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

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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.

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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.

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

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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, A β , and synaptic function

Synapses are a major target in the pathogenesis of AD [1], and the release of the amyloid-beta (A β) peptide at synaptic sites, triggered by neuronal activity, has a critical role in this process. The A β 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 A β depends on specific proteases that catalyze the cleavage of APP intramembranously and at different sites of its extracellular domain [45–47]. A β 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 length) [48] as well as mature fibrils with high β -sheet content [49,50] which can assemble and deposit 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 A β [54] may exert toxicity at synapses by various mechanisms, prominently by impairing glutamatergic synaptic transmission strength and plasticity [55,56] and causing synaptic loss [56]. A β secretion at synaptic sites is activity-dependent [57,58] and the available evidence supports the notion that APP and A β are part of a feedback loop that regulates neuronal excitability, in which secreted A β acts as a neuromodulator that suppresses excitatory synaptic activity postsynaptically [57,58]. A recent study, however, suggested that small increases in A β 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 A β on synaptic transmission, with picomolar and low nanomolar concentrations of A β having positive and negative effects on synaptic transmission

respectively, through mechanisms involving activation of the α 7-nicotinic acetylcholine receptor [60–62]. Higher levels of A β associated with pathology, however, impair long-term potentiation (LTP), a persistent strengthening 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 (AMPA) type regulate the changes in neuronal excitability associated with LTP and LTD. A β induces endocytosis of AMPARs postsynaptically, thus attenuating glutamatergic excitatory neurotransmission [57,58,64]. The mechanisms of A β -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 A β 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 A β and A β oligomers, however, will disrupt synaptic plasticity and ultimately lead to neuronal loss. Dyshomeostasis of A β 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 A β 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 electrolyte 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).

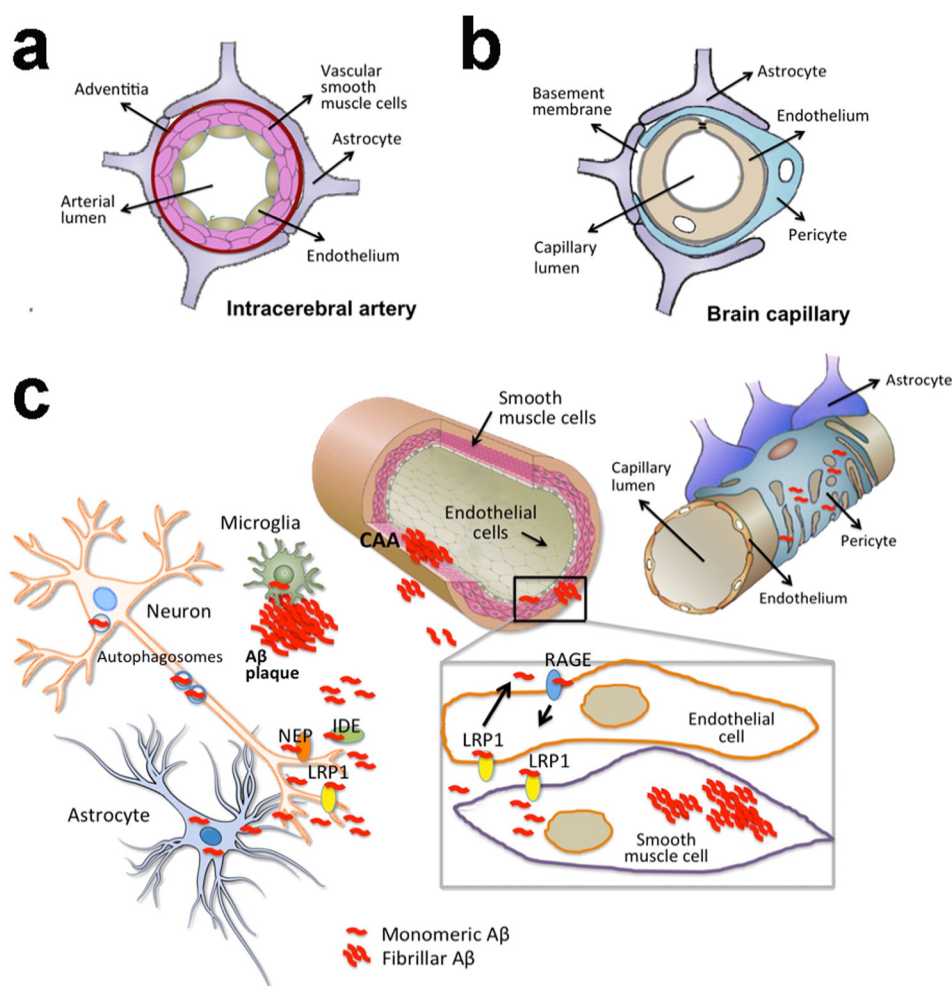


Fig. 1. Neurovascular cell components and their interactions with amyloid- β . **a.** Endothelium of cerebral arteries is surrounded by vascular smooth muscle cells surrounded by astrocytic end-feet that rest on a layer of connective tissue, the adventitia. **b.** The capillary wall is composed of endothelium and pericytes attached to a basement membrane and encased by astrocytic end-feet. **c.** After its generation by neurons and release at synaptic sites, $A\beta$ in the interstitial fluid (ISF) can be cleared by neuronal reuptake via LRP1; by neuronal autophagy (both intracellular $A\beta$ and potentially reuptaken $A\beta$); 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 $A\beta$ into the brain. Pericytes clear extracellular $A\beta$ 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.

After its generation by neurons and release at synaptic sites, $A\beta$ 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 $A\beta$ clearance from brain has been documented for AD [86] and slower clearance kinetics resulting in increased $A\beta$ 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 $A\beta$ 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 $A\beta$ [84]. However, most $A\beta$ 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 $A\beta$ and clear it from the synaptic space through reuptake followed by lysosomal degradation [91,92]. Endocytosis is critical for the generation of $A\beta$ [93,94] and its retrieval from the cell surface [95]. LRP1-dependent clearance of $A\beta$ [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 $A\beta$ half-life in ISF and exacerbated $A\beta$ plaque deposition [97].

The autophagy-lysosomal pathway also plays a central role in the regulation of intracellular and extracellular levels of $A\beta$ [98–101] (Fig. 1). In addition to being a major $A\beta$ degradative pathway, autophagy may contribute to $A\beta$ secretion [102]. Significant neuronal loss, as seen in AD, is rare in mutant APP transgenic mice that robustly deposit $A\beta$, but it is usually extensive in APP/ $A\beta$ models that accumulate $A\beta$ intraneuronally [103]. If the accumulation of $A\beta$ 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 $A\beta$ through autophagy may supersede the negative impact associated with release of $A\beta$ in ISF [104].

LRP1 also has a critical role in clearing A β 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 A β 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 A β (70 nM–100 nM [105]). High levels of interstitial A β , such as those observed in transgenic mouse models, may thus trigger further increases in ISF A β levels by saturation of LRP1-mediated transport, and promote A β aggregation. During its transport across the blood–brain barrier, neuronally-generated A β 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 A β initiates the process of A β clearance from brain to blood [108–112]. The critical role of LRP1 in the export of A β out of the brain is substantiated by multiple *in vitro* and *in vivo* studies. When A β 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) A β clearance [105]. This effect suggested the existence of

other transport mechanism(s) at the blood–brain barrier that operate to clear A β 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 A β 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 A β clearance, compared to iPSC-derived endothelial cells expressing a non-protective PICALM allele [116]. These data suggest that PICALM regulates A β transcytosis and clearance and that polymorphisms in the PICALM locus enhance A β clearance and thereby reduce the risk of AD.

LRP1 is also expressed in vascular smooth muscle cells (VSMC), where it mediates lysosomal degradation of A β [117–119]. Uptake and degradation of A β by VSMC is inhibited by serum-response factor and myocardin, which reduce clearance of A β by downregulating LRP1 [119]. Early in AD, VSMC develop a hypercontractile phenotype [120] that contributes to deficits in cerebral blood flow. In addition, A β

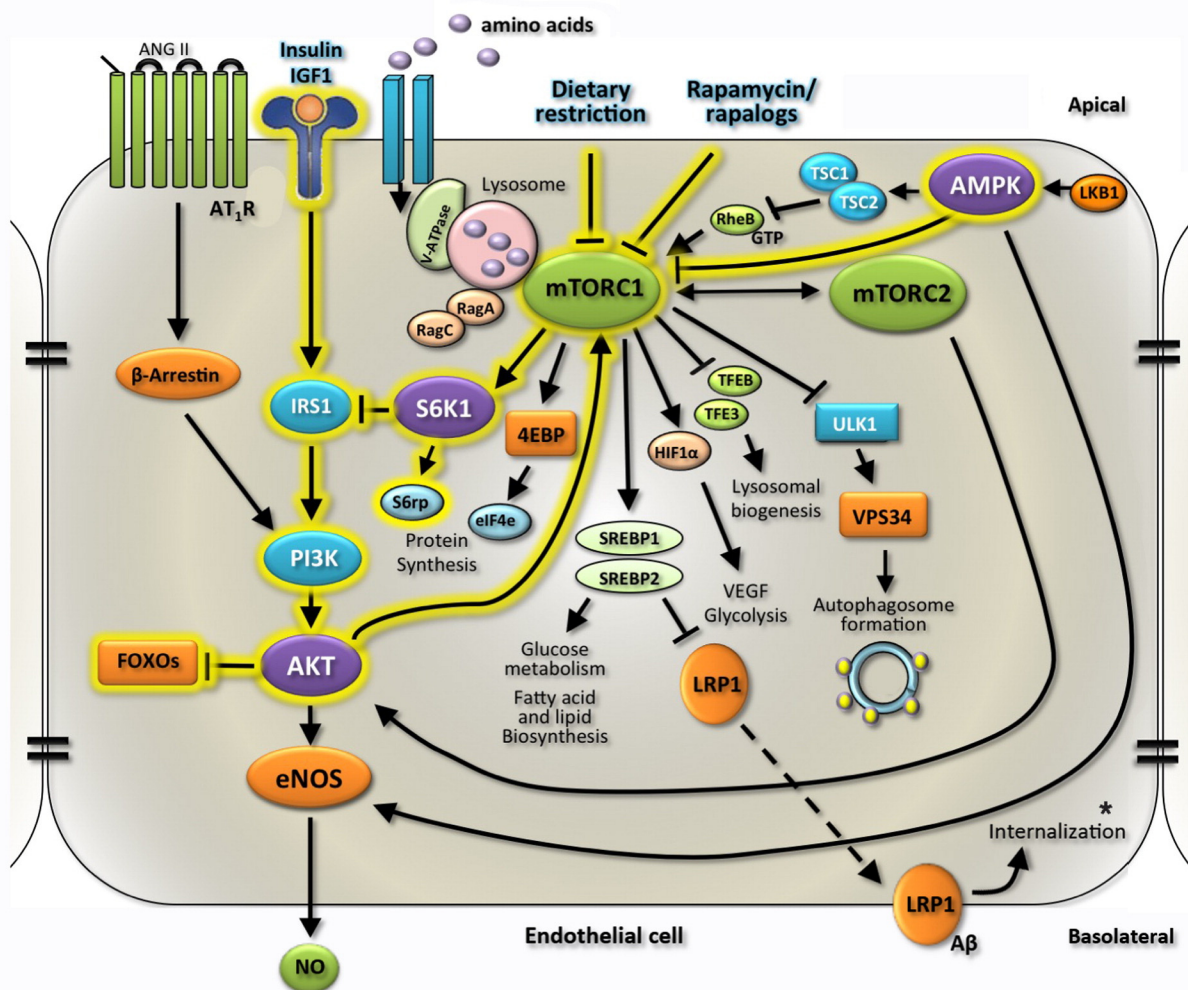


Fig. 2. Vascular mTOR-dependent mechanisms linking the control of aging to the pathogenesis of Alzheimer's disease. mTOR-driven pathways of aging (highlighted in yellow and limited to those for which evidence in mammals is available) as they interface with the regulation of eNOS-dependent NO generation and with pathways of A β clearance in brain vascular endothelial cells. *, first step in the clearance of A β 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.

degradation by VSMC becomes impaired [120]. Accumulation of A β and A β deposition in pial and intracerebral arteries and arterioles thus leads to cerebral amyloid angiopathy (CAA; Fig. 1c). In agreement with the critical role of LRP1 in VSMC-mediated A β clearance, conditional deletion of LRP1 in VSMC exacerbates A β plaque deposition and CAA in a mouse model of AD [117].

Microglia and astrocytes also have important roles in the removal of A β from ISF [78–80]. Both microglia [77,78,121,122] and astrocytes [79,123,124] have a prominent role in clearing A β from ISF by internalization and degradation. In addition, pericytes may also be integral in A β clearance, since *Pdgfr β* haploinsufficiency – which leads to pericyte loss – dramatically decreases A β clearance and increases A β deposition early in progression of AD-like pathogenesis in a mouse model of AD [125]. Pericytes clear extracellular A β 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 glycosylated end-products (RAGE), has been implicated in the movement of A β back into the CNS [85,130]. Re-entry of A β 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 A β 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 cerebrovascular endothelial cells [139]. Aging, in turn, exacerbates loss of pericyte coverage and subsequent cerebrovascular 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 A β 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) potentially 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-1 α by its phosphorylation and stabilization [154]. Stabilized HIF-1 α associates with its companion subunit HIF-1 β 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 thought 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 testosterone 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 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/6J 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 responses [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 AT₁R 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/reoxygenation 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 APOEε4 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 potentially influenced by other regulatory mechanisms, involving neurovascular coupling [244] and CO₂ 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 responses 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 catalyzes the conversion 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 Ca²⁺ 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 A β in ISF increase in conditions of low CBF as a consequence of diminished A β 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 A β), 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 A β levels that triggers abnormal A β accumulation, the second hit in the pathogenesis of AD [4] (Fig. 3). While A β -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 A β with the cerebral vasculature has specific consequences for different cerebrovascular cell components. CAA results from focal to widespread A β deposition in leptomeningeal and intracortical cerebral blood vessels (Fig. 1). CAA can be prominent in VSMC of pial and intracerebral arteries and arterioles; A β depositions in the glia limitans and adjacent neuropil are referred to as precapillary A β , and those in the capillary wall are referred to as capillary A β 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 A β sequence such as L34V and E22Q mutations have accelerated VSMC degeneration that leads to hemorrhagic stroke and dementia [274–276]. Furthermore, A β 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 A β 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

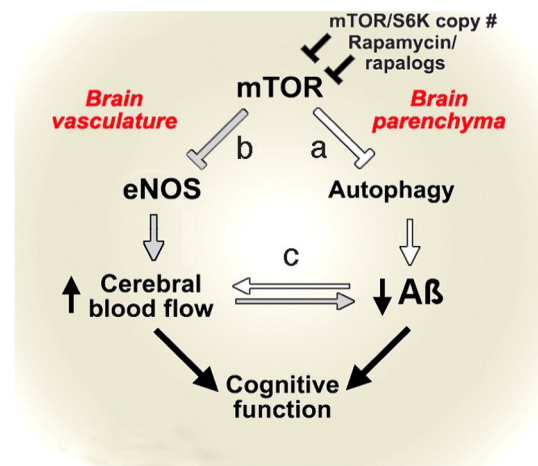


Fig. 3. Vascular and parenchymal mTOR-dependent mechanisms of Alzheimer's disease pathogenesis. mTOR mechanisms of AD pathogenesis impact both 'hits' in the 'two hit' hypothesis [4]. mTOR inhibits autophagy, whose activation has been associated with improved outcomes by reducing levels of neuronally-generated A β 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 A β 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 A β from ISF [(c), right-pointing shaded arrow]. In turn, keeping ISF A β levels low preserves brain vascular integrity and function [(c), left-pointing white arrow]. Concomitantly reducing net A β generation in parenchyma and preserving A β clearance through brain vasculature is expected to maintain low steady-state parenchymal A β levels.

leads to brain hypoperfusion, diminished functional hyperemia, and CAA [119,120].

In addition, A β 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 A β , 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 A β 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 A β in neurons [283,284], and it was demonstrated that enhancing autophagic-lysosomal function leads to substantially decreased A β levels and deposition in brain [98]. More recent studies suggest a dual role for autophagy in the degradation and secretion of A β [101,102]. Consistent with a critical role of the autophagy-lysosomal pathway in the regulation of A β levels, activation of AMP-activated protein kinase by resveratrol, which potently inhibits mTOR by phosphorylation, enhances autophagic-lysosomal degradation of A β 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 A β 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 activity or levels of mTOR in AD mouse brains are mechanistically linked to increased A β [193,296]. Consistent with these observations, recent studies showed that chronic (48 h) mTOR attenuation protects against synaptic failure induced by A β 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 A β is produced in neurons and a large proportion of neuronally-generated A β 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 A β production. In turn, relief of mTOR-mediated inhibition of endothelium-dependent nitric oxide release maintains cerebrovascular integrity and function, thus increasing the rate of A β 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 Parkinsonism linked to chromosome 17. Evidence suggests that tau is downstream of A β 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, hyperphosphorylated 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 S³⁵⁶, S²¹⁴ and T^{231/301}, Thr²³¹, and potentially Ser²¹⁴/Thr²¹². 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 phospho-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 A β 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 A β 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 A β -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 A β 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 A β 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.

Transparency Document

The [Transparency document](#) associated with this article can be found, in online version.

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