Mini-Review

TOR-dependent cerebrovascular aging in Alzheimer's disease

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ABSTRACT

Increasing evidence suggests that vascular dysfunction, a universal feature of aging, mechanistically contributes to the onset and pathogenesis of neurological diseases of aging. It was recently discovered that attenuating activity of the mammalian/mechanistic target of rapamycin (mTOR) extends both life- and health-span in mice by delaying aging. Here we review current evidence for a critical role of mTOR in age-associated vascular dysfunction and discuss potential mechanisms by which this pathway may lead to cognitive decline in Alzheimer's disease.

KEYWORDS: TOR, vascular, Alzheimer's disease, aging, cognitive decline

ABBREVIATIONS

mTOR, mammalian target of rapamycin; CBF, cerebral blood flow; AD, Alzheimer's disease; PD, Parkinson's disease; VD, vascular dementia; NO, nitric oxide; CAA, cerebral amyloid angiopathy; BBB, blood-brain barrier; NVU, neurovascular unit; TJ, tight junction; ZO, zona occludens; APP, amyloid precursor protein; T2DM, type 2 diabetes mellitus; eNOS, endothelial nitric oxide synthase; TSC, tumor suppressor complex; ADMA, asymmetric dimethylarginine.

INTRODUCTION

Vascular dysfunction is a universal feature of aging [1-3]. In the human brain, vascular changes

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associated with aging result in chronically decreased cerebral blood flow [4, 5] and a diminished response to vasodilatory stimuli [6-9]. Chronic reduction in CBF has been associated with decreased neuronal plasticity and a subsequent impairment of cognitive function [1, 10]. A growing body of evidence suggests that age-related decline in CBF is due to alterations in cerebral vasculature including reduction of microvascular density, decreased cellular stability, impaired plasticity, and altered vessel reactivity [1, 2]. As a reduction in both regional CBF and brain vessel density has been observed in so-called "healthy aging" when subjects are compared to young adults [4], decreased CBF is not necessarily indicative of a disease state, but of vascular changes that may underlie the susceptibility of aged brains to functional decline. Notably, reduced CBF and altered vascular permeability are prevalent pathologies in a multitude of age-related brain disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and vascular dementia (VD), whose risks are increased by peripheral metabolic conditions (e.g. type 2 diabetes mellitus and atherosclerosis), that prominently impair peripheral circulation. Thus, it is possible that a common mechanistic pathway underlies both central and peripheral vascular endothelium dysfunction associated with aging, and thereby contributes to various states of cognitive impairment as well as to peripheral organ dysfunction.

The mammalian target of rapamycin (mTOR) is a central regulator of cell growth and survival and an integrator of upstream pathways sensing metabolic states that is activated by inputs from nutrients, insulin, and growth factors. Recent studies

showed that decreasing mTOR activity increases lifespan by delaying aging in mice [11], and attenuation of the invertebrate TOR ortholog also extends lifespan in *D. melanogaster* [12] and *C. elegans* [13-15]. Notably, though, the mechanisms through which TOR controls organismal aging are still unknown. mTOR controls protein homeostasis by regulating cap-dependent translation and by inhibiting autophagy, a major mechanism of protein/organelle maintenance [16]. mTOR also regulates important aspects of metabolism by inhibiting cellular glycolysis autonomously, and via its role in the control of glucose metabolism by the liver [17]. In addition, prior studies have suggested a role of mTOR in the regulation of endothelium-dependent, NO-mediated vasodilation [18, 19]. The mechanisms by which mTOR regulates vascular endothelial function, however, are still largely unexplored. Given that inhibition of mTOR enhances both life- and health-span [11, 13, 15] and that vascular aging has been linked to varying degrees of cognitive decline from both 'normal' forgetfulness to progressive neurodegeneration, it is imperative that the link between mTOR activity and vascular aging be examined. Here we will review current evidence for a role of TOR in the regulation of specific processes associated with vascular aging and their relationship with the pathogenesis of Alzheimer's, a prominent age-associated brain disease.

Vascular endothelium and aging

The blood-brain barrier (BBB) is a highly selective permeability barrier that dynamically regulates the interactions between the peripheral circulation and the central nervous system (CNS). Cerebral capillaries are enclosed by a layer of single vascular endothelial cells connected by tight junctions (TJ), and these cells communicate and interact with neurons, astrocytes, pericytes and the extracellular matrix to form the neurovascular unit (NVU), the health of which is essential to the proper function of the CNS [20, 21]. Tight junctions are integral to NVU health as they form the primary seal of the BBB to restrict paracellular diffusion from the blood into the brain [20]. This barrier function is primarily mediated by the integral membrane proteins claudin and occludin, and cellular adhesion to the actin cytoskeleton and signal transduction at the TJ are mediated by junctional adhesion molecule (JAM-A) and the scaffolding protein family zonula occludens (ZO) [1, 20].

Disruption of the BBB leads to compromise of the CNS. Consistent with this hypothesis, decreased expression of many TJ proteins is a common feature of neurological diseases of aging, particularly those leading to dementia [3, 22, 23]. Decreased expression of occludin and ZO-1 has been observed in the microvasculature of Multiple Sclerosis (MS) [24, 25] and AD patients [26]. Furthermore, microvascular deficits result in reduced CBF which impairs oxygen supply to – and the clearance of neurotoxins from – the brain [3]. This dysfunction can lead to subsequent damage such as accumulation of amyloid-β in the vasculature [27], resulting in cerebral amyloid angiopathy (CAA), a common pathological feature of Alzheimer's disease [28]. Since aging is the primary risk factor for both BBB dysfunction [1] and neurodegenerative disease, recent efforts have been made to investigate the role of vascular factors in the development of dementia. Current evidence suggests that increased BBB permeability may be a precipitating event in a number of neurodegenerative pathologies, rather than a consequence of a disease state.

Cerebral blood flow in aging and in neurological diseases of aging

Under normal circumstances, regional blood flow correlates with metabolic demand, suggesting that these factors are tightly coupled in the CNS [2]. Neurovascular coupling, the rapid increase in oxygen and glucose delivery to active brain regions, is a process vital for proper brain function [29, 30]. In line with this observation, current evidence suggests that global CBF decreases with age and is associated with cognitive decline even in "healthy" subjects [4, 5], though these changes appear to be regionally distinct. For example, one study using human volunteers found reduced CBF in specific regions including the cingulate, superior temporal, and inferior frontal gyri in aged subjects compared to young controls [4]. It is noteworthy that these regions subserve higher order cognitive functions such as learning and memory. Similar findings have been reported in both rodents and non-human primates [31, 32], with one study reporting a nearly 40% decrease in cortical arteriole density in senescent animals compared to young adults [2].

These age-associated changes are amplified in disease states; arterial spin labeling MRI has shown that resting CBF is significantly reduced in AD patients [33], while fMRI studies confirm that patients with mild cognitive impairment (MCI) exhibit delayed increases in CBF and that these delays worsen with disease progression [34]. Furthermore, numerous studies have demonstrated that vessel/capillary rarefaction in the hippocampus and cortex results in cognitive dysfunction [2, 35, 36], while hypoperfusion of the brain has been demonstrated to increase neuronal β-amyloid levels and tau phosphorylation in mice modeling AD [37]. In addition to decreased vasculature and reduced blood flow, other vascular pathologies are present in AD. Cerebral amyloid angiopathy (CAA), the accumulation and deposition of β-amyloid within the vasculature, is present in more than 80% of AD cases [38]; similarly, mice expressing the amyloid precursor protein (APP) and transforming growth factor β1 (TGFβ1) exhibit progressive neurovascular uncoupling and cognitive decline concomitant with increased vascular deposition of β-amyloid [39].

Cardiovascular risk factors in Alzheimer's disease

It may not be surprising, then, that many of the known risk factors for AD – cardiovascular disease, diabetes, high blood pressure, heart disease – have profound effects on vascular integrity and perfusion of the brain. For example, a wealth of research has shown that the vascular impairments associated with obesity exacerbate the symptoms of AD [3, 40]. Similarly, individuals with severe atherosclerosis incur a substantially increased risk of developing dementia, with one study finding a 300% increase in the development of AD or vascular dementia in this population [41]. Furthermore, epidemiological studies consistently link type 2 diabetes, as well as intermediate stages of insulin resistance, with increased risk of developing AD [42-50] with T2DM patients having up to a 65% increased risk of developing AD [47]. It is noteworthy that many of the secondary complications from diabetes are related to damaged blood vessels; diabetics commonly suffer damage to vasculature in the retina leading to the development of retinopathy and vision loss (World Health Organization), they have double the risk of developing cardiovascular disease [51],

and a large portion (approximately 75%) of deaths in diabetics are attributed to coronary artery disease [52].

Interestingly, impaired neurovascular coupling precedes accumulation of β-amyloid in a mouse model of AD [53]. Similar findings have been reported in mice modeling ALS [54] and Parkinson's disease [55], wherein impaired BBB or endothelial function precedes symptomatic pathology. In the case of AD, vascular dysfunction may have a causal role in disease pathogenesis as β-amyloid is primarily cleared from the brain across the BBB by the low-density lipoprotein receptor-related protein 1 (LRP1) [56-59] and impaired clearance increases β-amyloid deposition in the brain. Age-associated cerebrovascular dysfunction may thus constitute the first "hit" in a two-hit mechanism [3] of disease pathogenesis in AD.

Conversely, lifestyle factors that reduce cardiovascular risk have been shown to reduce risk for both age-associated cognitive decline and AD. Studies in rats demonstrate that chronic exercise which improves overall vascular function also enhances microvascular growth in the cerebral cortex [60, 61]. Similarly, numerous human studies have shown a positive correlation between aerobic exercise capacity and cognitive function in healthy subjects regardless of age [62-64].

While it is clear that decreased CBF and vascular dysfunction contribute to cognitive impairment, the specific mechanisms that link endothelial dysfunction to decreased CBF are not yet fully understood. Alterations to CBF may be due to changes in microvascular density, vascular remodeling, impaired vessel reactivity, or any combination of these factors. It has been proposed that endothelial dysfunction may be directly responsible for decreases in cerebral capillary density that contribute to cognitive impairment. Since vascular dysfunction is primarily associated with aging and also associated with cognitive decline, and the mTOR inhibitor rapamycin delays aging, our and other laboratories are actively investigating the role of mTOR signaling in age-associated vascular changes such as the decline in endothelium-dependent vasodilation both for central and peripheral vasculature. Notably, mTOR hyperactivity has been suggested to be prevalent in AD brain as postmortem samples exhibit dysregulation of PTEN, S6K1, and Akt [65, 66],

all of which are part of the mTOR signaling cascade. Indeed, increased mTOR activity has been reported for AD brains [67].

TOR and the regulation of NO generation by endothelial nitric oxide synthase

Inhibition of mTOR has been demonstrated to delay endothelial cell senescence both *in vivo* [68] and *in vitro* [69], supporting the idea that mTOR may be causally involved in endothelial aging and thus may be a crucial target for disorders associated with vascular dysfunction. Similar findings have been reported in animal models of AD, wherein the activity of both mTOR and its downstream target p70S6K were shown to be increased in the hippocampus and cortex compared to controls [70, 71]. Our laboratory reported a pronounced decrease in global CBF as well as vessel rarefaction in the hAPP (J20) mouse model expressing human APP containing both the Swedish and Indiana familial mutations. Inhibition of mTOR by chronic rapamycin treatment abrogated these deficits [28]. Furthermore, these rapamycin-induced effects were mechanistically linked to increased endothelial NO synthase activity as determined by eNOS phosphorylation at Ser1176 [28], suggestive of increased local nitric oxide release and improved vasodilation. This point is of particular importance as a number of recent studies have implicated impaired NO signaling as a key mechanism of age-associated vascular dysfunction [7, 72]. This has been attributed to both decreased endothelial-dependent NO production and impaired sensitivity to NO [2].

Indeed, aged endothelial cells exhibit decreased NO production as well as diminished expression of eNOS [1], and multiple studies have confirmed impaired vessel reactivity in aging [8, 73, 74], particularly in regard to nitric oxide (NO)-induced vasodilation [6, 7]. Notably, eNOS deficiency has also been shown to facilitate atherosclerosis, insulin resistance, and general cardiac dysfunction [75-77], all of which are associated with decreases in CBF and subsequent cognitive dysfunction as discussed earlier. It has also been suggested that vessel constriction [78] or stiffening [79] may impair perivascular clearance of β-amyloid. All together, these findings suggest that unresponsive vascular endothelium is at least partially responsible for the long-term observed decrease in CBF in specific brain regions leading to subsequent cognitive dysfunction. In support of this idea, plasma levels of the endogenous eNOS inhibitor asymmetric dimethylarginine (ADMA) have been shown to increase during normal aging [80] and to be further elevated in subjects with AD [81] and elderly ischemic stroke victims [82]. Thus, it is becoming more clear that NO deficiency likely plays a significant role in vascular aging, and that mTOR may be mechanistically involved in this process, but the question remains: What are the molecular mechanisms by which mTOR modulates endothelial NO generation?

Though our understanding of how mTOR regulates NO production is incomplete, a number of potential links are currently being explored. A major target of mTOR is ribosomal protein S6 kinase beta-1 (S6K1). Mice fed a high fat diet [68] and old rats [83] have been shown to exhibit increased mTOR signaling or express hyperactive S6K1 in the aorta, respectively. Furthermore, overexpression of an active mutant S6K1 caused eNOS uncoupling and accelerated endothelial senescence in young cells [83]. Both rapamycin treatment and S6K1 silencing in these senescent cells enhanced NO production, suggesting that it is the downstream targets of mTOR activity that ultimately impinge upon eNOS and thereby regulate endothelial function. Interestingly, enhanced activity of the mTORC1 inhibitor AMPK, which facilitates TSC1/TSC2 activity and in turn inhibits the mTORC1 activator Rheb, activates eNOS [84]. Thus, it is possible that AMPK modulation of eNOS activity is accomplished through reduction of mTOR/S6K1 signaling. In support of this hypothesis, recent reports suggested that decreased AMPK activity is responsible for enhanced mTOR/S6K1 signaling and subsequent cardiac hypertrophy in spontaneously hypertensive rats [85, 86]. This observation suggests a potential mechanistic link between mTOR activity and endothelial dysfunction that could also be operant in vasculature of the CNS.

TOR-dependent vascular aging and age-associated neurological disease

It has been suggested [11] that organismal aging does not arise from gradual processes of functional decline operating uniformly in every organ and

physiological system, but that it is the result of specific age-associated changes involving a finite number of critical systems. The recent discovery of mTOR as a key regulator of mammalian aging has spurred research aimed at the identification of the mTOR-centered mechanisms that drive organismal aging. This research is crucial in combating aging-associated neurological diseases, most prominently AD, which is the most socially and economically costly disease in developed countries [87]. Thus, a mechanistic understanding of how TOR drives organismal aging is expected to be instrumental for interventions aimed at increasing healthspan, the period of life with good health and function [88]. Although the mechanisms through which TOR drives aging are still unknown, a wealth of prior data and our recent studies suggest that TOR-dependent vascular dysfunction may be central to the increased vulnerability of aged brains to specific neurological diseases.

CONCLUSION

The discovery of mTOR as a key regulator of mammalian aging has opened up multiple avenues for research aimed at identifying the specific age-associated mechanisms that increase vulnerability of aged individuals to Alzheimer's disease and other neurological diseases of aging. Recent evidence reviewed herein suggests that mTOR may be a critical mediator of brain vascular aging by mechanistically promoting endothelial dysfunction, a universal feature of mammalian aging [1-3]. mTOR-dependent vascular aging and dysfunction may thus constitute one mechanism by which mTOR promotes brain aging, and conceivably organismal aging [11]. This would not be unexpected, since changes associated with vascular aging have long been proposed to underlie decreased resilience and eventual loss of function associated with aging [89]. The discovery of mTOR as a specific molecular target involved in the determination of aging rate in mammals is a pivotal advance in the quest to elucidate mechanisms and design effective intervention strategies to attenuate, postpone, or even prevent a multitude of costly and devastating neurological diseases including atherosclerosis, Parkinson's and Alzheimer's disease. As endothelial dysfunction associated with aging is also prominent in peripheral tissues, mTOR could also potentially be targeted to alleviate peripheral vascular aging and thus provide novel interventions for age-associated ailments such as heart disease and complications of type 2 diabetes, among others. Future endeavors to elucidate the specific mechanism(s) by which mTOR promotes age-associated vascular dysfunction are imperative. The recent observation that mTOR inhibition with rapamycin achieves improved brain vessel reactivity and dilation through enhanced NO release [28] is a promising step forward in this effort.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest.

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