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Neurovascular mechanisms and blood–brain barrier disorder in Alzheimer’s disease

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Abstract

Vascular dysfunction has a critical role in Alzheimer’s disease (AD). Recent data from brain imaging studies in humans and animal models suggest that cerebrovascular dysfunction may precede cognitive decline and onset of neurodegenerative changes in AD and AD models. Cerebral hypoperfusion and impaired amyloid β -peptide ($A\beta$) clearance across the blood–brain barrier (BBB) may contribute to the onset and progression of dementia AD type. Decreased cerebral blood flow (CBF) negatively affects the synthesis of proteins required for memory and learning, and may eventually lead to neuritic injury and neuronal death. Impaired clearance of $A\beta$ from the brain by the cells of the neurovascular unit may lead to its accumulation on blood vessels and in brain parenchyma. The accumulation of $A\beta$ on the cerebral blood vessels, known as cerebral amyloid angiopathy (CAA), is associated with cognitive decline and is one of the hallmarks of AD pathology. CAA can severely disrupt the integrity of the blood vessel wall resulting in micro or macro intracerebral bleedings that exacerbates neurodegenerative process and inflammatory response and may lead to hemorrhagic stroke, respectively. Here, we review the role of the neurovascular unit and molecular mechanisms in vascular cells behind AD and CAA pathogenesis. First, we discuss apparent vascular changes, including the cerebral hypoperfusion and vascular degeneration that contribute to different stages of the disease process in AD individuals. We next discuss the role of the low-density lipoprotein receptor related protein-1 (LRP), a key $A\beta$ clearance receptor at the BBB and along the cerebrovascular system, whose expression is suppressed early in AD. We also discuss how brain-derived apolipoprotein E isoforms may influence $A\beta$ clearance across the BBB. We then review the role of two interacting transcription factors, myocardin and serum response factor, in cerebral vascular cells in controlling CBF responses and LRP-mediated $A\beta$ clearance. Finally, we discuss the role of microglia and perivascular macrophages in $A\beta$ clearance from the brain. The data reviewed here support an essential role of neurovascular and BBB mechanisms in contributing to both, onset and progression of AD.

Keywords

Alzheimer’s disease; Neurovascular; Blood–brain barrier; $A\beta$; Clearance

Neurovascular unit and blood–brain barrier

Dynamic communication between the cells of the neurovascular unit is required for normal brain functioning [45,105]. The neurovascular unit consists of all the major cellular

components of the brain including neurons, astrocytes, brain endothelium, pericytes, vascular smooth muscle cells (VSMC), microglia and perivascular macrophages.

The vascular tree of the brain originates from large arteries at the base of the brain at the circle of Willis. These large arteries travel through the dura mater branching into the leptomeningeal or pial arteries that travel on the surface of the brain in the subarachnoid space [104]. The surface pial arteries branch next into penetrating intracerebral arteries and arterioles (20–90 μm in diameter in human brain). The cerebral arteries consist of three layers: the tunica intima (endothelium), tunica media (mainly VSMC), and tunica adventitia (collagen, fibroblasts, running nerves). VSMC can control cerebral blood flow (CBF) [15], which is essential to the maintenance of the neurovascular unit.

It is generally thought that the penetrating intracerebral vessels are separated from brain parenchyma by the surrounding perivascular spaces also known as Virchow-Robin spaces [12,20,33,59]. It has been viewed for a long time that the Virchow-Robin space is an extension of the subarachnoid space and contains fluid that is close in composition to the cerebrospinal fluid [12]. However, more recent work has suggested that at the surface of the human brain there is a direct continuity of the subarachnoid space with these perivascular spaces, but the penetrating arterial perivascular spaces are separated from subpial and subarachnoid spaces by a membrane of leptomeningeal cells continuous with the pia mater [67,101]. It has been suggested that perivascular spaces are expandable in the white matter, central gray matter and midbrain [67]. Immunocompetent perivascular cells are normally found in these perivascular spaces [54].

The penetrating arteries further branch into the arterioles and capillary microvasculature (6–10 μm in diameter) composed of endothelium and pericytes that are partially separated by the basal lamina extracellular matrix that provides support for capillaries. The total length of capillaries in human brain is about 400 miles and the capillary surface area available for molecular transport is about 20 m^2 [7]. The tightly sealed monolayer of brain endothelial cells making these capillaries is the key component of the blood–brain barrier (BBB) which prevents the passive exchange of solutes between the blood and brain. Pericyte processes wrap the endothelium and communicate directly with endothelial cells through the specialized synapse-like “peg-socket” contacts [94]. Pericytes have an essential role in maintaining the stability of microvessels [94], and have also been shown to modulate CBF [65]. Astrocytic end feet also contact the abluminal capillary surface providing the brain with physical support. Sporadic microglia can also be found in the surrounding pericapillary area in normal brain.

A healthy brain relies on all of the cells of the neurovascular unit to function properly and communicate with each other in order for neuronal synapses and circuitries to maintain normal cognitive functions.

Alzheimer’s disease

Alzheimer’s disease (AD) is characterized by cerebrovascular and neuronal dysfunctions leading to a progressive decline in cognitive functions [105]. Pathological hallmarks of AD include neurofibrillary tangles consisting of hyper-phosphorylated microtubule-associated protein called tau and extracellular amyloid plaques. The main component of amyloid plaques in AD brains is amyloid β ($\text{A}\beta$) peptide. $\text{A}\beta$ (38–43 amino acids) is a proteolytic by-product from the amyloid precursor protein (APP) generated by the sequential β -secretase and γ -secretase cleavage. Recently, it has been shown that oligomeric $\text{A}\beta$ species (smallest of which are dimers) isolated from AD brains are the most synaptotoxic forms [79]. In the rodent hippocampus, oligomeric $\text{A}\beta$ species decrease neuronal long-term potentiation after high frequency stimulations and act through metabotropic glutamate receptors to enhance long-term depression and reduce neuronal dendritic spine density.

Rare, early onset AD (<1% of all AD cases) is caused by genetic mutations in the APP, presenilin 1 (PSEN1) or presenilin 2 (PSEN2) which lead to increased processing of APP [10]. Alternatively, mutations in the A β portion of APP, such as the Dutch (E693Q) [88], Iowa (D694N) [34], Flemish (A692G) [39], Arctic (E693G) [62] and Italian (E693K) [82], all lead to vasculotropic A β peptides that may cause cerebral hemorrhage without any evidence of increased APP processing. For instance, the Dutch mutant A β peptide preferentially accumulates on the cerebral vasculature, which is seemingly because of vascular clearance deficit of this mutant A β [19]. The majority of late-onset AD patients (>99%), however, do not have a mutation that cause an increase in APP processing [84]. This suggests that other pathogenic mechanisms besides increased proteolytic cleavage of APP can lead to the accumulation of A β and amyloid, such as impaired A β clearance [106].

Vascular changes in Alzheimer's disease

It was suggested 20 years ago that vascular defects present in AD may be important in the development of the disease [77]. More recently, data from clinical imaging, epidemiological and pharmacotherapy studies have indicated that vascular changes play an important role early in AD pathogenesis [21]. Magnetic resonance imaging (MRI), transcranial doppler measurements, and single photon excitation computed tomography (SPECT) in humans have established that the resting CBF is significantly reduced in AD patients, and this may be an early event in AD pathogenesis. Arterial spin-labeling MRI has demonstrated cerebral hypoperfusion in AD patients [48]. Functional MRI (fMRI) studies using blood oxygenation level dependent (BOLD) contrast to measure increases in CBF during a task that assess episodic memory have established that there is a delay in the CBF response in patients with mild cognitive impairment (MCI), and that this delay in fMRI-BOLD signal becomes more pronounced in AD patients [70]. This suggests that CBF reductions are present in the early stages of AD pathogenesis, as MCI is considered a potential transitional state between normal aging and dementia.

Longitudinal data from a large population-based study (1,730 participants of the Rotterdam Study), showed that higher CBF velocity, measured by transcranial doppler flowmetry, was related to a lower prevalence of cognitive decline [72]. MRI scans showed less hippocampal and amygdalar atrophy in the elderly patients with greater CBF. Furthermore, this study suggested that low CBF may contribute early to the progression of dementia, prior to the cognitive decline and cerebral atrophy. Another longitudinal study examining the conversion of MCI to AD using SPECT imaging showed significant CBF reductions in the parietal lobule, angular gyrus, and precuneus of MCI patients that had a high-predictive value of conversion to AD [41]. These data also suggest that regional reduction in CBF is an early event in AD.

Studies using 2-[18F] fluoro-2-deoxy-D-glucose (FDG)-PET, which measures cerebral glucose transport across the BBB, have shown reduction in cerebral glucose uptake in individuals with MCI or probable and possible AD prior to conversion to AD [26,44]. These studies have indicated that reduced brain glucose uptake is not a result of brain atrophy, but, on the contrary, it may precede neurodegeneration [75]. A longitudinal FDG-PET study has also suggested hippocampal reductions in glucose uptake during normal aging as a predictive factor of cognitive decline [61].

Several epidemiological and pathological studies have demonstrated positive links and overlap between cerebrovascular disorder, such as atherosclerosis and AD. For example, it was found that there is a threefold increase in the risk of developing AD or vascular dementia in people with severe atherosclerosis [42]. More recently, it was found in a large population-based study (678 participants of the Rotterdam Study) that atherosclerosis, primarily in the carotid arteries, is positively associated with the risk of developing dementia [89]. Furthermore, postmortem

grading of circle of Willis atherosclerotic lesions has showed that atherosclerosis was more severe in cases with AD and vascular dementia than in non-demented controls [6]. It has been suggested that the atherosclerotic changes in the arteries of the circle of Willis may account for the observed hemodynamic disturbances present in brains of AD individuals [52].

Another hypothesis of CBF reductions in AD has suggested that loss or abnormal cholinergic innervations of intracerebral blood vessels may contribute to brain hypoperfusion [29,47]. More recently, the upregulation of two transcription factors myocardin (MYOCD) and serum response factor (SRF) in AD cerebral VSMC has been shown to lead to arterial hypercontractility potentiating reduced CBF [15], as discussed below.

Vascular anatomical defects observed in AD further support the importance of vascular disorder in AD pathogenesis. These are atrophy and irregularities of arterioles and capillaries, swelling and increased number of pinocytotic vesicles in endothelial cells, increase in collagen IV, heparan sulfate proteoglycans and laminin deposition in the basement membrane, disruption of the basement membrane, reduced total microvascular density and occasional swelling of astrocytic end feets [5,11,29,35,51,90]. Reduced staining of endothelial markers CD34 and CD31 observed in AD brains suggests that there is an extensive degeneration of the endothelium during the disease progression [50]. Recent genomic profiling of brain endothelial cells has uncovered that extremely low expression of vascular-restricted mesenchyme homeobox 2 gene in AD individuals leads to aberrant angiogenesis and premature pruning of capillary networks resulting in reductions in cerebral microcirculation [99]. Thus, it is possible that brain endothelial morphological changes seen in AD are not caused necessarily by direct ischemic vascular injury, but may reflect a state of a failing vascular remodeling in the presence of overwhelming angiogenic stimuli and unresponsive endothelium.

Reduced smooth muscle alpha actin (SMA) expression has been suggested in AD vessels based on the immunostaining studies [28]. However, more recent quantitative Western blot analysis indicated that SMA expression may in fact be increased in AD VSMC when the SMA protein expression levels in cerebral VSMC were normalized to the levels of proteins whose expression was not altered by AD process [15].

Finally, cerebral amyloid angiopathy (CAA) with A β deposits in the VSMC layer of small cerebral arteries (Fig. 1) is a major pathological insult to the neurovascular unit in AD [85]. A β plaques also accumulate onto and around cerebral capillaries [16,92]. Decreased clearance of A β across the BBB and by cells of the neurovascular unit may contribute to CAA and parenchymal A β deposits, as discussed below. The prevalence of CAA in AD individuals and in the elderly population without AD is >80% and 10–40%, respectively [3,36]. A strong association between CAA and cognitive impairment has also been established [4]. CAA is a significant cause of cerebral hemorrhage in elderly population [46]. Clinical topographical imaging has shown cerebral microbleedings are present in roughly 30% of AD cases [66]. On the other hand, lobar cerebral hemorrhage occurs in 7–18% of AD cases [36], which may be due to a significant loss of the VSMC layer [53] leading to vessel rupture.

Matrix metalloproteinases, reactive oxygen species (ROS) and CAA

A β treatment of human VSMC has been shown to significantly increase the activation of matrix metalloproteinase 2 (MMP2) via increasing the mRNA expression of membrane type 1 (MT1)-MMP, the primary MMP2 activator at the cell surface [49]. MMP9 specifically has been shown to be significantly present in postmortem AD tissue [2]. Activated MMP9 can degrade basement membranes, extracellular matrix proteins and tight junction proteins subsequently damaging the integrity of the BBB [31,71,100] and potentially leading to spontaneous cerebral hemorrhages.

In vivo microdialysis administration of A β 40 in the rat brain has been shown to directly increase ROS, as measured by 2, 3-dihydroxybenzoic acid (reaction product of salicylate with hydroxyl radicals and/or peroxynitrite) [64]. Intracerebroventricular infusion of A β 42 in rats was also shown to increase ROS by downregulating important endogenous antioxidant enzymes such as mitochondrial magnesium-superoxide dismutase 2 (SOD2), glutathione peroxidase and glutathione-S-transferase [55].

High levels of ROS in AD may damage proteins essential for important neurovascular mechanisms. For example, Alzheimer's patients have been shown to have high levels of oxidized soluble form of the low-density lipoprotein receptor related protein 1 (sLRP), which under normal conditions is the key endogenous A β chaperone protein in plasma [73] as discussed below. 14-3-3 ζ and γ isoforms, proteins that are involved in cell growth, survival and differentiation, were also found to be highly oxidized in brain extracts from AD and CAA patients [76]. Interestingly, it has been shown in Tg2576 mice overexpressing Swedish mutant APP695 that vascular oxidative stress precedes parenchymal oxidative stress [63], suggesting that vascular insults may be an early event in the development of AD-like pathology in this model.

Transport of A β via the receptor for advanced glycation endproducts (RAGE) across the BBB provides a major source of A β that can deposit in the brain and can directly lead to neuroinflammation via activating nuclear factor- κ B mediated secretion of proinflammatory cytokines, such as tumor necrosis factor- α and interleukin 6 [23], that may reduce the BBB patency. The breakdown of the BBB may in turn disrupt the normal transport of nutrients, vitamins and electrolytes across the BBB, which are essential for proper neuronal functioning. Therefore, therapies that reduce ROS, MMP2, and MMP9, as for example with some forms of non-anticoagulant-activated protein C (APC) analogs [14,37], or that block RAGE-A β interaction may be potentially useful strategies to correct BBB dysfunction in AD.

A β clearance hypothesis

There is a significant body of evidence suggesting that A β accumulates in the wall of cerebral vessels and in the brains of AD individuals because of imbalances between its production and clearance from the brain [106]. Figure 2 illustrates some of the major A β clearance routes with emphasis on vascular clearance.

A β that is produced both in the brain and periphery by a number of different cell types is transported across the BBB via receptor-mediated transcytosis. RAGE is the key receptor that transports A β from the blood into the brain [23], which occurs at a rate that is five- to sixfold lower than the rate determined for the transport of large neutral amino acids [78]. LRP is the major cell surface A β clearance receptor that transports A β out of the brain across the BBB [9,25,80] and promotes A β clearance on VSMC [87] (Fig. 2). A β is not only cleared from the brain interstitial fluid (ISF) [9] as a soluble peptide, but can also be transported by its chaperone proteins in the ISF, such as apolipoprotein E (apoE), apolipoprotein J, and α 2-macroglobulin [103].

A β clearance directly into the blood seems to be the most prominent pathway, but it has also been suggested that there exists a perivascular route for the clearance of A β in the human brain [67] (Fig. 2). The pulsation force of cerebral blood vessels has been proposed to drive a A β drainage route along the perivascular spaces [97]. In this context, vessel constriction [15] and/or stiffening [97] may reduce A β clearance along the perivascular spaces by reducing the pulsatile flow which in turn would increase A β deposition in the arterial wall of AD patients.

A β can also be directly degraded by cells of the neurovascular unit. A vast body of evidence also suggests an important role of A β degrading enzymes such as insulin degrading enzyme

and neprilysin in the clearance of A β (Fig. 2), which is not discussed in detail here, but is reviewed elsewhere [74].

If any of the clearance pathways are disrupted, soluble A β will accumulate and promote the formation of toxic A β oligomeric and aggregated species, which have devastating effects on the neurovascular unit. Furthermore, vasculotropic A β mutations, such as the Dutch mutation, decrease the clearance rate of the peptide by approximately 6.8-fold when compared with the wild-type A β peptide clearance rates [60].

sLRP “sink” mechanism for A β clearance

Soluble LRP (sLRP) in plasma has recently been identified as a major endogenous peripheral “sink” agent of A β [73] (Fig. 2). The N-terminus of LRP can be cleaved at the cell surface by β -secretase [93] and this extracellular domain of LRP exists in plasma as sLRP [68]. A β can bind directly to sLRP in vitro and μ 70% of A β in human plasma is associated with sLRP [73]. Furthermore, sLRP in AD patient’s plasma is significantly oxidized and does not efficiently bind to A β . These data have suggested that sLRP in human plasma acts as peripheral sink pulling A β from the brain into the blood, and carrying it to the liver for degradation. Cell surface LRP also mediates systemic A β clearance by the liver [83]. Understanding the specific A β -LRP interactions will greatly advance our understanding of this A β clearance mechanism. It has been recently reported that A β binds to clusters II and IV of LRP via its C-terminal end [22].

ApoE and A β clearance across the BBB

ApoE is a reactive apolipoprotein that has a major function in the transport of lipids and cholesterol in our body. ApoE exists in 3 isoforms (apoE2, apoE3, and apoE4) in humans. Roughly, 40–65% of individuals that develop AD carry at least one apoE4 allele, and apoE4 homozygosity increases ones chance of developing AD from 20 to 90% [18]. On the other hand, carrying an apoE2 allele seems to reduce the risk of individuals to develop AD [17]. The exact relationship how apoE genotype influences AD pathogenesis and A β metabolism is still a subject of intense research.

With respect to vascular clearance, it has been shown that apoE is an A β chaperone protein [98] (Fig. 2). ApoE has been associated with impaired transport of A β across the BBB [9,24, 58]. Particularly, apoE2-A β and apoE3-A β complexes are cleared at the BBB at a substantially faster rate than apoE4-A β complexes, and lipidated apoE-A β complexes are cleared across the BBB significantly slower than their respective lipid-poor apoE-A β complexes [24]. It seems that while free A β can be rapidly cleared from the brain mainly via LRP, A β -ApoE complexes are cleared by the very low-density lipoprotein receptor at a much slower rate, causing A β retention in the brain.

Interestingly, pericytes with apoE2 or apoE3 genotype compared to pericytes with an apoE4 allele were more resistant to toxic effects of A β 40 carrying an E22Q mutation, as seen in hereditary cerebral hemorrhage with amyloidosis of the Dutch type [91]. The distribution of cerebrovascular amyloid in AD varies with apoE genotype and specifically the increasing dose of apoE4 alleles has been associated with increased CAA [1,86]. Future research understanding cellular and molecular mechanism by which apoE genotype influences the disease process in AD individuals may be useful in developing both the diagnostic tools and new therapeutic opportunities for AD.

Myocardin and serum response factor transcriptional control of A β clearance

MYOCD, a SAF-A/B, Acinus and PIAS domain family nuclear protein, is a smooth muscle specific transcriptional co-activator that binds SRF to induce gene expression [95]. SRF is a ubiquitously expressed transcription factor that binds to a 10 base pair cis element called a CArG box, which is located in the regulatory region of numerous target genes [81]. MYOCD and SRF constitute a molecular switch for the VSMC differentiation program [13,56]. We have shown that increased MYOCD and SRF expressed in AD VSMC contribute to arterial hypercontractility and cerebral hypoperfusion [15]. Specifically, MYOCD and SRF increase the levels of genes encoding contractile proteins and regulating calcium homeostasis causing arterial hypercontractility and reduced CBF (Fig. 3 white pathway). This chronic arterial hypercontractility may reduce the arterial pulsations because the vessel no longer properly relaxes, and therefore the contribution of A β clearance along the perivascular spaces may also be reduced.

Recently, CAA-positive cerebral blood vessels isolated from AD patients have also been shown to have a significant increase in MYOCD and SRF levels [8] confirming the above findings. However, neither A β nor ROS effect the expression of MYOCD or SRF [15], therefore it seems that the increase in MYOCD and SRF is not due to oxidant stress or A β -related VSMC injury. Therefore, the high expression of MYOCD and SRF in CAA-positive AD vessels may be just by coincidence or alternatively, high levels of MYOCD and SRF may negatively affect the clearance of A β from the brain. Recent data, suggesting the latter, show that increased levels of MYOCD and SRF in AD VSMC may suppress A β clearance and thus exacerbate CAA. We have shown that high levels of MYOCD and SRF in vitro and in vivo directly lead to increased expression of sterol response element binding protein 2 (SREBP2), which mediates LRP transcriptional repression, causing CAA and focal brain A β accumulations due to reduced LRP-dependent clearance (Fig. 3 yellow pathway).

MYOCD and SRF activate SREBP2 transcription in a CArG dependent mechanism. SREBP2 represses LRP transcription via binding to the sterol response element in the LRP 5' untranslated region [57]. Low levels of LRP in VSMC reduce the clearance of A β and therefore potentiate CAA and focal A β accumulation. It is of note that small pial and intracerebral arteries in AD do not typically develop atherosclerotic changes and accumulation of aggregated low-density lipoproteins and cholesterol esters [43]. According to the present data this could be due to MYOCD-SRF-mediated SREBP-2 stabilization and SREBP-2 dependent LRP downregulation. However, a potential side effect of such an "anti-atherosclerotic" VSMC phenotype in small resistant brain arteries, may be the loss of the LRP-dependent A β clearing function and development of CAA with focal brain amyloidosis.

Hypoxia has been shown to increase MYOCD levels in VSMC [8,15,69]. The molecular mechanism by which hypoxia increases MYOCD is not yet completely understood. It has been suggested that midkine upregulation under hypoxic conditions is responsible for MYOCD overexpression [69]. However, we have recently identified a putative conserved hypoxia response element in the MYOCD promoter, suggesting possible direct activation of MYOCD by hypoxia inducible factor 1 α . More experimental data will be required to fully elucidate how hypoxia induces MYOCD.

A β clearance by microglia and perivascular macrophages

Microglial and perivascular macrophages may play role in clearing A β from the brain (Fig. 2) and in enhancing AD-related inflammation. Microglia have been shown to be significantly activated in AD brains and localized at sites of amyloid deposition [96]. Early activation of microglia in early AD pathogenesis has been show to be beneficial in scavenging and clearing toxic A β from the brain. Specifically, decreased microglia accumulation in CC-chemokine

receptor deficient Tg-2576 AD transgenic mice increased mortality and A β accumulation particularly around blood vessels [27]. However, as the disease progresses the microglial activation may lead to inflammation, reduced A β clearance and severe neurodegeneration [40].

Perivascular macrophages are CD163 (hemoglobin-haptoglobin scavenger receptor) and CD206 (mannose receptor) positive immune cells located in outer aspects of blood vessels within the brain. Perivascular macrophages are antigen-presenting phagocytotic cells, and have been shown to respond to CNS inflammation. Interestingly, blood-derived macrophages from AD patients were shown to be less effective at phagocytosing A β compared with cells derived from non-demented control patients [30]. It has also been shown that A β induces migration of monocytes across the human BBB via RAGE and platelet endothelial cell adhesion molecule 1 [32]. Clodronate-mediated depletion of perivascular macrophages in vivo significantly increased CAA in TgCRND8 AD transgenic mice, whereas chitin stimulated perivascular macrophages turnover and promoted A β clearance [38]. These data suggest an important role of microglia and perivascular macrophages in A β clearance, and provide additional evidence that A β is cleared along perivascular spaces. Whether the role of these cells in AD is more beneficial or harmful still remains controversial. Further experimental data are needed to resolve this issue.

Conclusions

The elaborate interactions between the different cell types of the neurovascular unit provide the brain with a healthy environment that is required for proper cognitive functioning. The role of the neurovasculature and BBB in the progression of neurodegenerative diseases, such as AD reviewed here, are now becoming more fully appreciated. Recently, breakdown of the blood spinal cord barrier and reductions in microcirculation have been implicated in the early disease onset prior to motor neuron degeneration in mutant SOD1 expressing transgenic mice, a model of familial amyotrophic lateral sclerosis (ALS) [102]. It will be important to continue evaluating the degree of neurovascular and BBB involvement in other neurodegenerative diseases, such as ALS, multiple sclerosis, and Parkinson's disease. Ultimately understanding the different molecular mechanisms of the neurovascular uncoupling and BBB breakdown present in neurodegenerative diseases will allow for the development of more effective diagnostic procedures and treatment strategies for different forms of neurodegeneration.

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SMA Thioflavin S

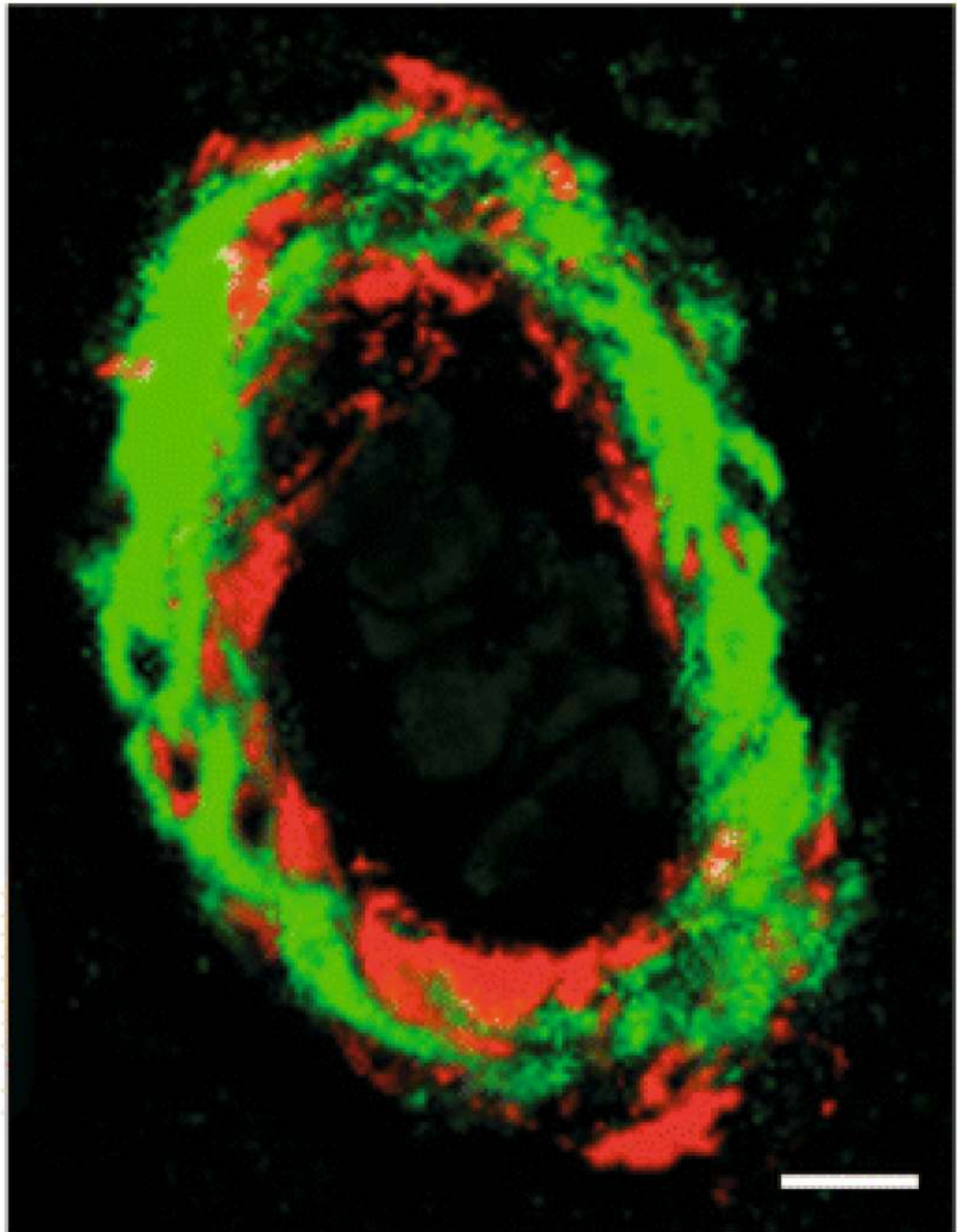


Fig. 1. Cerebral amyloid angiopathy in AD. Immunofluorescent staining of smooth muscle α actin (SMA; *red*) and amyloid staining (thioflavin S, *green*) in an AD cerebral vessel from Brodmann area 9. Staining shows significant amyloid accumulation in the vascular smooth muscle cell (VSMC) layer of this blood vessel. Amyloid accumulation may result from decreased $A\beta$ clearance along the perivascular spaces caused by a decreased low-density lipoprotein receptor related protein-1 (LRP)-mediated $A\beta$ clearance by VSMC, faulty $A\beta$ clearance by perivascular macrophages and/or reduced passive $A\beta$ drainage due to reductions in the arterial pulsatile blood flow. CAA can lead to spontaneous hemorrhage and rupture of the vessel wall due to a

loss of the VSMC layer, enzymatically-induced breakdown of the vessel wall, oxidant stress and cytokine-mediated vascular injury. *Scale bar 25 μm*

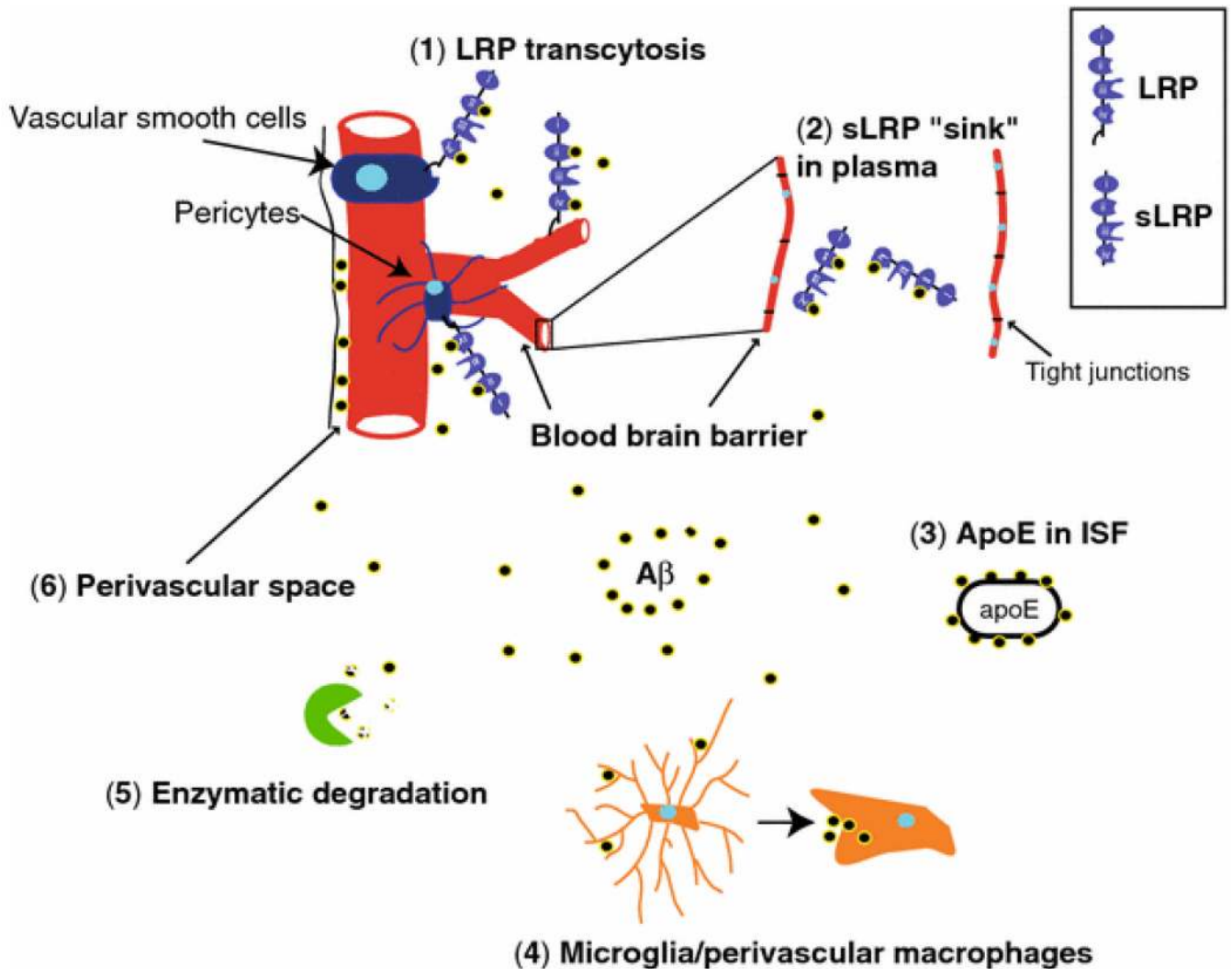


Fig. 2.

Essential A β clearance vascular and other routes. A β clearance can occur via several routes: 1 LRP-mediated transcytosis (purple, receptor) across the blood–brain barrier (red, capillaries) removes A β from brain interstitial fluid to blood and LRP-mediated degradation of A β on vascular smooth muscle cells and pericytes lowers A β levels in perivascular spaces (blue, cells), 2 soluble LRP, sLRP-mediated (purple, soluble receptor) endogenous A β “sink” action in plasma increases peripheral A β clearance and lowers the levels of free A β in the circulation which in turn promotes the cell surface LRP-mediated clearance of brain-derived A β across the blood–brain barrier, 3 A β chaperones in brain interstitial fluid such as ApoE isoforms may reduce clearance of brain-derived A β in an isoform-specific manner, i.e., apoE4 > apoE3 or apoE2, 4 clearance of A β by microglia and perivascular brain macrophages (orange, cells) from brain parenchyma and perivascular spaces, respectively, 5 direct enzymatic degradation of A β in the brain (green, enzymes), and 6 elimination of A β along the perivascular spaces by passive drainage that is influenced by the arterial pulsatile flow. The illustrated pathways by all means do not cover in detail all possible routes that control A β levels in the brain

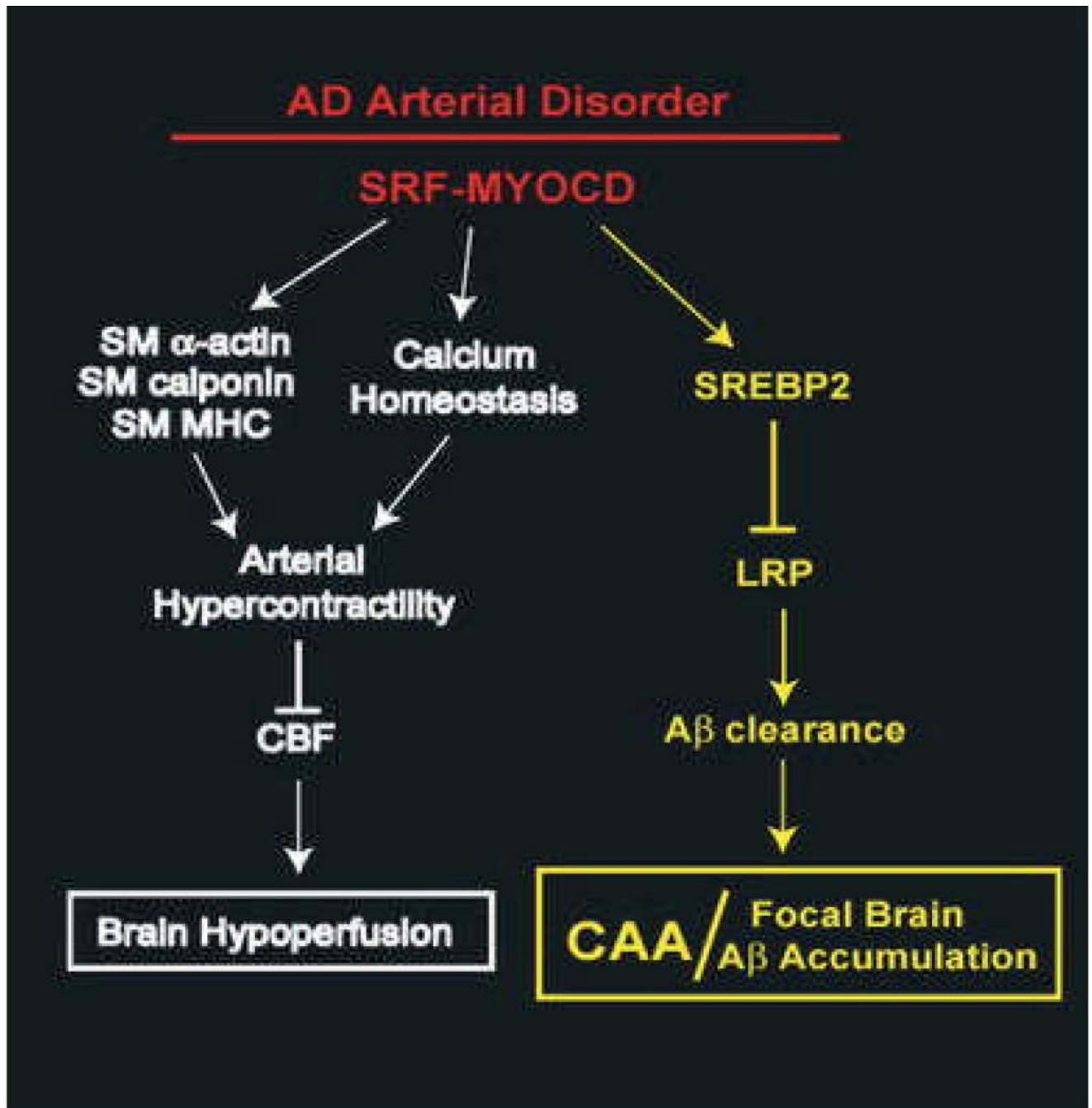


Fig. 3. MYOCD and SRF hypothesis of AD arterial pathology. High levels of SRF-MYOCD in AD VSMC contribute to brain hypoperfusion (*white pathway*) by increasing the expression of contractile proteins, such as smooth muscle (SM) α -actin, calponin, and myosin heavy chain (MHC) and by increasing the expression of genes that regulate calcium homeostasis. This leads to arterial hypercontractility, reduced resting cerebral blood flow (CBF) and attenuated CBF responses to brain activation, which ultimately creates a chronic hypoperfusion state. Furthermore, SRF-MYOCD potentiate CAA and focal brain A β accumulation (*yellow pathway*) via CArG-box dependent activation of SREBP2, which acts as transcriptional suppressor of LRP, a key A β clearance receptor