,39]. Interestingly, angiotensin II (AngII) impairs insulin-stimulated phosphorylation of eNOS through activation of its receptor AT_1R and by transactivation of epidermal growth factor receptor, resulting in the activation of mTOR/S6K1 and phosphorylation of IRS-1 at $Ser^{636/639}$, which blocks Akt-dependent phosphorylation of eNOS [209]. These studies delineate a mechanism that may contribute to explain the mechanistic link between mTOR pathway activity and the regulation of endothelium-dependent nitric oxide release and vasodilation (Fig. 2).

The regulation of eNOS gene expression and protein activation, and many other key aspects of endothelial homeostasis is controlled by hemodynamic shear stress, the blood flow-generated frictional force acting on endothelial cells [161,211]. Further evidence that the mTOR/S6K1 signaling pathway has a key role in the modulation of eNOS function was provided by studies that used a perivascular ‘cast’ consisting of a cylinder with a tapered lumen to create a high shear stress field in the carotid artery of eNOS-GFP transgenic mice, with concomitant regions of low shear stress and oscillatory shear stress upstream and downstream of the perivascular ‘cast’ device [39]. mTOR attenuation with rapamycin dose-dependently increased low basal eNOS expression levels in regions of the casted carotid under low shear stress, and conversely decreased high basal eNOS expression levels in regions under high shear stress, suggesting that mTOR regulates the shear stress responsiveness of the vessel wall [39].

In addition to releasing NO, brain vascular endothelial cells are connected by tight junctions to form the blood–brain barrier. Emerging evidence suggests that mTOR may have a critical role in endothelial injury arising from ischemia-reperfusion, the initial phase of blood–brain barrier disruption. In an *in vitro* model of ischemia-reperfusion using oxygen-glucose deprivation/reoxigenation in brain microvascular endothelial cells (BMVEC), and *in vivo* by transient middle cerebral artery occlusion/reperfusion in rat, attenuation of mTOR with

rapamycin attenuated BMVEC apoptosis and increase in reactive oxygen species, reversed a decrease in levels and promoted the redistribution of tight junction protein zonula occludens-1 to the cell membrane, and reduced Evans blue extravasation in the ischemic hemisphere [212]. mTOR inhibition by rapamycin also decreased progression of brain edema after focal cerebral ischemia-reperfusion injury by preserving blood–brain barrier integrity and inhibiting MMP9 and AQP4 expression [213]. Similar results were reported for short-term outcomes of ischemic stroke followed by reperfusion, with rapamycin providing protective effects on percent infarct area, apparent diffusion coefficient, signal intensity, and motor function compared to the vehicle-treated group [214]. Protective effects of mTOR attenuation with rapamycin have also been reported for *in vitro* models of oxygen-glucose deprivation and reoxygenation by attenuation of astrocytic migration and decreased production of inflammatory mediators by these cells [215]. These studies are in agreement with the notion that inhibition of the mTOR pathway may induce neuroprotective autophagy in models of ischemia and ischemia protection by preconditioning [216–218].

Recent studies, however, suggested that activity of the Akt/mTOR/S6K1 pathway may be necessary for *in vitro* preconditioning and protection against oxygen and glucose deprivation [219]. An active Akt/mTOR pathway appears required for the protective effects of ischemic post conditioning, both *in vitro* and in *in vivo* models [220]. Although the role of mTOR in stroke is not yet clear, differences observed between different models may be related to differences in the time scale for treatment and outcome measurement (short- or long-term), and from cell type-specific roles of mTOR.

In addition to its role in regulating key aspects of vascular endothelial function, available evidence indicates a role of mTOR in the regulation of vascular smooth muscle cell proliferation and phenotypic conversion from contractile to synthetic phenotypes [221] (Fig. 2b). Neointima formation, the leading cause of restenosis following coronary stenting, is caused by the proliferation of smooth muscle cells in the coronary artery. Restenosis is associated with infiltration by monocytes. Rapamycin inhibits neointima formation and reduces the adhesiveness of smooth muscle cells in the coronary artery, reducing infiltration of monocytes, thus inhibiting restenosis in humans [222]. mTOR inhibition also blocks arginine vasopressin-induced down regulation of autophagy in vascular smooth muscle cells [223], and promotes VSMC fate [224, 225]. Although distal EC dysfunction has been observed with rapamycin-eluting stents implanted in coronary heart disease patients [226,227], recent studies have shown that this effect may be at least partially due to delayed or absent re-endothelization of the stent [228], or to effects of the stent itself [229]. *In vitro* studies using primary mouse VSMC [225,230] have shown that mTOR is required for chondrogenic/osteogenic transdifferentiation of vascular smooth muscle cells that contributes significantly to medial arterial calcification and that this pathway is inhibited by adiponectin. Furthermore, *in vivo* studies of vascular calcification in mice modeling chronic renal failure showed that Klotho, a protein implicated in the regulation of aging, is required for mTOR-dependent vascular calcification in kidney [231]. Consistent with these observations, it was recently shown that mTOR attenuation with rapamycin blocks plaque progression in ApoE knockout mice fed a diet supplemented with cholesterol by inhibition of monocyte chemotaxis [232].

Taken together, the evidence discussed above suggests that mTOR is a negative regulator of eNOS-dependent NO generation and of tight junction integrity at the blood–brain barrier, both critical aspects of brain vascular endothelial cell function. In addition, the evidence discussed suggests a critical role of mTOR in driving vascular smooth muscle cell proliferation as well as their phenotypic conversion from a contractile to a secretory phenotype as well as in their chondrogenic/osteogenic transdifferentiation.

7. Cerebrovascular dysfunction in Alzheimer's disease

In epidemiological studies, conditions with cerebrovascular functional disturbances such as diabetes mellitus [233,234], hypertension [235], cerebral small vessel disease [236] transient ischemia, stroke and microvascular pathologies [237] increase the risk for AD. Conversely, controlling vascular dysfunction reduces the risk for AD. *APOE* genotype is associated with risk for AD [238,239]. ApoEε2, ε3, and ε4 alleles strongly modify the likelihood of developing AD and CAA in a dose-dependent manner. ApoEε4 and ApoEε2 increase and decrease the risk for AD respectively. *APOE* genotype may act by modulating the likelihood that Aβ begins to deposit. A current hypothesis is that APOEe4 increases Aβ accumulation in the brain and its vasculature, or impairs clearance as compared to other isoforms, or both [240].

A steady supply of blood and oxygen delivery to brain is critical for brain function and essential for life. In normal physiological conditions, brain blood flow is kept remarkably constant. One important aspect of cerebral blood flow regulation is cerebral autoregulation, a process that ensures constant brain blood flow under conditions of variable arterial blood pressure. The mechanisms of cerebral autoregulation are not completely understood, and evidence suggests that the regulatory mechanisms are possibly different for responses elicited by increases versus decreases in pressure [241]. Reductions in cerebral blood flow stimulate the release of specific vasoactive substances in the brain and recent evidence suggests that intrinsic innervation may have a role in this response [242]. Compensatory mechanisms activated by increases in pressure involve the myogenic response of cerebral smooth muscle, that constricts when subject to elevated pressure and dilates in response to decreased pressure [243]. The regulation of cerebral blood flow is also potently influenced by other regulatory mechanisms, involving neurovascular coupling [244] and CO_2 reactivity [245] and by cholinergic hemodynamic regulation [246]. Large arteries as well as parenchymal arterioles contribute prominently to vascular resistance in brain [247]. Large artery resistance in brain provides a mechanism to ensure constant blood flow and can help attenuate changes in microvascular pressure when arterial blood pressure increases, thus ensuring microvasculature integrity and protecting the brain from vasogenic edema [241].

The local increase of cerebral blood flow during brain activity as a result neurovascular coupling involves the communication between neurons, glia, vascular cells [244]. During functional hyperemia, blood flow through parenchymal microvessels increases focally at the activated area as a response to the activation of neurons, only during the period of activation. This response is coordinated by the interaction between endothelial cells, pericytes and smooth muscle cells. There is general consensus that the regulation of neurovascular coupling involves many vasoactive factors acting simultaneously to increase local blood

flow by targeting these vascular cell types. Please see Girouard and Iadecola [248] for a detailed review of this topic. Endothelial cells play an important role in the regulation of vascular tone through the release of vasoactive substances such as nitric oxide (NO) [249], endothelium-derived hyperpolarizing factor [250] and endothelin [251]. NO can be biosynthesized by NO synthases (NOS). NO contributes significantly to functional hyperemia elicited by neuronal network activation [244, 252–256]. In mammals, there are three genes that encode neuronal (nNOS, NOS1), endothelial (eNOS, NOS3) and cytokine-inducible (iNOS, NOS2) forms of the enzyme respectively. iNOS is involved in antimicrobial reponses and in the regulation of specific T cell subsets of the immune system [257]. Significant evidence exists for a role of nNOS in the regulation of functional hyperemia [244,252] and recent studies suggest a role of eNOS [253–255]. eNOS is protective against focal ischemia-induced injury [258] and mediates the increases in CBF elicited by exercise [259]. Endothelial dysfunction as impaired NO bioavailability is also associated with impaired neurovascular coupling in conditions associated with microvascular aging such as hypertension and obesity [127,260–264]. The relative contribution of eNOS and nNOS to the regulation of functional hyperemia, however, is not yet fully understood [253,255,265]. While it has been shown functional hyperemia may be independent of eNOS [265], recent evidence suggests that genetic ablation of eNOS decreases CBF responses in somatosensory cortex evoked by whisker stimulation or the administration of ATP [266]. Once released, NO induces vasodilation by binding to the heme moiety of guanylyl cyclase in smooth muscle cells of arteries and arterioles, activating the enzyme that then catalizes the coversion of GTP to cGMP [267]. cGMP serves as a second messenger in smooth muscle to ultimately result in smooth muscle relaxation *via* decrease in smooth muscle Ca^{2+} concentration [268].

Cerebrovascular dysfunction includes microvascular deficits and focal disruption of microcirculation, leading to a decrease in microcapillary density; neurovascular uncoupling; loss of blood–brain barrier integrity; and endothelial and vascular smooth muscle cell dysfunction, leading to decreased responsiveness to vasodilating stimuli [4–6,119]. Neurovascular dysfunction is one of the earliest events in AD and other types of neurodegeneration that lead to diminished CBF [5–7]. Neurovascular uncoupling, demonstrated as diminished CBF in response to brain network activation, is also prominent and precedes neurodegenerative changes in AD patients [7,269]. Decreased CBF diminishes the brain's supply of oxygen and nutrients and reduces effective clearance of toxic products of brain metabolism from ISF. In addition, levels of Aß in ISF increase in conditions of low CBF as a consequence of diminished Aß vascular clearance [86]. Cerebrovascular dysfunction and disintegration lead to loss of neuronal networks by insufficient delivery of oxygen and nutrients to glial/neuronal networks, failure to clear products of metabolism (including Aβ), and leakage of blood-borne molecules [4,6]. Although some blood-borne proteins are cleared in the interstitial space, accumulation of serum proteins in parenchyma can lead to brain edema and suppression of capillary perfusion [4].

The importance of vascular dysfunction in AD has long been recognized, but vascular pathways to AD remain understudied. Cerebrovascular dysfunction may be the first 'hit' in a 'two-hit' process that leads to AD, triggering the imbalance in brain Aß levels that triggers abnormal Aß accumulation, the second hit in the pathogenesis of AD [4] (Fig. 3). While Aß-

induced toxicity has been extensively studied, there is still a significant gap in our understanding of the molecular mechanisms through which vascular dysfunction is linked to AD.

The interactions of Aβ with the cerebral vasculature has specific consequences for different cerebrovascular cell components. CAA results from focal to widespread Aß deposition in leptomeningeal and intracortical cerebral blood vessels (Fig. 1). CAA can be prominent in VSMC of pial and intracerebral arteries and arterioles; Aß depositions in the glia limitans and adjacent neuropil are referred to as precapillary Aß, and those in the capillary wall are referred to as capillary Aß depositions [270]. CAA is present in roughly 80% of patients with AD [4,270, 271]. However, CAA is frequently observed in the elderly, even in those without AD [270]. In CAA of small arteries and arterioles, the smooth muscle layer can atrophy and rupture, leading to intracerebral hemorrhage, which contributes to and also aggravates brain damage [272, 273]. Indeed, patients carrying specific variants in the Aβ sequence such as L34V and E22Q mutations have accelerated VSMC degeneration that leads to hemorrhagic stroke and dementia [274–276]. Furthermore, Aβ itself constricts cerebral arteries [277]. In a recent study, cognitively normal APOE4 carriers (who have a higher risk for AD) showed impaired CBF responses to brain activation before detectable Aß accumulation [278,279]. Both patients with AD and mouse models of AD develop high levels of serum response factor (SRF) and MYOCD, two transcription factors that control VSMC differentiation. These abnormal levels of SRF and MYOCD induce a hypercontractile phenotype that leads to brain hypoperfusion, diminished functional hyperemia, and CAA [119,120].

In addition, Aβ induces toxicity and dysfunction in vascular endothelial cells including the activation of programmed cell death pathways [52] as well as by inducing oxidative-nitrosative stress, which activates the DNA repair enzyme poly(ADP)-ribose polymerase (PARP) [280]. The resulting increase in ADP-ribose opens transient receptor potential melastatin-2 (TRPM2) channels in endothelial cells, leading to intracellular calcium ion overload [280]. Exposure to a mixture of monomeric and oligomeric Aβ, but not to each species separately, reduced LRP1 and increased RAGE levels in cultured endothelial cells modeling the blood–brain barrier [281]. Furthermore, recent studies demonstrated that oligomeric Aβ interacts with TRAIL DR4 and DR5 death receptors on endothelial cells, triggering mitochondrial-dependent activation of multiple caspases associated with programmed cell death [282].

8. mTOR in Alzheimer's and other age-associated neurodegenerations

Initial evidence that mTOR is involved in the pathogenesis of AD came from studies that examined the relevance of macroautophagy for the generation of Aß in neurons [283,284], and it was demonstrated that enhancing autophagic-lysosomal function leads to substantially decreased Aß levels and deposition in brain [98]. More recent studies suggest a dual role for autophagy in the degradation and secretion of Aß [101,102]. Consistent with a critical role of the autophagy-lysosomal pathway in the regulation of Aß levels, activation of AMP-activated protein kinase by resveratrol, which potently inhibits mTOR by phosphorylation,

enhances autophagic-lysosomal degradation of Aβ in a manner dependent on the AMPK-mediated inhibition of mTOR [285] (Fig. 1).

mTOR is involved in the pathogenesis of other neurodegenerative diseases of aging through its inhibition of apoptosis and proteostasis, the latter prominently through autophagy, in polyglutamine-expanded huntingtin models of Huntington's disease [286] and alpha synuclein [287], parkin [288] and L-DOPA dyskinesia [289] models of Parkinson's and Lewy body diseases [189], and in the tau P301S model of tauopathy [199]. Of note, recent studies from one of our laboratories showed that proteins with reported chaperone-like activity were over-represented among the proteins upregulated in brains of rapamycin-fed mice modeling Alzheimer's disease, and that this was associated with increased activity of the master regulator of the heat shock response, heat-shock factor 1 [290]. Thus, mTOR-dependent inhibition of proteostasis in brain may involve both autophagy and aspects of the chaperone response. In addition to inhibiting autophagy, mTOR may also be involved in aberrant activation of the cell cycle, which caused neurodegeneration in a fly model of tauopathy [291] and contributes to toxicity of Aß oligomers in models of AD [292]. Hyperactive or upregulated mTOR and some of its downstream effectors have been observed in brains of patients with AD [293,294] and in some AD mouse models [40,191,192,295]. Furthermore, studies have suggested that the increased acitivity or levels of mTOR in AD mouse brains are mechanistically linked to increased Aß [193,296]. Consistent with these observations, recent studies showed that chronic (48 h) mTOR attenuation protects against synaptic failure induced by Aß in cultured neurons by increasing the frequency of miniature postsynaptic currents through a presynaptic mechanism [195].

In the first *in vivo* mechanistic demonstration that mTOR was mechanistically involved in the pathogenesis of AD-like disease as modeled in mice, chronic treatment of AD mice with enterically-delivered rapamycin, at doses that increase lifespan by delaying aging [25,26, 28], blocked the progression of AD-like cognitive deficits and decreased brain histopathological hallmarks of the disease in two independent models of AD, hAPP(J20) [191] and 3xTg-AD mice [192]. This involved the activation of autophagy, at least in neurons and potentially in other parenchymal cell types. Late intervention with rapamycin at 15 months of age, after plaques and tangles appear in the 3xTg-AD model, was shown not to be effective in this model [297]. More recent studies however, demonstrated that in the hAPP(J20) model of AD, chronic systemic attenuation of mTOR with rapamycin can treat established AD-like cognitive deficits even when treatment is started *after* robust AD-like memory impairments occur [40]. The mechanisms of action involve restoration of cerebrovascular function and integrity through preservation of endothelium-dependent nitric oxide-mediated vasodilation [40]. Thus, mTOR attenuation in brain both activates autophagy in parenchyma and restores cerebrovascular integrity and function, suggesting that mTOR has critical roles in the regulation of autophagy in neurons and in the regulation of nitric oxide release in brain vasculature (Fig. 3). These compartments are functionally linked, because Aß is produced in neurons and a large proportion of neuronally-generated Aß is cleared through the vasculature [4,67,85, 111]. Thus, attenuation of mTOR in brain may establish a feedforward loop linking neurons and the cerebrovascular compartment, in which relief of mTOR-mediated inhibition of neuronal autophagy lowers the rate of Aß production. In turn, relief of mTOR-mediated inhibition of endothelium-dependent nitric oxide release

maintains cerebrovascular integrity and function, thus increasing the rate of Aß clearance from brain (Fig. 3). While the acute effects of mTOR attenuation on endothelium-dependent vasodilation are consistent with prior studies [31,33,34,39] and may be explained by the activation of eNOS and subsequent release of nitric oxide [40], it is unknown how acute nitric oxide-dependent vasodilation caused by mTOR attenuation leads to the long-term restoration of vascular density and the maintenance of CBF in AD mice.

Hyperphosphorylated forms of the microtubule-associated protein, tau, form neurofibrillary tangles, a histological marker of Alzheimer's disease. Neurofibrillary tangles are also histological hallmarks of other tauopathies such as frontotemporal dementia and Parkisonism linked to chromosome 17. Evidence suggests that tau is downstream of Aß in a pathway of toxicity associated with the pathogenesis of AD [298]. In agreement with this notion, recent studies showed that mTOR attenuation blocks hyperphosphorylated tau-induced neurodegeneration in the perforant pathway [299]. Furthermore, hyperphophorylated tau levels and its localization may be regulated by mTOR [300]. An important yet largely unexplored output of mTOR signaling is the mTOR-dependent phosphorylation of tau at S^{356}, S^{214} and T^{231301}, Thr^{231}, and potentially Ser^{214}/Thr^{212}. mTOR-dependent tau phosphorylation sites are critical for generation of abnormally hyperphosphorylated and misfolded tau [302], mechanistically linked to AD neurodegeneration [301]. Further, attenuating mTOR activity with rapamycin blocks tau phosphorylation and restores cognitive deficits in streptozotocin-induced diabetic mice [198]. Attenuation of mTOR with rapamycin also decreased tau levels and phosphorylation in hippocampus, restored hippocampal volume, reduced demyelination, and improved behavioral outcomes in a rat model of accelerated aging [303]. Fredrick *et al.* [304] and Caccamo *et al.* [305] recently showed that rapamycin or a rapamycin analog decreased brain phopho-tau, insoluble tau, and neurofibrillary tangles, and restored behavioral deficits in tau mutant mice. In light of this evidence, mTOR inhibitors are being actively pursued as therapies for tauopathies [306].

9. Vascular TOR-centered pathways may link the regulation of aging to the pathogenesis of Alzheimer's disease

Organismal aging does not arise from gradual processes of functional decline operating uniformly in every organ and physiological system, but comes from specific age-associated changes involving a finite number of critical systems [26]. There is a wealth of correlative data on age-associated physiological changes and the pathology of age-associated diseases. However, until recently, the rate of aging could not be experimentally manipulated other than by caloric restriction; thus, mechanistic studies to determine the causality of relationships between aging and the pathogenesis age-associated diseases were very difficult. With the discovery that specific TOR-centered molecular pathways control the rate of aging, we can now seek answers to the key question: Which molecules are at the interface between regulation of the rate of aging and the mechanisms of specific age-associated disease? Because aging contributes over 90% of the risk for Alzheimer's disease, the identification of the specific molecular mechanisms that link the regulation of brain aging to the pathogenesis of AD are imperative.

Because mTOR controls key metabolic functions in most cell types and pharmacologically inhibiting mTOR extends lifespan and healthspan [25–28], retarding multiple, but not all, aspects of aging in mice, mTOR may be involved in several different, specific processes of complex disease mechanisms mediating neurodegeneration. Based on the evidence reviewed above, TOR-dependent neurovascular dysfunction may be a critical event in the pathogenesis of AD-like disease as modeled in mice (Figs. 1–3). In addition to models of AD, chronic mTOR attenuation improves disease-specific outcomes in models of Huntington's [286], Parkinson's and Lewy body diseases [189,288], and cognitive outcomes in a rat model of accelerated aging [303]. In addition to inhibiting autophagy, mTOR may also be involved in aberrant activation of the cell cycle, which caused neurodegeneration in a fly model of tauopathy [291] and contributes to toxicity of Aß oligomers in models of AD [292].

mTOR attenuation activates autophagy in brain parenchyma, including neurons of mouse models of AD [191,192,307] (Fig. 3) and models of tauopathy [197–199,305]. The activation of autophagy in neurons is linked to delayed disease progression in these models. The involvement of autophagy may be limited to parenchymal cells, including but not restricted to neurons undergoing disease-specific proteostatic stress via accumulation of misfolded and aggregated proteins such as Aß and tau [191,192,197–199,305,307]. Evidence for activated macroautophagy, however, was also found in brain vascular endothelial cells in association with neuritic plaques [308]. Furthermore, mTOR attenuation blocks Aß-induced toxicity while increasing autophagy in cultured vascular endothelial cells [309]. Whether the activation of autophagy as a result of mTOR attenuation in brain vascular cells contributes to its beneficial effects, however, remains unknown.

We have only begun to approach the identification of specific molecules at the interface between the regulation of aging and the mechanisms of specific age-associated diseases ('age-pathogenic' mechanisms). Emerging evidence suggests that mTOR-driven inhibition of endothelium-dependent nitric oxide-mediated vasodilation may constitute a mechanistic link between aging and the pathogenesis of Alzheimer's disease and potentially a critical age-pathogenic mechanism in brain (Fig. 2).

10. Potential therapeutic approaches

A mechanistic understanding of age-pathogenic mechanisms as defined above will be crucial for interventions aimed at increasing healthspan, the period of life with good health and function. This aim was singled out as an important area of future endeavor by the trans-NIH Geroscience Interest Group Summit [310]. Although how TOR drives aging is still unknown, a wealth of prior data and our recent studies suggest that TOR-dependent brain vascular dysfunction may be critical event in the pathogenesis of AD-like deficits in mouse models, and a key age-pathogenic mechanism underlying the increased vulnerability of aged brains to specific neurological diseases.

Closing the gap in our understanding of the mechanisms that link brain vascular dysfunction to AD will lead to the development of pharmacological strategies to treat AD, including but not limited to drugs currently approved for clinical use such as rapamycin and rapalogs.

Importantly, the elucidation of mTOR-centered age-pathogenic mechanisms may have major implications for treatment of neurological diseases of aging beyond AD alone. Emerging evidence suggests that one of these critical age-pathogenic mechanisms may reside at the brain vascular endothelium and may be common to all age-associated neurological diseases of aging that share vascular dysfunction as a key mechanistic component. As these mechanisms are mapped, many other potential targets for pharmacological intervention will be uncovered. Once age-pathogenic mechanisms have been mapped and therapeutic strategies have been devised, there is the promise that AD and other dementias could be treated to retard or halt functional decline.

At present, because mTOR inhibitors are available for clinical use, translational studies of mTOR inhibition as a therapy for moderate-stage AD are possible in the very short term. It is also expected that therapies in which mTOR inhibition is used in ‘on-off’ schedules in combination with other drugs may be developed. Furthermore, it is becoming increasingly apparent that more than one drug will be needed to effectively treat AD. Immunization-based therapies to reduce Aß have shown detrimental vascular effects [311,312]. An important implication of the elucidation of mTOR-dependent pathways of vascular damage in AD is that this knowledge may lead to using mTOR inhibitors with drugs to reduce Aß while preserving cerebrovascular integrity and function. However, because of the multiple effects expected of systemic inhibition of mTOR, mechanisms of rapamycin-induced neuroprotection and vasculoprotection must be elucidated to enable the design of better strategies, such as the use of existing drugs, or development of new ones, that target key effectors of rapamycin-induced neuroprotection and/or vasculoprotection while avoiding potential undesirable side effects. As these studies are performed, rapamycin-based therapies to treat AD can be designed that take advantage of strategies such as intermittent administration, personalized dosage, and tailored frequency of treatment.

Acknowledgements

This work was funded by I01 BX002211–01A2 Veterans Administration Research and Development Merit Award, NIA 2 P30 AG013319–21 Nathan Shock Center of Excellence in the Biology of Aging, the Robert L. Bailey and Daughter Lisa K. Bailey Alzheimer’s Fund, the William & Ella Owens Medical Research Foundation and the JMR Barker Foundation, and the San Antonio Medical Foundation to V.G.; M.J.H acknowledges the support of Grant UL TR001120 from the National Center for Advancing Translational Sciences, NIH, and the Robert A. Welch Distinguished Chair Endowment in the Department of Biochemistry (AQ0039).

References

[1]. Selkoe DJ, Alzheimer’s disease is a synaptic failure, Science 298 (2002) 789–791. [PubMed: 12399581]

[2]. Tanzi RE, The genetics of Alzheimer disease, Cold Spring Harb. Perspect. Med 2 (2012) 10.1101/cshperspect.a006296.

[3]. Sonntag WE, Eckman DM, Ingraham J, Riddle DR, in: Riddle DR (Ed.), Brain Aging: Models, Methods, and Mechanisms Frontiers in Neuroscience, 2007.

[4]. Zlokovic BV, Neurovascular pathways to neurodegeneration in Alzheimer’s disease and other disorders, Nat. Rev. Neurosci 12 (2011) 723–738, 10.1038/nrn3114. [PubMed: 22048062]

[5]. Cantin S, et al., Impaired cerebral vasoreactivity to CO2 in Alzheimer’s disease using BOLD fMRI, NeuroImage 58 (2011) 579–587, 10.1016/j.neuroimage.2011.06.070. [PubMed: 21745581]

[6]. Iadecola C, Neurovascular regulation in the normal brain and in Alzheimer's disease, Nat. Rev. Neurosci 5 (2004) 347–360, 10.1038/nrn1387. [PubMed: 15100718]

[7]. Ruitenberg A, et al., Cerebral hypoperfusion and clinical onset of dementia: the rotterdam study, Ann. Neurol 57 (2005) 789–794, 10.1002/ana.20493. [PubMed: 15929050]

[8]. Marin J, Rodriguez-Martinez MA, Age-related changes in vascular responses, Exp. Gerontol 34 (1999) 503–512. [PubMed: 10817806]

[9]. Al-Shaer MH, et al., Effects of aging and atherosclerosis on endothelial and vascular smooth muscle function in humans, Int. J. Cardiol 109 (2006) 201–206, http://dx.doi.org/10.1016/j.ijcard . http://dx.doi.org/10.1016/j.ijcard2005.06.002. 2005.06.002 . [PubMed: 16054252]

[10]. Hatake K, Kakishita E, Wakabayashi I, Sakiyama N, Hishida S, Effect of aging on endothelium-dependent vascular relaxation of isolated human basilar artery to thrombin and bradykinin, Stroke 21 (1990) 1039–1043. [PubMed: 2114673]

[11]. Martin AJ, Friston KJ, Colebatch JG, Frackowiak RS, Decreases in regional cerebral blood flow with normal aging, J. Cereb. Blood Flow Metab 11 (1991) 684–689, [PubMed: 2050757]

[12]. Miners S, Moulding H, de Silva R, Love S, Reduced vascular endothelial growth factor and capillary density in the occipital cortex in dementia with lewy bodies, Brain Pathol 24 (2014) 334–343, 10.1111/bpa.12130. [PubMed: 24521289]

[13]. Kalaria RN, Vascular basis for brain degeneration: faltering controls and risk factors for dementia, Nutr. Rev 68 (Suppl. 2) (2010) S74–S87, 10.1111/j.1753-4887.2010.00352.x. [PubMed: 21091952]

[14]. Tolppanen AM, Solomon A, Soininen H, Kivipelto M, Midlife vascular risk factors and Alzheimer's disease: evidence from epidemiological studies, J. Alzheimers Dis 32 (2012) 531–540, [PubMed: 22842867]

[15]. Voisine C, Pedersen JS, Morimoto RI, Chaperone networks: tipping the balance in protein folding diseases, Neurobiol. Dis 40 (2010) 12–20, 10.1016/j.nbd.2010.05.007. [PubMed: 20472062]

[16]. Buxton RB, The physics of functional magnetic resonance imaging (fMRI), Rep. Prog. Phys. Phys. Soc 76 (2013) 096601, 10.1088/0034-4885/76/9/096601.

[17]. Dazert E, Hall MN, MTOR signaling in disease, Curr. Opin. Cell Biol 23 (2011) 744–755, http://dx.doi.org/10.1016/j.ceb.2011.09 . http://dx.doi.org/10.1016/j.ceb.2011.09003. 003 . [PubMed: 21963299]

[18]. Laplante M, Sabatini DM, MTOR signaling at a glance, J. Cell Sci 122 (2009) 3589–3594, 10.1242/jcs.051011. [PubMed: 19812304]

[19]. Stanfel MN, Shamieh LS, Kaeberlein M, Kennedy BK, The TOR pathway comes of age, Biochim. Biophys. Acta 1790 (2009) 1067–1074, 10.1016/j.bbagen.2009.06.007. [PubMed: 19539012]

[20]. Hansen M, et al., Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans, Aging Cell 6 (2007) 95–110, 10.1111/j.1474-9726.2006.00267.x. [PubMed: 17266679]

[21]. Honjoh S, Yamamoto T, Uno M, Nishida E, Signalling through RHEB-1 mediates intermittent fasting-induced longevity in C. elegans, Nature 457 (2009) 726–730, 10.1038/nature07583. [PubMed: 19079239]

[22]. Vellai T, et al., Genetics: influence of TOR kinase on lifespan in C. elegans, Nature 426 (2003) 620, 10.1038/426620a.

[23]. Katewa SD, Kapahi P, Role of TOR signaling in aging and related biological processes in Drosophila melanogaster, Exp. Gerontol 46 (2011) 382–390, 10.1016/j.exger.2010.11.036. [PubMed: 21130151]

[24]. Kaeberlein M, Lessons on longevity from budding yeast, Nature 464 (2010) 513–519, 10.1038/nature08981. [PubMed: 20336133]

[25]. Harrison DE, et al., Rapamycin fed late in life extends lifespan in genetically heterogeneous mice, Nature 460 (2009) 392–395, [PubMed: 19587680]

[26]. Wilkinson JE, et al., Rapamycin slows aging in mice, Aging Cell 11 (2012) 675–682, 10.1111/j.1474-9726.2012.00832.x. [PubMed: 22587563]

[27]. Miller RA, et al., Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice, J. Gerontol. A Biol. Sci. Med. Sci 66 (2011) 191–201, [PubMed: 20974732]

[28]. Miller RA, et al., Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction, Aging Cell 13 (2014) 468–477, [PubMed: 24341993]

[29]. Dunlop EA, Tee AR, MTOR and autophagy: a dynamic relationship governed by nutrients and energy, Semin. Cell Dev. Biol 36 (2014) 121–129, 10.1016/j.semcdb.2014.08.006. [PubMed: 25158238]

[30]. Bar-Peled L, Sabatini DM, Regulation of mTORC1 by amino acids, Trends Cell Biol 24 (2014) 400–406, 10.1016/j.tcb.2014.03.003. [PubMed: 24698685]

[31]. Corbin F, Blaise GA, Parent M, Chen H, Daloze PM, Effect of rapamycin on rat aortic ring vasomotion, J. Cardiovasc. Pharmacol 24 (1994) 813–817. [PubMed: 7532760]

[32]. Jabs A, et al., Sirolimus-induced vascular dysfunction. Increased mitochondrial and nicotinamide adenosine dinucleotide phosphate oxidase-dependent superoxide production and decreased vascular nitric oxide formation, J. Am. Coll. Cardiol 51 (2008) 2130–2138, 10.1016/j.jacc.2008.01.058. [PubMed: 18510959]

[33]. Milliard S, et al., Rapamycin's effect on vasomotion in the rat, Transplant. Proc 30 (1998) 1036–1038. [PubMed: 9636418]

[34]. Parlar A, Can C, Erol A, Ulker S, Posttransplantation therapeutic rapamycin concentration protects nitric oxide-related vascular endothelial function: comparative effects in rat thoracic aorta and coronary endothelial cell culture, Transplant. Proc 42 (2010) 1923–1930, 10.1016/j.transproceed.2010.03.134. [PubMed: 20620549]

[35]. Rajapakse AG, et al., Hyperactive S6K1 mediates oxidative stress and endothelial dysfunction in aging: inhibition by resveratrol, PLoS One 6 (2011), e19237, 10.1371/journal.pone.0019237. [PubMed: 21544240]

[36]. Wang CY, et al., Obesity increases vascular senescence and susceptibility to ischemic injury through chronic activation of Akt and mTOR, Sci. Signal 2 (2009) ra11, 10.1126/scisignal.2000143.

[37]. Yepuri G, et al., Positive crosstalk between arginase-II and S6K1 in vascular endothelial inflammation and aging, Aging Cell 11 (2012) 1005–1016, 10.1111/acel.12001. [PubMed: 22928666]

[38]. Ramzy D, et al., Role of endothelin-1 and nitric oxide bioavailability in transplant-related vascular injury: comparative effects of rapamycin and cyclosporine, Circulation 114 (2006) I214–I219, 10.1161/CIRCULATIONAHA.105.000471. [PubMed: 16820575]

[39]. Cheng C, et al., Rapamycin modulates the eNOS vs. shear stress relationship, Cardiovasc. Res 78 (2008) 123–129, 10.1093/cvr/cvm103. [PubMed: 18079107]

[40]. Lin AL, et al., Chronic rapamycin restores brain vascular integrity and function through NO synthase activation and improves memory in symptomatic mice modeling Alzheimer's disease, J. Cereb. Blood Flow Metab 33 (2013) 1412–1421, 10.1038/jcbfm.2013.82. [PubMed: 23801246]

[41]. Ho A, Sudhof TC, Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 2548–2553. [PubMed: 14983046]

[42]. Rice HC, Young-Pearse TL, Selkoe DJ, Systematic evaluation of candidate ligands regulating ectodomain shedding of amyloid precursor protein, Biochemistry 52 (2013) 3264–3277, 10.1021/bi400165f. [PubMed: 23597280]

[43]. Young-Pearse TL, Chen AC, Chang R, Marquez C, Selkoe DJ, Secreted APP regulates the function of full-length APP in neurite outgrowth through interaction with integrin beta1, Neural Dev 3 (2008) 15, 10.1186/1749-8104-3-15. [PubMed: 18573216]

[44]. Bai Y, et al., The in vivo brain interactome of the amyloid precursor protein, Mol. Cell. Proteomics 7 (2008) 15–34, 10.1074/mcp.M700077-MCP200. [PubMed: 17934213]

[45]. Nhan HS, Chiang K, Koo EH, The multifaceted nature of amyloid precursor protein and its proteolytic fragments: friends and foes, Acta Neuropathol 129 (2015) 1–19, 10.1007/s00401-014-1347-2. [PubMed: 25287911]

[46]. Chow VW, Mattson MP, Wong PC, Gleichmann M, An overview of APP processing enzymes and products, Neruomol. Med 12 (2010) 1–12, 10.1007/s12017-009-8104-z.

[47]. De Strooper B, Vassar R, Golde T, The secretases: enzymes with therapeutic potential in Alzheimer disease, Nat. Rev. Neurol 6 (2010) 99–107, 10.1038/nrneurol.2009.218. [PubMed: 20139999]

[48]. Ahmed M, et al., Structural conversion of neurotoxic amyloid-beta(1–42) oligomers to fibrils, Nat. Struct. Mol. Biol 17 (2010) 561–567, 10.1038/nsmb.1799. [PubMed: 20383142]

[49]. Kirschner DA, Abraham C, Selkoe DJ, X-ray diffraction from intraneuronal paired helical filaments and extraneuronal amyloid fibers in Alzheimer disease indicates cross-beta conformation, Proc. Natl. Acad. Sci. U. S. A 83 (1986) 503–507. [PubMed: 3455785]

[50]. Luhrs T, et al., 3D structure of alzheimer's amyloid-beta(1–42) fibrils, Proc. Natl. Acad. Sci. U. S. A 102 (2005) 17342–17347, 10.1073/pnas.0506723102. [PubMed: 16293696]

[51]. Crews L, Masliah E, Molecular mechanisms of neurodegeneration in Alzheimer's disease, Hum. Mol. Genet 19 (2010) R12–R20, 10.1093/hmg/ddq160. [PubMed: 20413653]

[52]. Ghiso J, Fossati S, Rostagno A, Amyloidosis associated with cerebral amyloid angiopathy: cell signaling pathways elicited in cerebral endothelial cells, J. Alzheimers Dis 42 (Suppl. 3) (2014) S167–S176, 10.3233/JAD-140027. [PubMed: 24670400]

[53]. Revesz T, et al., Sporadic and familial cerebral amyloid angiopathies, Brain Pathol 12 (2002) 343–357. [PubMed: 12146803]

[54]. Mucke L, Selkoe DJ, Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction, Cold Spring Harb. Perspect. Med 2 (2012), a00633810.1101/cshperspect.a006338. [PubMed: 22762015]

[55]. Chapman PF, et al., Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice, Nat. Neurosci 2 (1999) 271–276, 10.1038/6374. [PubMed: 10195221]

[56]. Walsh DM, et al., Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo, Nature 416 (2002) 535–539, 10.1038/416535a. [PubMed: 11932745]

[57]. Hsieh H, et al., AMPAR removal underlies abeta-induced synaptic depression and dendritic spine loss, Neuron 52 (2006) 831–843, [PubMed: 17145504]

[58]. Kamenetz F, et al., APP processing and synaptic function, Neuron 37 (2003) 925–937. [PubMed: 12670422]

[59]. Abramov E, et al., Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses, Nat. Neurosci 12 (2009) 1567–1576, 10.1038/nn.2433. [PubMed: 19935655]

[60]. Dineley KT, Bell KA, Bui D, Sweatt JD, Beta -amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in xenopus oocytes, J. Biol. Chem 277 (2002) 25056–25061, 10.1074/jbc.M200066200. [PubMed: 11983690]

[61]. Hernandez CM, Kayed R, Zheng H, Sweatt JD, Dineley KT, Loss of alpha7 nicotinic receptors enhances beta-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septohippocampal pathology in a mouse model of Alzheimer's disease, J. Neurosci 30 (2010) 2442–2453, 10.1523/JNEUROSCI.5038-09.2010. [PubMed: 20164328]

[62]. Dineley KT, et al., Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: in vitro and in vivo mechanisms related to Alzheimer's disease, J. Neurosci 21 (2001) 4125–4133. [PubMed: 11404397]

[63]. Nicholls RE, et al., Transgenic mice lacking NMDAR-dependent LTD exhibit deficits in behavioral flexibility, Neuron 58 (2008) 104–117, 10.1016/j.neuron.2008.01.039. [PubMed: 18400167]

[64]. Malinow R, New developments on the role of NMDA receptors in Alzheimer's disease, Curr. Opin. Neurobiol 22 (2012) 559–563, 10.1016/j.conb.2011.09.001. [PubMed: 21962484]

[65]. Shankar GM, et al., Amyloid-beta protein dimers isolated directly from alzheimer's brains impair synaptic plasticity and memory, Nat. Med 14 (2008) 837–842, 10.1038/nm1782. [PubMed: 18568035]

[66]. Cirrito JR, et al., Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo, Neuron 58 (2008) 42–51, [PubMed: 18400162]

[67]. Zlokovic BV, The blood–brain barrier in health and chronic neurodegenerative disorders, Neuron 57 (2008) 178–201, 10.1016/j.neuron.2008.01.003. [PubMed: 18215617]

[68]. Daneman R, Zhou L, Kebede AA, Barres BA, Pericytes are required for blood– brain barrier integrity during embryogenesis, Nature 468 (2010) 562–566, 10.1038/nature09513. [PubMed: 20944625]

[69]. Helms HC, Madelung R, Waagepetersen HS, Nielsen CU, Brodin B, In vitro evidence for the brain glutamate efflux hypothesis: brain endothelial cells cocultured with astrocytes display a polarized brain-to-blood transport of glutamate, Glia 60 (2012) 882–893, 10.1002/glia.22321. [PubMed: 22392649]

[70]. Takahashi H, et al., Brain pericyte-derived soluble factors enhance insulin sensitivity in GT1–7 hypothalamic neurons, Biochem. Biophys. Res. Commun 457 (2015) 532–537, 10.1016/j.bbrc.2015.01.016. [PubMed: 25597994]

[71]. Korn J, Christ B, Kurz H, Neuroectodermal origin of brain pericytes and vascular smooth muscle cells, J. Comp. Neurol 442 (2002) 78–88. [PubMed: 11754368]

[72]. Bandopadhyay R, et al., Contractile proteins in pericytes at the blood–brain and blood-retinal barriers, J. Neurocytol 30 (2001) 35–44. [PubMed: 11577244]

[73]. Hill RA, et al., Regional blood flow in the normal and ischemic brain is controlled by arteriolar smooth muscle cell contractility and not by capillary pericytes, Neuron 87 (2015) 95–110, 10.1016/j.neuron.2015.06.001. [PubMed: 26119027]

[74]. Hubbard JA, Hsu MS, Seldin MM, Binder DK, Expression of the astrocyte water channel aquaporin-4 in the mouse brain, ASN Neuro 7 (2015) 10.1177/1759091415605486.

[75]. Petzold GC, Murthy VN, Role of astrocytes in neurovascular coupling, Neuron 71 (2011) 782–797, 10.1016/j.neuron.2011.08.009. [PubMed: 21903073]

[76]. Streit WJ, Graeber MB, Heterogeneity of microglial and perivascular cell populations: insights gained from the facial nucleus paradigm, Glia 7 (1993) 68–74, http://dx.doi.org/10.1002/glia . http://dx.doi.org/10.1002/glia440070112. 440070112 . [PubMed: 8423064]

[77]. Wilcock DM, et al., Intracranially administered anti-abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation, J. Neurosci 23 (2003) 3745–3751. [PubMed: 12736345]

[78]. Wang Y, et al., TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model, Cell 160 (2015) 1061–1071, 10.1016/j.cell.2015.01.049. [PubMed: 25728668]

[79]. Wyss-Coray T, et al., Adult mouse astrocytes degrade amyloid-beta in vitro and in situ, Nat. Med 9 (2003) 453–457, 10.1038/nm838. [PubMed: 12612547]

[80]. Koistinaho M, et al., Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides, Nat. Med 10 (2004) 719–726, 10.1038/nm1058. [PubMed: 15195085]

[81]. Saido T, Leissring MA, Proteolytic degradation of amyloid beta-protein, Cold Spring Harb. Perspect. Med 2 (2012), a006379, 10.1101/cshperspect.a006379. [PubMed: 22675659]

[82]. Yin KJ, et al., Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism, J. Neurosci 26 (2006) 10939–10948, 10.1523/JNEUROSCI.2085-06.2006. [PubMed: 17065436]

[83]. Hernandez-Guillamon M, et al., Sequential amyloid-beta degradation by the matrix metalloproteases MMP-2 and MMP-9, J. Biol. Chem 290 (2015) 15078–15091, 10.1074/jbc.M114.610931. [PubMed: 25897080]

[84]. Iliff JJ, et al., A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta, Sci. Transl. Med 4 (2012) 147ra111, 10.1126/scitranslmed.3003748.

[85]. Mackic JB, et al., Human blood–brain barrier receptors for alzheimer's amyloid-beta 1–40. Asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer, J. Clin. Invest 102 (1998) 734–743, 10.1172/JCI2029. [PubMed: 9710442]

[86]. Mawuenyega KG, et al., Decreased clearance of CNS beta-amyloid in Alzheimer's disease, Science 330 (2010) 1774, 10.1126/science.1197623. [PubMed: 21148344]

[87]. Patterson BW, et al., Age and amyloid effects on human central nervous system amyloid-beta kinetics, Ann. Neurol (2015)10.1002/ana.24454.

[88]. Sagare AP, Winkler EA, Bell RD, Deane R, Zlokovic BV, From the liver to the blood–brain barrier: an interconnected system regulating brain amyloid-beta levels, J. Neurosci. Res 89 (2011) 967–968, 10.1002/jnr.22670. [PubMed: 21544850]

[89]. Kanekiyo T, Bu G, The low-density lipoprotein receptor-related protein 1 and amyloid-beta clearance in Alzheimer's disease, Front. Aging Neurosci 6 (2014) 93, [PubMed: 24904407]

[90]. Strickland DK, et al., Sequence identity between the alpha 2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor, J. Biol. Chem 265 (1990) 17401–17404. [PubMed: 1698775]

[91]. Qiu Z, Strickland DK, Hyman BT, Rebeck GW, Alpha2-macroglobulin enhances the clearance of endogenous soluble beta-amyloid peptide via low-density lipoprotein receptor-related protein in cortical neurons, J. Neurochem 73 (1999) 1393–1398. [PubMed: 10501182]

[92]. Li J, et al., Differential regulation of amyloid-beta endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms, J. Biol. Chem 287 (2012) 44593–44601, 10.1074/jbc.M112.420224. [PubMed: 23132858]

[93]. Koo EH, Squazzo SL, Evidence that production and release of amyloid beta-protein involves the endocytic pathway, J. Biol. Chem 269 (1994) 17386–17389. [PubMed: 8021238]

[94]. Perez RG, Squazzo SL, Koo EH, Enhanced release of amyloid beta-protein from codon 670/671 "Swedish" mutant beta-amyloid precursor protein occurs in both secretory and endocytic pathways, J. Biol. Chem 271 (1996) 9100–9107. [PubMed: 8621560]

[95]. Soriano S, et al., Expression of beta-amyloid precursor protein-CD3gamma chimeras to demonstrate the selective generation of amyloid beta(1–40) and amyloid beta(1–42) peptides within secretory and endocytic compartments, J. Biol. Chem 274 (1999) 32295–32300. [PubMed: 10542269]

[96]. Narita M, Holtzman DM, Schwartz AL, Bu G, Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein, J. Neurochem 69 (1997) 1904–1911. [PubMed: 9349534]

[97]. Kanekiyo T, et al., Neuronal clearance of amyloid-beta by endocytic receptor LRP1, J. Neurosci 33 (2013) 19276–19283, 10.1523/JNEUROSCI.3487-13.2013. [PubMed: 24305823]

[98]. Yang DS, et al., Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits, Brain 134 (2011) 258–277, 10.1093/brain/awq341. [PubMed: 21186265]

[99]. Yang DS, et al., Therapeutic effects of remediating autophagy failure in a mouse model of Alzheimer disease by enhancing lysosomal proteolysis, Autophagy 7 (2011) 788–789. [PubMed: 21464620]

[100]. Nixon RA, Autophagy, amyloidogenesis and Alzheimer disease, J. Cell Sci 120 (2007) 4081–4091, 10.1242/jcs.019265. [PubMed: 18032783]

[101]. Nixon RA, The role of autophagy in neurodegenerative disease, Nat. Med 19 (2013) 983–997, 10.1038/nm.3232. [PubMed: 23921753]

[102]. Nilsson P, et al., Abeta secretion and plaque formation depend on autophagy, Cell Rep 5 (2013) 61–69, http://dx.doi. org/10.1016/j.celrep.2013.08.042. [PubMed: 24095740]

[103]. Wirths O, Bayer TA, Intraneuronal abeta accumulation and neurodegeneration: lessons from transgenic models, Life Sci 91 (2012) 1148–1152, 10.1016/j.lfs.2012.02.001. [PubMed: 22401905]

[104]. Nixon RA, Alzheimer neurodegeneration, autophagy, and abeta secretion: the ins and outs (comment on DOI 10.1002/bies.201400002), BioEssays 36 (2014) 547, http://dx.doi.org/10.1002/bies . http://dx.doi.org/10.1002/bies201400064. 201400064 . [PubMed: 24819351]

[105]. Shibata M, et al., Clearance of alzheimer's amyloid-ss(1–40) peptide from brain by LDL receptor-related protein-1 at the blood–brain barrier, J. Clin. Invest 106 (2000) 1489–1499, [PubMed: 11120756]

[106]. Jaeger LB, et al., Testing the neurovascular hypothesis of Alzheimer's disease: LRP-1 antisense reduces blood–brain barrier clearance, increases brain levels of amyloid-beta protein, and impairs cognition, J. Alzheimers Dis 17 (2009) 553–570, [PubMed: 19433890]

[107]. Deane R, Bell RD, Sagare A, Zlokovic BV, Clearance of amyloid-beta peptide across the blood–brain barrier: implication for therapies in Alzheimer's disease, CNS Neurol. Disord. Drug Targets 8 (2009) 16–30. [PubMed: 19275634]

[108]. Bell RD, et al., Transport pathways for clearance of human alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system, J. Cereb. Blood Flow Metab 27 (2007) 909–918, 10.1038/sj.jcbfm.9600419. [PubMed: 17077814]

[109]. Deane R, et al., LRP/amyloid beta-peptide interaction mediates differential brain efflux of abeta isoforms, Neuron 43 (2004) 333–344, 10.1016/j.neuron.2004.07.017. [PubMed: 15294142]

[110]. Zlokovic BV, Deane R, Sagare AP, Bell RD, Winkler EA, Low-density lipoprotein receptor-related protein-1: a serial clearance homeostatic mechanism controlling alzheimer's amyloid beta-peptide elimination from the brain, J. Neurochem 115 (2010) 1077–1089, 10.1111/j.1471-4159.2010.07002.x. [PubMed: 20854368]

[111]. Tanzi RE, Moir RD, Wagner SL, Clearance of alzheimer's abeta peptide: the many roads to perdition, Neuron 43 (2004) 605–608, 10.1016/j.neuron.2004.08.024. [PubMed: 15339642]

[112]. Ramanathan A, Nelson AR, Sagare AP, Zlokovic BV, Impaired vascular-mediated clearance of brain amyloid beta in Alzheimer's disease: the role, regulation and restoration of LRP1, Front. Aging Neurosci 7 (2015) 136, 10.3389/fnagi.2015.00136. [PubMed: 26236233]

[113]. Tebar F, Bohlander SK, Sorkin A, Clathrin assembly lymphoid myeloid leukemia (CALM) protein: localization in endocytic-coated pits, interactions with clathrin, and the impact of overexpression on clathrin-mediated traffic, Mol. Biol. Cell 10 (1999) 2687–2702. [PubMed: 10436022]

[114]. Harold D, et al., Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease, Nat. Genet 41 (2009) 1088–1093, 10.1038/ng.440. [PubMed: 19734902]

[115]. Lambert JC, et al., Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease, Nat. Genet 45 (2013) 1452–1458, 10.1038/ng.2802. [PubMed: 24162737]

[116]. Zhao Z, et al., Central role for PICALM in amyloid-beta blood–brain barrier transcytosis and clearance, Nat. Neurosci 18 (2015) 978–987, 10.1038/nn.4025. [PubMed: 26005850]

[117]. Kanekiyo T, Liu CC, Shinohara M, Li J, Bu G, LRP1 in brain vascular smooth muscle cells mediates local clearance of alzheimer's amyloid-beta, J. Neurosci 32 (2012) 16458–16465, http://dx.doi.org/10.1523/JNEUROSCI.3987-12 . http://dx.doi.org/10.1523/JNEUROSCI.3987-122012.2012 . [PubMed: 23152628]

[118]. Urmoneit B, et al., Cerebrovascular smooth muscle cells internalize alzheimer amyloid beta protein via a lipoprotein pathway: implications for cerebral amyloid angiopathy, Lab. Investig 77 (1997) 157–166. [PubMed: 9274858]

[119]. Bell RD, et al., SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells, Nat. Cell Biol 11 (2009) 143–153, 10.1038/ncb1819. [PubMed: 19098903]

[120]. Chow N, et al., Serum response factor and myocardin mediate arterial hypercontractility and cerebral blood flow dysregulation in alzheimer's phenotype, Proc. Natl. Acad. Sci. U. S. A 104 (2007) 823–828, 10.1073/pnas.0608251104. [PubMed: 17215356]

[121]. Wilcock DM, et al., Microglial activation facilitates abeta plaque removal following intracranial anti-abeta antibody administration, Neurobiol. Dis 15 (2004) 11–20. [PubMed: 14751766]

[122]. Griciuc A, et al., Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta, Neuron 78 (2013) 631–643, 10.1016/j.neuron.2013.04.014. [PubMed: 23623698]

[123]. Xiao Q, et al., Enhancing astrocytic lysosome biogenesis facilitates abeta clearance and attenuates amyloid plaque pathogenesis, J. Neurosci 34 (2014) 9607–9620, 10.1523/JNEUROSCI.3788-13.2014. [PubMed: 25031402]

[124]. Basak JM, Verghese PB, Yoon H, Kim J, Holtzman DM, Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of abeta uptake and degradation by astrocytes, J. Biol. Chem 287 (2012) 13959–13971, 10.1074/jbc.M111.288746. [PubMed: 22383525]

[125]. Sagare AP, et al., Pericyte loss influences alzheimer-like neurodegeneration in mice, Nat. Commun 4 (2013) 2932, 10.1038/ncomms3932. [PubMed: 24336108]

[126]. Candela P, et al., In vitro discrimination of the role of LRP1 at the BBB cellular level: focus on brain capillary endothelial cells and brain pericytes, Brain Res 1594 (2015) 15–26, 10.1016/j.brainres.2014.10.047. [PubMed: 25451130]

[127]. Tucsek Z, et al., Aging exacerbates obesity-induced cerebromicrovascular rarefaction, neurovascular uncoupling, and cognitive decline in mice, J. Gerontol. A Biol. Sci. Med. Sci (2014) 10.1093/gerona/glu080.

[128]. Montagne A, et al., Blood–brain barrier breakdown in the aging human hippocampus, Neuron 85 (2015) 296–302, 10.1016/j.neuron.2014.12.032. [PubMed: 25611508]

[129]. Halliday MR, et al., Accelerated pericyte degeneration and blood–brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease, J. Cereb. Blood Flow Metab (2015)10.1038/jcbfm.2015.44.

[130]. Deane R, et al., RAGE mediates amyloid-beta peptide transport across the blood– brain barrier and accumulation in brain, Nat. Med 9 (2003) 907–913, 10.1038/nm890. [PubMed: 12808450]

[131]. El Assar M, et al., Mechanisms involved in the aging-induced vascular dysfunction, Front. Physiol 3 (2012) 132, 10.3389/fphys.2012.00132. [PubMed: 22783194]

[132]. Kotsis V, Stabouli S, Karafillis I, Nilsson P, Early vascular aging and the role of central blood pressure, J. Hypertens 29 (2011) 1847–1853, 10.1097/HJH.0b013e32834a4d9f. [PubMed: 21799443]

[133]. Donato AJ, Morgan RG, Walker AE, Lesniewski LA, Cellular and molecular biology of aging endothelial cells, J. Mol. Cell. Cardiol (2015)10.1016/j.yjmcc.2015.01.021.

[134]. Toda N, Age-related changes in endothelial function and blood flow regulation, Pharmacol. Ther 133 (2012) 159–176, 10.1016/j.pharmthera.2011.10.004. [PubMed: 22079549]

[135]. Csiszar A, et al., Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function, Circ. Res 90 (2002) 1159–1166. [PubMed: 12065318]

[136]. van Bussel BC, et al., Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period, Hypertension 58 (2011) 588–595, 10.1161/HYPERTENSIONAHA.111.174557. [PubMed: 21859964]

[137]. Csiszar A, Ungvari Z, Koller A, Edwards JG, Kaley G, Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries, FASEB J 17 (2003) 1183–1185, http://dx.doi.org/10.1096/fj . http://dx.doi.org/10.1096/fj02-1049fje. 02-1049fje . [PubMed: 12709402]

[138]. Fontana L, Partridge L, Promoting health and longevity through diet: from model organisms to humans, Cell 161 (2015) 106–118, 10.1016/j.cell.2015.02.020. [PubMed: 25815989]

[139]. Csiszar A, et al., Caloric restriction confers persistent anti-oxidative, proangiogenic, and anti-inflammatory effects and promotes anti-aging miRNA expression profile in cerebromicrovascular endothelial cells of aged rats, Am. J. Physiol. Heart Circ. Physiol 307 (2014) H292–H306, 10.1152/ajpheart.00307.2014. [PubMed: 24906921]

[140]. Luo S, Lei H, Qin H, Xia Y, Molecular mechanisms of endothelial NO synthase uncoupling, Curr. Pharm. Des 20 (2014) 3548–3553. [PubMed: 24180388]

[141]. Rodriguez-Manas L, et al., Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation, Aging Cell 8 (2009) 226–238, 10.1111/j.1474-9726.2009.00466.x. [PubMed: 19245678]

[142]. Johnson SC, Rabinovitch PS, Kaeberlein M, MTOR is a key modulator of ageing and age-related disease, Nature 493 (2013) 338–345, 10.1038/nature11861. [PubMed: 23325216]

[143]. Yang H, et al., MTOR kinase structure, mechanism and regulation, Nature 497 (2013) 217–223, 10.1038/nature12122. [PubMed: 23636326]

[144]. Sarbassov DD, et al., Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB, Mol. Cell 22 (2006) 159–168, 10.1016/j.molcel.2006.03.029. [PubMed: 16603397]

[145]. Liu Y, et al., Rapamycin-induced metabolic defects are reversible in both lean and obese mice, Aging 6 (2014) 742–754. [PubMed: 25324470]

[146]. Duvel K, et al., Activation of a metabolic gene regulatory network downstream of mTOR complex 1, Mol. Cell 39 (2010) 171–183, 10.1016/j.molcel.2010.06.022. [PubMed: 20670887]

[147]. Ricoult SJ, Manning BD, The multifaceted role of mTORC1 in the control of lipid metabolism, EMBO Rep 14 (2013) 242–251, 10.1038/embor.2013.5. [PubMed: 23399656]

[148]. Li S, Oh YT, Yue P, Khuri FR, Sun SY, Inhibition of mTOR complex 2 induces GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1) and suppresses lipogenesis in cancer cells, Oncogene (2015)10.1038/onc.2015.123.

[149]. Llorente-Cortes V, Costales P, Bernues J, Camino-Lopez S, Badimon L, Sterol regulatory element-binding protein-2 negatively regulates low density lipoprotein receptor-related protein transcription, J. Mol. Biol 359 (2006) 950–960, 10.1016/j.jmb.2006.04.008. [PubMed: 16697011]

[150]. Perluigi M, Di Domenico F, Butterfield DA, MTOR signaling in aging and neurodegeneration: at the crossroad between metabolism dysfunction and impairment of autophagy, Neurobiol. Dis (2015)10.1016/j.nbd.2015.03.014.

[151]. Jung CH, et al., ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery, Mol. Biol. Cell 20 (2009) 1992–2003, 10.1091/mbc.E08-12-1249. [PubMed: 19225151]

[152]. Roczniak-Ferguson A, et al., The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis, Sci. Signal 5 (2012) ra42, 10.1126/scisignal.2002790.

[153]. Alexander A, et al., ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS, Proc. Natl. Acad. Sci. U. S. A 107 (2010) 4153–4158, 10.1073/pnas.0913860107. [PubMed: 20160076]

[154]. Hudson CC, et al., Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin, Mol. Cell. Biol 22 (2002) 7004–7014. [PubMed: 12242281]

[155]. Brocato J, Chervona Y, Costa M, Molecular responses to hypoxia-inducible factor 1alpha and beyond, Mol. Pharmacol 85 (2014) 651–657, 10.1124/mol.113.089623. [PubMed: 24569087]

[156]. Shaw RJ, Cantley LC, Ras PI(3)K and mTOR signalling controls tumour cell growth, Nature 441 (2006) 424–430, 10.1038/nature04869. [PubMed: 16724053]

[157]. Haruta T, et al., A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1, Mol. Endocrinol 14 (2000) 783–794, 10.1210/mend.14.6.0446. [PubMed: 10847581]

[158]. Shah OJ, Wang Z, Hunter T, Inappropriate activation of the TSC/rheb/mTOR/ S6K cassette induces IRS½ depletion, insulin resistance, and cell survival deficiencies, Curr. Biol 14 (2004) 1650–1656, 10.1016/j.cub.2004.08.026. [PubMed: 15380067]

[159]. Hresko RC, Mueckler M, MTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3 T3-L1 adipocytes, J. Biol. Chem 280 (2005) 40406–40416, 10.1074/jbc.M508361200. [PubMed: 16221682]

[160]. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (2005) 1098–1101, 10.1126/science.1106148. [PubMed: 15718470]

[161]. Sessa WC, ENOS at a glance, J. Cell Sci 117 (2004) 2427–2429, 10.1242/jcs.01165. [PubMed: 15159447]

[162]. Fulton D, et al., Regulation of endothelium-derived nitric oxide production by the protein kinase Akt, Nature 399 (1999) 597–601, 10.1038/21218. [PubMed: 10376602]

[163]. Wu YT, et al., MTOR complex 2 targets Akt for proteasomal degradation via phosphorylation at the hydrophobic motif, J. Biol. Chem 286 (2011) 14190–14198, http://dx.doi.org/10.1074/jbc . http://dx.doi.org/10.1074/jbcM111.219923. M111.219923 . [PubMed: 21321111]

[164]. Jia K, Chen D, Riddle DL, The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span, Development 131 (2004) 3897–3906, 10.1242/dev.01255. [PubMed: 15253933]

[165]. Kapahi P, et al., Regulation of lifespan in drosophila by modulation of genes in the TOR signaling pathway, Curr. Biol 14 (2004) 885–890, 10.1016/j.cub.2004.03.059. [PubMed: 15186745]

[166]. Kaeberlein M, et al., Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients, Science 310 (2005) 1193–1196, 10.1126/science.1115535. [PubMed: 16293764]

[167]. Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S, Extension of chronological life span in yeast by decreased TOR pathway signaling, Genes Dev 20 (2006) 174–184, 10.1101/gad.1381406. [PubMed: 16418483]

[168]. Robida-Stubbs S, et al., TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO, Cell Metab 15 (2012) 713–724, 10.1016/j.cmet.2012.04.007. [PubMed: 22560223]

[169]. Fontana L, Partridge L, Longo VD, Extending healthy life span–from yeast to humans, Science 328 (2010) 321–326, 10.1126/science.1172539. [PubMed: 20395504]

[170]. Mattson MP, Dietary factors, hormesis and health, Ageing Res. Rev 7 (2008) 43–48, 10.1016/j.arr.2007.08.004. [PubMed: 17913594]

[171]. Kenyon CJ, The genetics of ageing, Nature 464 (2010) 504–512, 10.1038/nature08980. [PubMed: 20336132]

[172]. Warner HR, NIA's intervention testing program at 10 years of age, Age 37 (2015) 22, 10.1007/s11357-015-9761-5. [PubMed: 25726185]

[173]. Richardson A, Galvan V, Lin AL, Oddo S, How longevity research can lead to therapies for Alzheimer's disease: the rapamycin story, Exp. Gerontol 68 (2015) 51–58, 10.1016/j.exger.2014.12.002. [PubMed: 25481271]

[174]. Selman C, et al., Ribosomal protein S6 kinase 1 signaling regulates mammalian life span, Science 326 (2009) 140–144, 10.1126/science.1177221. [PubMed: 19797661]

[175]. Linford NJ, et al., Transcriptional response to aging and caloric restriction in heart and adipose tissue, Aging Cell 6 (2007) 673–688, 10.1111/j.1474-9726.2007.00319.x. [PubMed: 17874999]

[176]. Rice KM, Kinnard RS, Wright GL, Blough ER, Aging alters vascular mechanotransduction: pressure-induced regulation of p70S6k in the rat aorta, Mech. Ageing Dev 126 (2005) 1213–1222, http://dx.doi.org/10.1016/j . http://dx.doi.org/10.1016/jmad.2005.07.001. mad.2005.07.001 . [PubMed: 16087221]

[177]. Houtkooper RH, et al., The metabolic footprint of aging in mice, Sci. Rep 1 (2011) 134, 10.1038/srep00134. [PubMed: 22355651]

[178]. Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM, mTORC1 controls fasting-induced ketogenesis and its modulation by ageing, Nature 468 (2010) 1100–1104, 10.1038/nature09584. [PubMed: 21179166]

[179]. Yang SB, et al., Rapamycin ameliorates age-dependent obesity associated with increased mTOR signaling in hypothalamic POMC neurons, Neuron 75 (2012) 425–436, 10.1016/j.neuron.2012.03.043. [PubMed: 22884327]

[180]. Baar EL, Carbajal KA, Ong IM, Lamming DW, Sex- and tissue-specific changes in mTOR signaling with age in C57BL/6 J mice, Aging Cell (2015) (in press).

[181]. Sharp ZD, Aging and TOR: interwoven in the fabric of life, Cell. Mol. Life Sci 68 (2011) 587–597, 10.1007/s00018-010-0542-0. [PubMed: 20960025]

[182]. Blagosklonny MV, Revisiting the antagonistic pleiotropy theory of aging: TOR-driven program and quasi-program, Cell Cycle 9 (2010) 3151–3156, 10.4161/cc.9.16.13120. [PubMed: 20724817]

[183]. Kapahi P, Protein synthesis and the antagonistic pleiotropy hypothesis of aging, Adv. Exp. Med. Biol 694 (2010) 30–37. [PubMed: 20886754]

[184]. Ingram DK, Roth GS, Calorie restriction mimetics: can you have your cake and eat it, too? Ageing Res. Rev 20 (2015) 46–62, 10.1016/j.arr.2014.11.005. [PubMed: 25530568]

[185]. Colman RJ, et al., Caloric restriction delays disease onset and mortality in rhesus monkeys, Science 325 (2009) 201–204, 10.1126/science.1173635. [PubMed: 19590001]

[186]. Colman RJ, et al., Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys, Nat. Commun 5 (2014) 3557, 10.1038/ncomms4557. [PubMed: 24691430]

[187]. Malagelada C, Jin ZH, Jackson-Lewis V, Przedborski S, Greene LA, Rapamycin protects against neuron death in in vitro and in vivo models of Parkinson's disease, J. Neurosci 30 (2010) 1166–1175, 10.1523/JNEUROSCI.3944-09.2010. [PubMed: 20089925]

[188]. Crews L, et al., Selective molecular alterations in the autophagy pathway in patients with lewy body disease and in models of alpha-synucleinopathy, PLoS One 5 (2010), e9313, 10.1371/journal.pone.0009313. [PubMed: 20174468]

[189]. Spencer B, et al., Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of parkinson's and lewy body diseases, J. Neurosci 29 (2009) 13578–13588, 10.1523/JNEUROSCI.4390-09.2009. [PubMed: 19864570]

[190]. Tain LS, et al., Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss, Nat. Neurosci 12 (2009) 1129–1135, 10.1038/nn.2372. [PubMed: 19684592]

[191]. Spilman P, et al., Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease, PLoS One 5 (2010), e997910.1371/journal.pone.0009979. [PubMed: 20376313]

[192]. Caccamo A, Majumder S, Richardson A, Strong R, Oddo S, Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and tau: effects on cognitive impairments, J. Biol. Chem 285 (2010) 13107–13120, 10.1074/jbc.M110.100420. [PubMed: 20178983]

[193]. Caccamo A, et al., Naturally secreted amyloid-beta increases mammalian target of rapamycin (mTOR) activity via a PRAS40-mediated mechanism, J. Biol. Chem 286 (2011) 8924–8932, 10.1074/jbc.M110.180638. [PubMed: 21266573]

[194]. Jiang T, et al., Temsirolimus promotes autophagic clearance of amyloid-beta and provides protective effects in cellular and animal models of Alzheimer's disease, Pharmacol. Res 81 (2014) 54–63, 10.1016/j.phrs.2014.02.008. [PubMed: 24602800]

[195]. Ramirez AE, Pacheco CR, Aguayo LG, Opazo CM, Rapamycin protects against abeta-induced synaptotoxicity by increasing presynaptic activity in hippocampal neurons, Biochim. Biophys. Acta 1842 (2014) 1495–1501, 10.1016/j.bbadis.2014.04.019. [PubMed: 24794719]

[196]. Orr ME, Salinas A, Buffenstein R, Oddo S, Mammalian target of rapamycin hyperactivity mediates the detrimental effects of a high sucrose diet on Alzheimer's disease pathology, Neurobiol. Aging 35 (2014) 1233–1242, [PubMed: 24411482]

[197]. Jiang T, et al., Temsirolimus attenuates tauopathy in vitro and in vivo by targeting tau hyperphosphorylation and autophagic clearance, Neuropharmacology 85 (2014) 121–130, 10.1016/j.neuropharm.2014.05.032. [PubMed: 24880087]

[198]. Wang S, et al., MTOR-mediated hyperphosphorylation of tau in the hippocampus is involved in cognitive deficits in streptozotocin-induced diabetic mice, Metab. Brain Dis 29 (2014) 729–736, 10.1007/s11011-014-9528-1. [PubMed: 24682776]

[199]. Ozcelik S, et al., Rapamycin attenuates the progression of tau pathology in P301S tau transgenic mice, PLoS One 8 (2013), e62459, 10.1371/journal.pone.0062459. [PubMed: 23667480]

[200]. Nalbandian A, Llewellyn KJ, Nguyen C, Yazdi PG, Kimonis VE, Rapamycin and chloroquine: the in vitro and in vivo effects of autophagy-modifying drugs show promising results in valosin containing protein multisystem proteinopathy, PLoS One 10 (2015), e012288810.1371/journal.pone.0122888. [PubMed: 25884947]

[201]. Wang IF, Tsai KJ, Shen CK, Autophagy activation ameliorates neuronal pathogenesis of FTLD-U mice: a new light for treatment of TARDBP/TDP-43 proteinopathies, Autophagy 9 (2013) 239–240, 10.4161/auto.22526. [PubMed: 23108236]

[202]. Wang IF, et al., Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43, Proc. Natl. Acad. Sci. U. S. A 109 (2012) 15024–15029, 10.1073/pnas.1206362109. [PubMed: 22932872]

[203]. Caccamo A, et al., Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability, J. Biol. Chem 284 (2009) 27416–27424, 10.1074/jbc.M109.031278. [PubMed: 19651785]

[204]. Halloran J, et al., Chronic inhibition of mammalian target of rapamycin by rapamycin modulates cognitive and non-cognitive components of behavior throughout lifespan in mice, Neuroscience 223 (2012) 102–113, 10.1016/j.neuroscience.2012.06.054. [PubMed: 22750207]

[205]. Majumder S, et al., Lifelong rapamycin administration ameliorates age-dependent cognitive deficits by reducing IL-1beta and enhancing NMDA signaling, Aging Cell 11 (2012) 326–335, 10.1111/j.1474-9726.2011.00791.x. [PubMed: 22212527]

[206]. Liu D, et al., The mitochondrial uncoupler DNP triggers brain cell mTOR signaling network reprogramming and CREB pathway up-regulation, J. Neurochem 134 (2015) 677–692, 10.1111/jnc.13176. [PubMed: 26010875]

[207]. Neff F, et al., Rapamycin extends murine lifespan but has limited effects on aging, J. Clin. Invest 123 (2013) 3272–3291, 10.1172/JCI67674. [PubMed: 23863708]

[208]. Kim JH, et al., Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats, J. Appl. Physiol 107 (2009) 1249–1257, 10.1152/japplphysiol.91393.2008. [PubMed: 19661445]

[209]. Kim JA, Jang HJ, Martinez-Lemus LA, Sowers JR, Activation of mTOR/p70S6 kinase by ANG II inhibits insulin-stimulated endothelial nitric oxide synthase and vasodilation, Am. J. Physiol. Endocrinol. Metab 302 (2012) E201–E208, 10.1152/ajpendo.00497.2011. [PubMed: 22028412]

[210]. Neto Mde M, Di Marco GS, Casarini DE, Lima VC, Campos AH, Orally administered rapamycin does not modify rat aortic vascular tone, J. Cardiovasc. Pharmacol 49 (2007) 96–99, 10.1097/FJC.0b013e31802e6420. [PubMed: 17312450]

[211]. Davis ME, Grumbach IM, Fukai T, Cutchins A, Harrison DG, Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding, J. Biol. Chem 279 (2004) 163–168, 10.1074/jbc.M307528200. [PubMed: 14570928]

[212]. Li H, et al., Evaluation of the protective potential of brain microvascular endothelial cell autophagy on blood–brain barrier integrity during experimental cerebral ischemia-reperfusion injury, Transl. Stroke Res 5 (2014) 618–626, 10.1007/s12975-014-0354-x. [PubMed: 25070048]

[213]. Guo W, Feng G, Miao Y, Liu G, Xu C, Rapamycin alleviates brain edema after focal cerebral ischemia reperfusion in rats, Immunopharmacol. Immunotoxicol 36 (2014) 211–223, 10.3109/08923973.2014.913616. [PubMed: 24773551]

[214]. Chauhan A, Sharma U, Jagannathan NR, Reeta KH, Gupta YK, Rapamycin protects against middle cerebral artery occlusion induced focal cerebral ischemia in rats, Behav. Brain Res 225 (2011) 603–609, 10.1016/j.bbr.2011.08.035. [PubMed: 21903138]

[215]. Li CY, et al., Inhibition of mTOR pathway restrains astrocyte proliferation, migration and production of inflammatory mediators after oxygen-glucose deprivation and reoxygenation, Neurochem. Int 83–84 (2015) 9–18, 10.1016/j.neuint.2015.03.001.

[216]. Sheng R, et al., Autophagy activation is associated with neuroprotection in a rat model of focal cerebral ischemic preconditioning, Autophagy 6 (2010) 482–494, 10.4161/auto.6.4.11737. [PubMed: 20400854]

[217]. Jiang T, et al., Ischemic preconditioning provides neuroprotection by induction of AMP-activated protein kinase-dependent autophagy in a rat model of ischemic stroke, Mol. Neurobiol 51 (2015) 220–229, 10.1007/s12035-014-8725-6. [PubMed: 24809692]

[218]. Papadakis M, et al., Tsc1 (hamartin) confers neuroprotection against ischemia by inducing autophagy, Nat. Med 19 (2013) 351–357, [PubMed: 23435171]

[219]. Dutta S, Rutkai I, Katakam PV, Busija DW, The mechanistic target of rapamycin (mTOR) pathway and S6 kinase mediate diazoxide preconditioning in primary rat cortical neurons, J. Neurochem 134 (2015) 845–856, 10.1111/jnc.13181. [PubMed: 26016889]

[220]. Xie R, et al., Mammalian target of rapamycin cell signaling pathway contributes to the protective effects of ischemic postconditioning against stroke, Stroke 45 (2014) 2769–2776, 10.1161/STROKEAHA.114.005406. [PubMed: 25013017]

[221]. Ha JM, et al., Platelet-derived growth factor regulates vascular smooth muscle phenotype via mammalian target of rapamycin complex 1, Biochem. Biophys. Res. Commun 464 (2015) 57–62, 10.1016/j.bbrc.2015.05.097. [PubMed: 26032503]

[222]. Zohlnhofer D, et al., Rapamycin effects transcriptional programs in smooth muscle cells controlling proliferative and inflammatory properties, Mol. Pharmacol 65 (2004) 880–889, [PubMed: 15044617]

[223]. Montes DK, et al., Vasopressin activates Akt/mTOR pathway in smooth muscle cells cultured in high glucose concentration, Biochem. Biophys. Res. Commun 441 (2013) 923–928, 10.1016/j.bbrc.2013.10.169. [PubMed: 24216105]

[224]. Xie Y, et al., Phosphorylation of GATA-6 is required for vascular smooth muscle cell differentiation after mTORC1 inhibition, Sci. Signal 8 (2015) ra44, 10.1126/scisignal.2005482.

[225]. Zhan JK, et al., Adiponectin attenuates the osteoblastic differentiation of vascular smooth muscle cells through the AMPK/mTOR pathway, Exp. Cell Res 323 (2014) 352–358, 10.1016/j.yexcr.2014.02.016. [PubMed: 24607448]

[226]. Horigome M, et al., Noninvasive evaluation of coronary endothelial function following sirolimus-eluting stent implantation by using positron emission tomography, Cardiology 114 (2009) 157–163, 10.1159/000226093. [PubMed: 19556789]

[227]. Togni M, et al., Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction, J. Am. Coll. Cardiol 46 (2005) 231–236, 10.1016/j.jacc.2005.01.062. [PubMed: 16022947]

[228]. Kotani J, et al., Incomplete neointimal coverage of sirolimus-eluting stents: angioscopic findings, J. Am. Coll. Cardiol 47 (2006) 2108–2111, 10.1016/j.jacc.2005.11.092. [PubMed: 16697331]

[229]. Brugaletta S, et al., Endothelial-dependent vasomotion in a coronary segment treated by ABSORB everolimus-eluting bioresorbable vascular scaffold system is related to plaque composition at the time of bioresorption of the polymer: indirect finding of vascular reparative therapy? Eur. Heart J 33 (2012) 1325–1333, 10.1093/eurheartj/ehr466. [PubMed: 22507972]

[230]. Zhan JK, et al., The mammalian target of rapamycin signalling pathway is involved in osteoblastic differentiation of vascular smooth muscle cells, Can. J. Cardiol 30 (2014) 568–575, 10.1016/j.cjca.2013.11.005. [PubMed: 24518659]

[231]. Zhao Y, et al., Mammalian target of rapamycin signaling inhibition ameliorates vascular calcification via klotho upregulation, Kidney Int 88 (2015) 711–721, 10.1038/ki.2015.160. [PubMed: 26061549]

[232]. Pakala R, Stabile E, Jang GJ, Clavijo L, Waksman R, Rapamycin attenuates atherosclerotic plaque progression in apolipoprotein E knockout mice: inhibitory effect on monocyte chemotaxis, J. Cardiovasc. Pharmacol 46 (2005) 481–486. [PubMed: 16160601]

[233]. Xu W, Qiu C, Winblad B, Fratiglioni L, The effect of borderline diabetes on the risk of dementia and Alzheimer's disease, Diabetes 56 (2007) 211–216, 10.2337/db06-0879. [PubMed: 17192484]

[234]. Xu WL, Qiu CX, Wahlin A, Winblad B, Fratiglioni L, Diabetes mellitus and risk of dementia in the kungsholmen project: a 6-year follow-up study, Neurology 63 (2004) 1181–1186. [PubMed: 15477535]

[235]. Barnes DE, Yaffe K, The projected effect of risk factor reduction on Alzheimer's disease prevalence, Lancet Neurol 10 (2011) 819–828, 10.1016/S1474-4422(11)70072-2. [PubMed: 21775213]

[236]. Chui HC, Zheng L, Reed BR, Vinters HV, Mack WJ, Vascular risk factors and Alzheimer's disease: are these risk factors for plaques and tangles or for concomitant vascular pathology that increases the likelihood of dementia? An evidence-based review, Alzheimers Res. Ther 4 (2012) 1, 10.1186/alzrt98. [PubMed: 22182734]

[237]. Gorelick PB, et al., Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American heart association/ American stroke association, Stroke 42 (2011) 2672–2713, 10.1161/STR.0b013e3182299496. [PubMed: 21778438]

[238]. Viticchi G, et al., Framingham risk score can predict cognitive decline progression in Alzheimer's disease, Neurobiol. Aging (2015) 10.1016/j.neurobiolaging.2015.07.023.

[239]. Breteler MM, Vascular risk factors for Alzheimer's disease: an epidemiologic perspective, Neurobiol. Aging 21 (2000) 153–160. [PubMed: 10867200]

[240]. Verghese PB, Castellano JM, Holtzman DM, Apolipoprotein E in Alzheimer's disease and other neurological disorders, Lancet Neurol 10 (2011) 241–252, 10.1016/S1474-4422(10)70325-2. [PubMed: 21349439]

[241]. Cipolla MJ, The Cerebral Circulation, 5, Morgan & Claypool Life, 2009.

[242]. Talman WT, Dragon D. Nitschke, Neuronal nitric oxide mediates cerebral vasodilatation during acute hypertension, Brain Res 1139 (2007) 126–132, 10.1016/j.brainres.2007.01.008. [PubMed: 17291465]

[243]. Kontos HA, et al., Role of tissue hypoxia in local regulation of cerebral microcirculation, Am. J. Physiol 234 (1978) H582–H591. [PubMed: 645924]

[244]. Lindauer U, Megow D, Matsuda H, Dirnagl U, Nitric oxide: a modulator, but not a mediator, of neurovascular coupling in rat somatosensory cortex, Am. J. Physiol 277 (1999) H799–H811. [PubMed: 10444508]

[245]. Kety SS, Schmidt CF, The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men, J. Clin. Invest 27 (1948) 484–492, 10.1172/JCI101995. [PubMed: 16695569]

[246]. Lecrux C, Kocharyan A, Sandoe CH, Tong XK, Hamel E, Pyramidal cells and cytochrome P450 epoxygenase products in the neurovascular coupling response to basal forebrain cholinergic input, J. Cereb. Blood Flow Metab 32 (2012) 896–906, 10.1038/jcbfm.2012.4. [PubMed: 22293985]

[247]. Heistad DD, Marcus ML, Abboud FM, Role of large arteries in regulation of cerebral blood flow in dogs, J. Clin. Invest 62 (1978) 761–768, 10.1172/JCI109187. [PubMed: 701475]

[248]. Girouard H, Iadecola C, Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease, J. Appl. Physiol 100 (2006) 328–335, 10.1152/japplphysiol.00966.2005. [PubMed: 16357086]

[249]. Cocks TM, Angus JA, Campbell JH, Campbell GR, Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture, J. Cell. Physiol 123 (1985) 310–320, 10.1002/jcp.1041230304. [PubMed: 3886674]

[250]. Li J, Bian KA, Bukoski RD, A non-cyclo-oxygenase, non-nitric oxide relaxing factor is present in resistance arteries of normotensive but not spontaneously hypertensive rats, Am. J. Med. Sci 307 (1994) 7–14. [PubMed: 8291511]

[251]. O'Brien RF, Robbins RJ, McMurtry IF, Endothelial cells in culture produce a vasoconstrictor substance, J. Cell. Physiol 132 (1987) 263–270, 10.1002/jcp.1041320210. [PubMed: 3114270]

[252]. Northington FJ, Matherne GP, Berne RM, Competitive inhibition of nitric oxide synthase prevents the cortical hyperemia associated with peripheral nerve stimulation, Proc. Natl. Acad. Sci. U. S. A 89 (1992) 6649–6652. [PubMed: 1378632]

[253]. Stobart JL, Lu L, Anderson HD, Mori H, Anderson CM, Astrocyte-induced cortical vasodilation is mediated by D-serine and endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A 110 (2013) 3149–3154, 10.1073/pnas.1215929110. [PubMed: 23386721]

[254]. Toth P, et al., Resveratrol treatment rescues neurovascular coupling in aged mice: role of improved cerebromicrovascular endothelial function and downregulation of NADPH oxidase, Am. J. Physiol. Heart Circ. Physiol 306 (2014) H299–H308, 10.1152/ajpheart.00744.2013. [PubMed: 24322615]

[255]. Chen BR, Kozberg MG, Bouchard MB, Shaik MA, Hillman EM, A critical role for the vascular endothelium in functional neurovascular coupling in the brain, J. Am. Heart Assoc 3 (2014), e000787, 10.1161/JAHA.114.000787. [PubMed: 24926076]

[256]. Toth P, et al., IGF-1 deficiency impairs neurovascular coupling in mice: implications for cerebromicrovascular aging, Aging Cell (2015)10.1111/acel.12372.

[257]. Muhl H, Bachmann M, Pfeilschifter J, Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity, Cell. Microbiol 13 (2011) 340–348, 10.1111/j.1462-5822.2010.01559.x. [PubMed: 21199257]

[258]. Endres M, et al., Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A 95 (1998) 8880–8885. [PubMed: 9671773]

[259]. Endres M, et al., Mechanisms of stroke protection by physical activity, Ann. Neurol 54 (2003) 582–590, 10.1002/ana.10722. [PubMed: 14595647]

[260]. Dunn KM, Nelson MT, Neurovascular signaling in the brain and the pathological consequences of hypertension, Am. J. Physiol. Heart Circ. Physiol 306 (2014) H1–14, http://dx.doi.org/10.1152/ajpheart . http://dx.doi.org/10.1152/ajpheart00364.2013. 00364.2013 . [PubMed: 24163077]

[261]. Kazama K, et al., Angiotensin II impairs neurovascular coupling in neocortex through NADPH oxidase-derived radicals, Circ. Res 95 (2004) 1019–1026, 10.1161/01.RES.0000148637.85595.c5. [PubMed: 15499027]

[262]. Park L, Anrather J, Girouard H, Zhou P, Iadecola C, Nox2-derived reactive oxygen species mediate neurovascular dysregulation in the aging mouse brain, J. Cereb. Blood Flow Metab 27 (2007) 1908–1918, 10.1038/sj.jcbfm.9600491. [PubMed: 17429347]

[263]. Park L, et al., Nox2-derived radicals contribute to neurovascular and behavioral dysfunction in mice overexpressing the amyloid precursor protein, Proc. Natl. Acad. Sci. U. S. A 105 (2008) 1347–1352, http://dx. doi.org/10. 1073/pnas.0711568105. [PubMed: 18202172]

[264]. Tucsek Z, et al., Obesity in aging exacerbates blood–brain barrier disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: effects on expression of genes involved in beta-amyloid generation and Alzheimer's disease, J. Gerontol. A Biol. Sci. Med. Sci 69 (2014) 1212–1226, 10.1093/gerona/glt177. [PubMed: 24269929]

[265]. Ayata C, Ma J, Meng W, Huang P, Moskowitz MA, L-NA-sensitive rCBF augmentation during vibrissal stimulation in type III nitric oxide synthase mutant mice, J. Cereb. Blood Flow Metab 16 (1996) 539–541, 10.1097/00004647-199607000-00002. [PubMed: 8964791]

[266]. Toth P, et al., Purinergic glio-endothelial coupling during neuronal activity: role of P2Y1 receptors and eNOS in functional hyperemia in the mouse somatosensory cortex, Am. J. Physiol. Heart Circ. Physiol (2015) 10.1152/ajpheart.00463. 2015.

[267]. Peach MJ, Loeb AL, Singer HA, Saye J, Endothelium-derived vascular relaxing factor, Hypertension 7 (1985) I94–100. [PubMed: 2987129]

[268]. Grange RW, et al., Nitric oxide contributes to vascular smooth muscle relaxation in contracting fast-twitch muscles, Physiol. Genomics 5 (2001) 35–44. [PubMed: 11161004]

[269]. Smith CD, et al., Altered brain activation in cognitively intact individuals at high risk for Alzheimer's disease, Neurology 53 (1999) 1391–1396. [PubMed: 10534240]

[270]. Attems J, Jellinger K, Thal DR, Van Nostrand W, Review: sporadic cerebral amyloid angiopathy, Neuropathol. Appl. Neurobiol 37 (2011) 75–93, 10.1111/j.1365-2990.2010.01137.x. [PubMed: 20946241]

[271]. Jellinger KA, Prevalence and impact of cerebrovascular lesions in alzheimer and lewy body diseases, Neurodegener. Dis 7 (2010) 112–115, 10.1159/000285518. [PubMed: 20173339]

[272]. Viswanathan A, Greenberg SM, Cerebral amyloid angiopathy in the elderly, Ann. Neurol 70 (2011) 871–880, 10.1002/ana.22516. [PubMed: 22190361]

[273]. Smith EE, et al., Correlations between MRI white matter lesion location and executive function and episodic memory, Neurology 76 (2011) 1492–1499, 10.1212/WNL.0b013e318217e7c8. [PubMed: 21518999]

[274]. Levy E, et al., Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type, Science 248 (1990) 1124–1126. [PubMed: 2111584]

[275]. Obici L, et al., A novel AbetaPP mutation exclusively associated with cerebral amyloid angiopathy, Ann. Neurol 58 (2005) 639–644, 10.1002/ana.20571. [PubMed: 16178030]

[276]. Fossati S, et al., Differential activation of mitochondrial apoptotic pathways by vasculotropic amyloid-beta variants in cells composing the cerebral vessel walls, FASEB J 24 (2010) 229–241, 10.1096/fj.09-139584. [PubMed: 19770225]

[277]. Thomas T, Thomas G, McLendon C, Sutton T, Mullan M, Beta-amyloid-mediated vasoactivity and vascular endothelial damage, Nature 380 (1996) 168–171, 10.1038/380168a0. [PubMed: 8600393]

[278]. Thambisetty M, Beason-Held L, An Y, Kraut MA, Resnick SM, APOE epsilon4 genotype and longitudinal changes in cerebral blood flow in normal aging, Arch. Neurol 67 (2010) 93–98, 10.1001/archneurol.2009.913. [PubMed: 20065135]

[279]. Scarmeas N, Habeck CG, Stern Y, Anderson KE, APOE genotype and cerebral blood flow in healthy young individuals, JAMA 290 (2003) 1581–1582, 10.1001/jama.290.12.1581. [PubMed: 14506116]

[280]. Park L, et al., The key role of transient receptor potential melastatin-2 channels in amyloid-beta-induced neurovascular dysfunction, Nat. Commun 5 (2014) 5318, 10.1038/ncomms6318. [PubMed: 25351853]

[281]. Qosa H, LeVine H III, Keller JN, Kaddoumi A, Mixed oligomers and monomeric amyloid-beta disrupts endothelial cells integrity and reduces monomeric amyloid-beta transport across hCMEC/D3 cell line as an in vitro blood–brain barrier model, Biochim. Biophys. Acta 1842 (2014) 1806–1815, 10.1016/j.bbadis.2014.06.029. [PubMed: 24997450]

[282]. Fossati S, Ghiso J, Rostagno A, TRAIL death receptors DR4 and DR5 mediate cerebral microvascular endothelial cell apoptosis induced by oligomeric alzheimer's abeta, Cell Death Dis 3 (2012), e321, 10.1038/cddis.2012.55. [PubMed: 22695614]

[283]. Yu WH, et al., Macroautophagy–a novel beta-amyloid peptide-generating pathway activated in Alzheimer's disease, J. Cell Biol 171 (2005) 87–98, 10.1083/jcb.200505082. [PubMed: 16203860]

[284]. Yu WH, et al., Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease, Int. J. Biochem. Cell Biol 36 (2004) 2531–2540, 10.1016/j.biocel.2004.05.010. [PubMed: 15325590]

[285]. Vingtdeux V, et al., AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism, J. Biol. Chem 285 (2010) 9100–9113, 10.1074/jbc.M109.060061. [PubMed: 20080969]

[286]. Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC, Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies, Cell Death Differ 16 (2009) 46–56, http://dx.doi.org/10.1038/cdd.2008 . http://dx.doi.org/10.1038/cdd.2008110. 110 . [PubMed: 18636076]

[287]. Bai X, et al., Rapamycin improves motor function, reduces 4-hydroxynonenal adducted protein in brain, and attenuates synaptic injury in a mouse model of synucleinopathy, Pathobiol. Aging Age Relat. Dis 5 (2015) 28743, 10.3402/pba.v5.28743. [PubMed: 26306821]

[288]. Siddiqui A, et al., Mitochondrial quality control via the PGC1alpha-TFEB signaling pathway is compromised by parkin Q311X mutation but independently restored by rapamycin, J. Neurosci 35 (2015) 12833–12844, 10.1523/JNEUROSCI.0109-15.2015. [PubMed: 26377470]

[289]. Decressac M, Bjorklund A, MTOR inhibition alleviates L-DOPA-induced dyskinesia in parkinsonian rats, J. Park. Dis 3 (2013) 13–17, 10.3233/JPD-120155.

[290]. Pierce A, et al., Overexpression of heat shock factor 1 phenocopies the effect of chronic inhibition of TOR by rapamycin and is sufficient to ameliorate alzheimer's-like deficits in mice modeling the disease, J. Neurochem (2012) (in press).

[291]. Khurana V, et al., TOR-mediated cell-cycle activation causes neurodegeneration in a drosophila tauopathy model, Curr. Biol 16 (2006) 230–241, 10.1016/j.cub.2005.12.042. [PubMed: 16461276]

[292]. Bhaskar K, et al., The PI3K-Akt-mTOR pathway regulates abeta oligomer induced neuronal cell cycle events, Mol. Neurodegener 4 (2009) 14, 10.1186/1750-1326-4-14. [PubMed: 19291319]

[293]. Tramutola A, et al., Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD, J. Neurochem 133 (2015) 739–749, 10.1111/jnc.13037. [PubMed: 25645581]

[294]. Sun YX, et al., Differential activation of mTOR complex 1 signaling in human brain with mild to severe Alzheimer's disease, J. Alzheimers Dis 38 (2014) 437–444, 10.3233/JAD-131124. [PubMed: 23979023]

[295]. Bove J, Martinez-Vicente M, Vila M, Fighting neurodegeneration with rapamycin: mechanistic insights, Nat. Rev. Neurosci 12 (2011) 437–452, 10.1038/nrn3068. [PubMed: 21772323]

[296]. Morel M, et al., Evidence of molecular links between PKR and mTOR signalling pathways in abeta neurotoxicity: role of p53, Redd1 and TSC2, Neurobiol. Dis 36 (2009) 151–161, 10.1016/j.nbd.2009.07.004. [PubMed: 19631745]

[297]. Majumder S, Richardson A, Strong R, Oddo S, Inducing autophagy by rapamycin before, but not after, the formation of plaques and tangles ameliorates cognitive deficits, PLoS One 6 (2011), e25416, 10.1371/journal.pone.0025416. [PubMed: 21980451]

[298]. Roberson ED, et al., Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model, Science 316 (2007) 750–754, 10.1126/science.1141736. [PubMed: 17478722]

[299]. Siman R, Cocca R, Dong Y, The mTOR inhibitor rapamycin mitigates perforant pathway neurodegeneration and synapse loss in a mouse model of early-stage alzheimer-type tauopathy, PLoS One 10 (2015), e0142340, 10.1371/journal.pone.0142340. [PubMed: 26540269]

[300]. Tang Z, et al., MTor mediates tau localization and secretion: implication for Alzheimer's disease, Biochim. Biophys. Acta 1853 (2015) 1646–1657, 10.1016/j.bbamcr.2015.03.003. [PubMed: 25791428]

[301]. Tang Z, et al., Mammalian target of rapamycin (mTor) mediates tau protein dyshomeostasis: implication for Alzheimer disease, J. Biol. Chem 288 (2013) 15556–15570, 10.1074/jbc.M112.435123. [PubMed: 23585566]

[302]. Tepper K, et al., Oligomer formation of tau protein hyperphosphorylated in cells, J. Biol. Chem 289 (2014) 34389–34407, 10.1074/jbc.M114.611368. [PubMed: 25339173]

[303]. Kolosova NG, et al., Rapamycin suppresses brain aging in senescence-accelerated OXYS rats, Aging 5 (2013) 474–484. [PubMed: 23817674]

[304]. Frederick C, et al., Rapamycin ester analog CCI-779/temsirolimus alleviates tau pathology and improves motor deficit in mutant tau transgenic mice, J. Alzheimers Dis (2014)10.3233/JAD-142097.

[305]. Caccamo A, et al., MTOR regulates tau phosphorylation and degradation: implications for Alzheimer's disease and other tauopathies, Aging Cell 12 (2013) 370–380, 10.1111/acel.12057. [PubMed: 23425014]

[306]. Sarnat HB, Flores-Sarnat L, Infantile tauopathies: hemimegalencephaly; tuberous sclerosis complex; focal cortical dysplasia 2; ganglioglioma, Brain Dev (2014)10.1016/j.braindev.2014.08.010.

[307]. Caccamo A, De Pinto V, Messina A, Branca C, Oddo S, Genetic reduction of mammalian target of rapamycin ameliorates Alzheimer's disease-like cognitive and pathological deficits by restoring hippocampal gene expression signature, J. Neurosci 34 (2014) 7988–7998, 10.1523/JNEUROSCI.0777-14.2014. [PubMed: 24899720]

[308]. Ma JF, Huang Y, Chen SD, Halliday G, Immunohistochemical evidence for macroautophagy in neurones and endothelial cells in Alzheimer's disease, Neuropathol. Appl. Neurobiol 36 (2010) 312–319, 10.1111/j.1365-2990.2010.01067.x. [PubMed: 20102518]

[309]. Fonseca AC, Oliveira CR, Pereira CF, Cardoso SM, Loss of proteostasis induced by amyloid beta peptide in brain endothelial cells, Biochim. Biophys. Acta 1843 (2014) 1150–1161, 10.1016/j.bbamcr.2014.02.016. [PubMed: 24589617]

[310]. Kennedy BK, et al., Geroscience: linking aging to chronic disease, Cell 159 (2014) 709–713, 10.1016/j.cell.2014.10.039. [PubMed: 25417146]

[311]. Morgan D Immunotherapy for Alzheimer's disease. J. Intern. Med 269, 54–63, doi: 10.1111/j.1365-2796.2010.02315.x.

[312]. Meyer-Luehmann M, et al., T cell mediated cerebral hemorrhages and microhemorrhages during passive Abeta immunization in APPPS1 transgenic mice, Mol. Neurodegener 6 (2011) 22, 10.1186/1750-1326-6-22. [PubMed: 21388539]

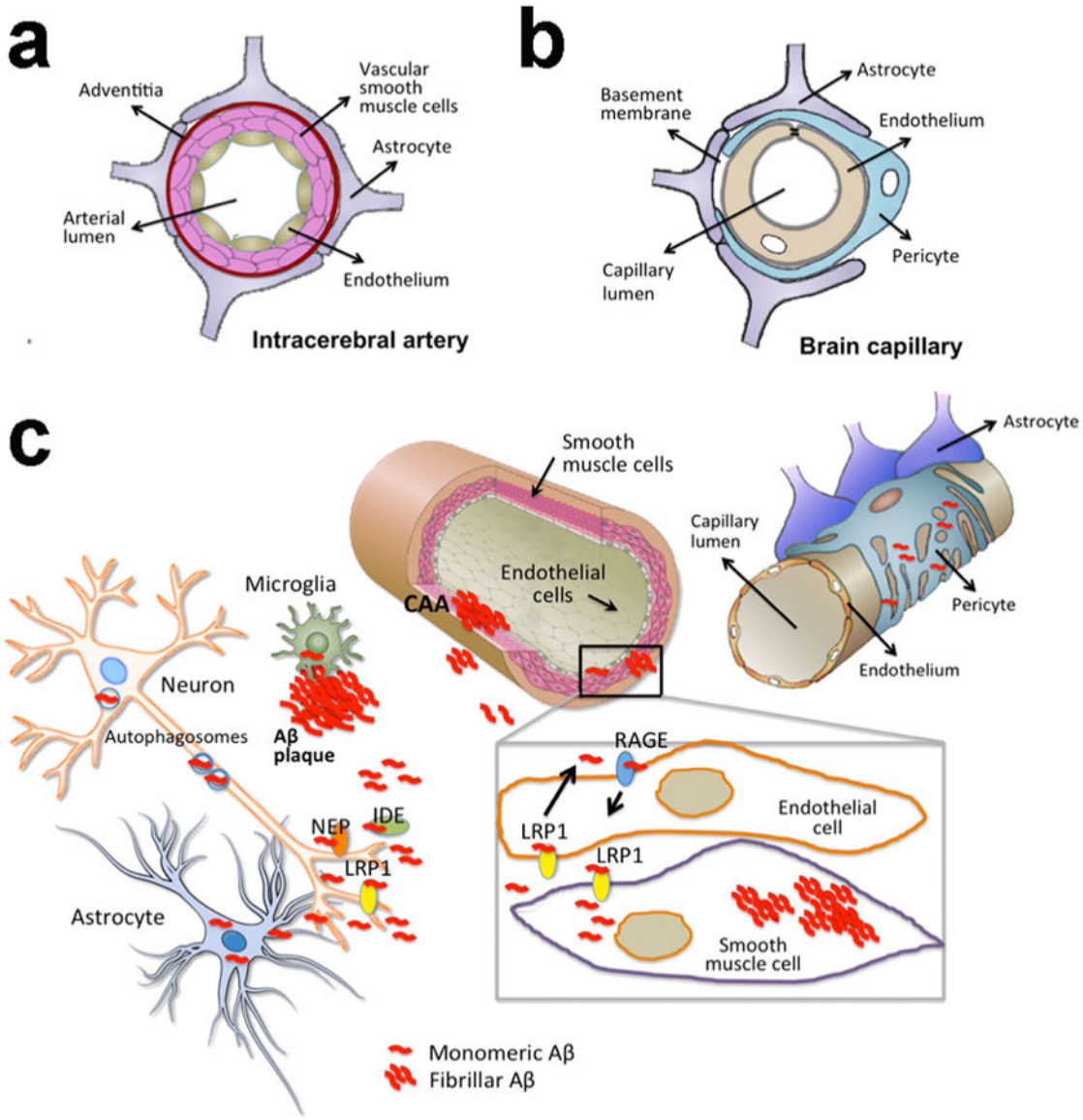

Fig. 1. Neurovascular cell components and their interactions with amyloid-ß.

a. Endothelium of cerebral arteries is surrrounded by vascular smooth muscle cells surrounded by astrocytic end-feet that rest on a layer of connective tissue, the adventitia. **b.** The capillary wall is composed of endothelium and pericytes attached to a basement membrane and encased by astrocytic end-feet. **c.** After its generation by neurons and release at synaptic sites, Aβ in the interstitial fluid (ISF) can be cleared by neuronal reuptake via LRP1; by neuronal autophagy (both intracellular Aβ and potentially reuptaken Aβ); through proteolytic degradation by insulin-degrading enzyme (IDE) and neprilysin (NEP) [81–83]; through uptake and degradation by microglia and astrocytes [77–80]; through LRP1-mediated uptake and degradation by VSMC; by transport to the CSF and reabsorption into venous circulation [84] (not shown), and by direct transport via LRP1 across the blood–brain barrier [67,85]. RAGE (or ApoJ, not shown) mediate re-entry of Aβ into the brain. Pericytes clear extracellular Aβ via LRP1 [125,126]. CAA, cerebral amyloid angiopathy. RAGE,

receptor for advanced glycation end-products. Illustrations of vessels in panel **c** are modified from Patel and Honoré 2010 Nat Rev. Nephrol 6:530 and from Hoffman and Calabrese 2010 Nat Rev. Rheumatol 10:454. Schematic representations of brain capillaries and arteries in panels a and b are adapted from Zlokovic BV 2011 Nat Rev Neurosci 12:723.

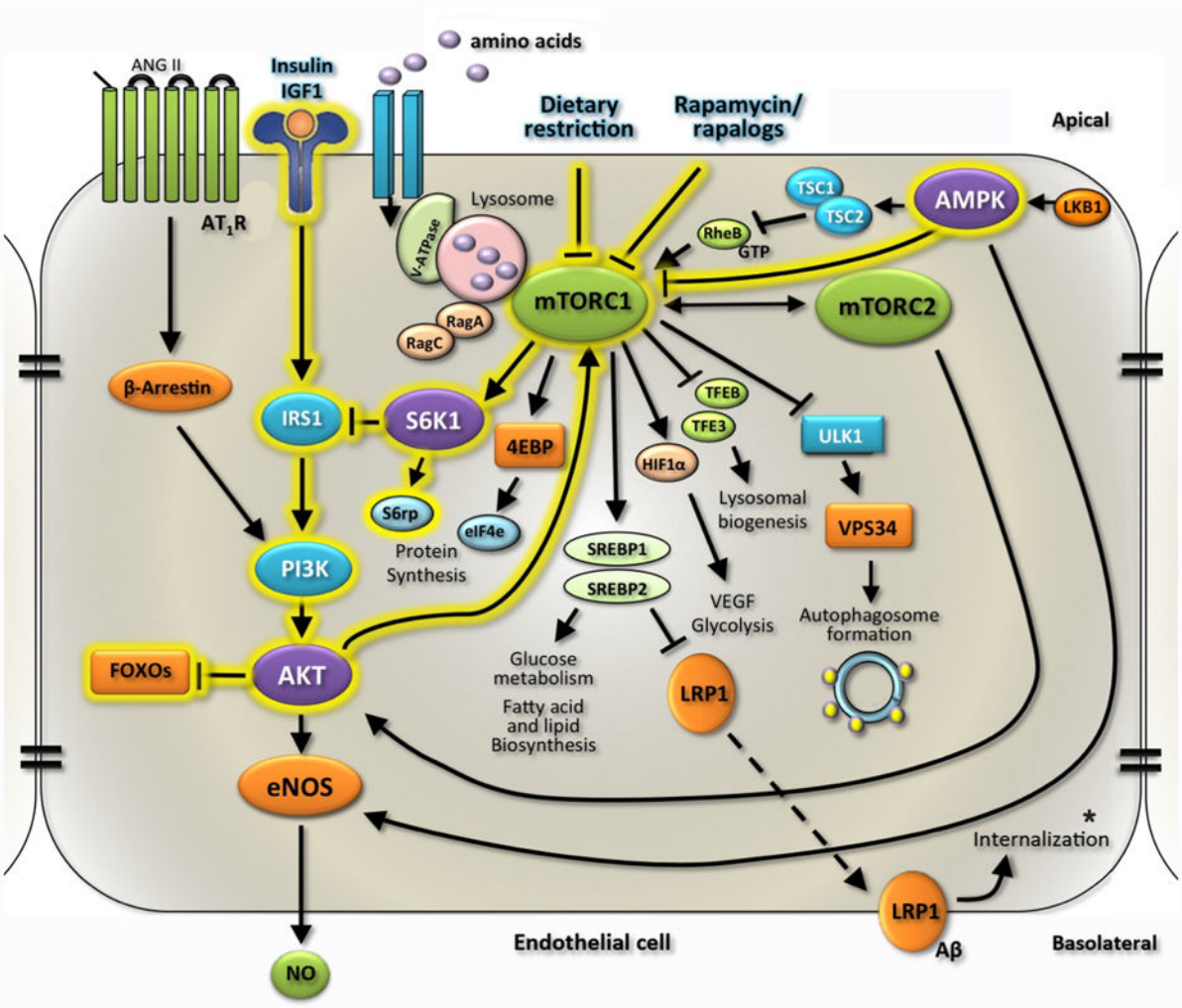

Fig. 2. Vascular mTOR-dependent mechanisms linking the control of aging to the pathogenesis of Alzheimer's disease.

mTOR-driven pathways of aging (highlighted in yellow and limited to those for which evidence in mammals is available) as they interface with the regulation of eNOS-dependent NO generation and with pathways of Aß clearance in brain vascular endothelial cells. *, first step in the clearance of Aß by transcytosis across the blood–brain barrier. NB: There is evidence for a role of mTOR in the regulation of specific aspects of function in VSMC, discussed in the '*mTOR and vascular function*' section. Because those mechanisms are less understood, we have limited our schematic representation of the intersection between of mTOR-driven pathways of aging and those of AD pathogenesis to mechanisms acting in endothelial cells, that have been more extensively examined.

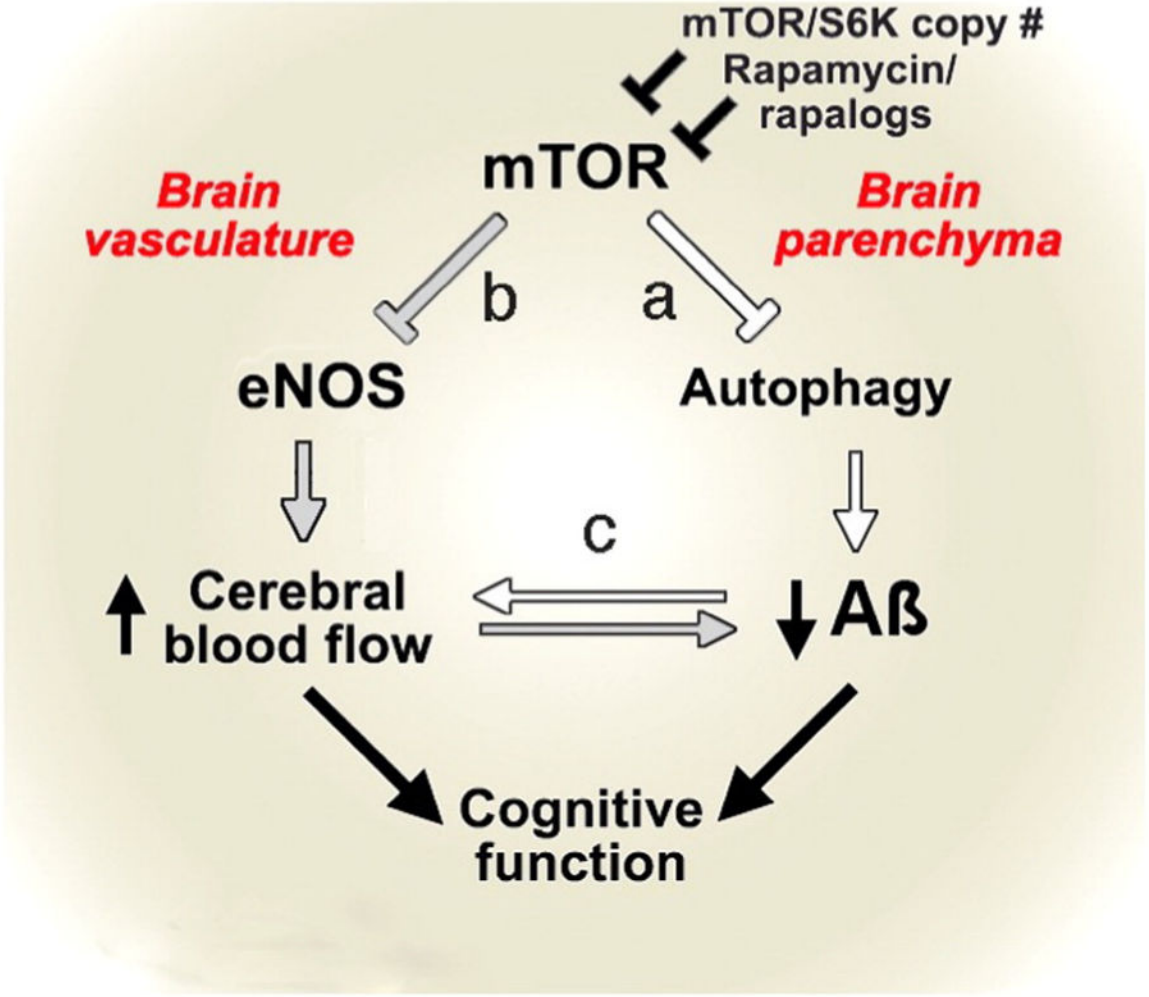

Fig. 3. Vascular and parenchymal mTOR-dependent mechanisms of Alzheimer's disease pathogenesis.

mTOR mechanisms of AD pathogenesis impact both 'hits' in the 'two hit' hypothesis [4]. mTOR inhibits autophagy, whose activation has been associated with improved outcomes by reducing levels of neuronally-generated Aß in ISF **(a)** [98,102,191,192,307]. mTOR also inhibits eNOS activation in vascular endothelial cells **(b)** [31,33,34,39,40], possibly through the phosphorylation of IRS1 [37,156,157], and thus impedes NO release and vasodilation. Attenuation of mTOR activity may improve cognitive function in AD by releasing mTOR inhibition of autophagy in brain parenchyma [191,192], to reduce ISF Aß levels **(a;** Hit two in the two-hit hypothesis [4]), and by releasing mTOR inhibition of eNOS in vascular endothelial cells, to restore vascular integrity and function **(b;** Hit one in the two-hit hypothesis [4]). This enables effective clearance of Aß from ISF [**(c),** right-pointing shaded arrow]. In turn, keeping ISF Aß levels low preserves brain vascular integrity and function [**(c,** left-pointing white arrow]. Concomitantly reducing net Aß generation in parenchyma and preserving Aß clearance through brain vasculature is expected to maintain low steady-state parenchymal Aß levels.