Neurovascular Dysfunction and Faulty Amyloid β-Peptide Clearance in Alzheimer Disease

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Neurovascular dysfunction is an integral part of Alzheimer disease (AD). Changes in the brain vascular system may contribute in a significant way to the onset and progression of cognitive decline and the development of a chronic neurodegenerative process associated with accumulation of amyloid β-peptide (Aβ) in brain and cerebral vessels in AD individuals and AD animal models. Here, we review the role of the neurovascular unit and molecular mechanisms in cerebral vascular cells behind the pathogenesis of AD. In particular, we focus on blood–brain barrier (BBB) dysfunction, decreased cerebral blood flow, and impaired vascular clearance of Aβ from brain. The data reviewed here support an essential role of the neurovascular and BBB mechanisms in AD pathogenesis.

Alzheimer disease (AD) is a neurodegenerative disorder associated with neurovascular dysfunction (Zlokovic 2005, 2010; de la Torre 2010; Marchesi 2011), cognitive decline (Cummins 2004), and accumulation in brain of amyloid β peptide (Aβ; Querfurth and LaFerla 2010) and tau-related lesions in neurons termed neurofibrillary tangles (Battatore et al. 2007; Ittner and Gotz 2011). Multiple epidemiological studies have demonstrated a remarkable overlap among risk factors for cerebrovascular disorder and sporadic, late-onset AD (de la Torre 2010; Jellinger 2010; Kalaria 2010). For example, mid-life diabetes (Luchsinger et al. 2007; Knopman and Roberts 2010), hypertension (ladecola and Davison 2008), and obesity (Whitmer et al. 2008) have all been shown to increase the risk for both AD and vascular dementia. It is now generally acknowledged that most AD cases have mixed vascular pathology and small-vessel disease (Jellinger 2010; Marchesi 2011). Moreover, reduced brain blood perfusion (Ruitenber et al. 2005), silent infarcts (Vermeer et al. 2003), and the presence of one or more infarctions (Snowdon et al. 1997) all increase the risk of AD.

The amyloid hypothesis states that Aβ initiates a cascade of events leading to neuronal injury and loss (Hardy and Selkoe 2002) associated with cognitive decline (Cummins 2004). According to an alternative two-hit vascular hypothesis of AD (Zlokovic 2005, 2010; de la Torre 2010; Marchesi 2011), Aβ accumulation in the brain is a second insult (hit 2) that is initiated by vascular damage (hit 1; Fig. 1). Although the molecular and cellular events for
each step in the disease process and for each risk factor are not absolutely clear, all vascular factors might share a common final disease pathway, involving brain microvascular dysfunction and/or degeneration, as well as Aβ and tau pathology (Zlokovic 2011), as discussed below. The vascular hypothesis maintains that reduced cerebral blood flow (CBF) and hypoxia, from one end, and blood–brain barrier (BBB) dysfunction associated with accumulation of different vasculotoxic and neurotoxic macromolecules in the brain, from the other, can initiate neuronal dysfunction and neurodegenerative changes independently and/or prior to Aβ deposition (Zlokovic 2005, 2010; Bell et al. 2010; de la Torre 2010; Marchesi 2011). Moreover, several studies have suggested that cerebrovascular dysfunction and injury lead to faulty Aβ clearance from brain (Deane et al. 2003; Eisele et al. 2010), and/or elevated expression of β-amyloid precursor protein (APP; Atwood et al. 2002; Kumar-Singh et al. 2005; Cullen et al. 2006; Weller et al. 2008), resulting in Aβ accumulation in the brain and around
cerebral blood vessels. Elevated levels of Aβ in brain may in turn accelerate neurovascular (Deane et al. 2003; Bell et al. 2009) and neuronal (Yan et al. 1996; Walsh et al. 2002; Takuma et al. 2009) dysfunction and promote self-propagation (Meyer-Luehmann et al. 2006, 2008; Eisele et al. 2010), as in prion diseases (Prusiner 1996), leading to cerebral β-amyloidosis (Zlokovic 2008).

Here we will review the role of the neurovascular unit and molecular mechanisms within cerebral vascular cells behind the pathogenesis of AD. In particular, we will focus on BBB dysfunction, decreased CBF, and impaired vascular clearance of Aβ from brain.

**NEUROVASCULAR UNIT**

The neurovascular unit (NVU) consists of different cell types, including (1) vascular cells such as brain endothelial cells, a site of the anatomical BBB in vivo, pericytes, and vascular smooth muscle cells (VSMCs), (2) glial cells such as astrocytes, microglia and oligodendroglia, and (3) neurons (Fig. 2A; Zlokovic 2008; Guo and Lo 2009; Moskowitz et al. 2010). The close proximity of nonneuronal neighboring cells with each other and with neurons allows for effective cell-to-cell cross-communications that are critical for normal functions in the healthy central nervous system (CNS) and are increasingly recognized as important in the disease process in multiple neurological disorders (Boilee et al. 2006; Zhong et al. 2008; Zlokovic 2008).

The NVU functions in the healthy brain include control of neurovascular coupling and BBB permeability, matrix interactions, inactivation of neurotransmitters, signaling through angioneurins (i.e., growth factors that have both the neurotrophic and vasculotrophic functions) (Zacchigna et al. 2008), and clearance of toxins from brain (Fig. 2B).

The BBB is a highly specialized, continuous endothelial cell membrane that normally prevents the entry of plasma components, red blood cells, and leukocytes into the brain. In addition, through specific transporters in brain endothelium, the BBB regulates delivery of energy metabolites and essential nutrients that are required for proper neuronal and synaptic functions. The BBB is responsible for maintaining the constant “chemical” composition of brain interstitial fluid required for optimal brain function. Under physiological conditions, the BBB and pericytes control entry from blood and promote clearance from brain of various potentially neurotoxic and vasculotoxic macromolecules (Zlokovic 2008).

Alzheimer disease is associated with microvascular dysfunction, defective BBB, and vascular factors (Bailey et al. 2004; Wu et al. 2005;...
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Zlokovic 2005, 2008; Paul et al. 2007; Zipser et al. 2007; Kalaria 2010; Knopman and Roberts 2010; Miyazaki et al. 2011; Neuwelt et al. 2011). Microvascular degeneration diminishes CBF, resulting in shortages in oxygen supply, energy substrates, and nutrients to the brain. On the other hand, microvascular defects compromise clearance of neurotoxic molecules from brain, resulting in accumulation of pathological deposits in brain interstitial fluid, nonneuronal cells, and neurons. According to the recent evidence, microvascular injury ultimately leads to neuronal dysfunction and development of neurodegenerative changes. Vascular damage may also contribute to the development of cerebral β-amyloidosis and cerebral amyloid angiopathy (CAA) caused by accumulation of Aβ in brain and the vessel wall, respectively (Zlokovic 2008).

CEREBRAL BLOOD FLOW DYSREGULATION AND REDUCTIONS

An adequate blood supply is ensured by a tight coupling between local tissue metabolic demands and blood perfusion of the active neuronal site. The link between regional synaptic activity and a CBF increase is known as functional hyperemia. Neurovascular coupling requires intact and effectively innervated pial and intracerebral arteries and normal responsiveness of brain endothelium, VSMCs, and pericytes to vasoactive stimuli (Iadecola 2004; Peppiatt et al. 2006; Bell et al. 2010). In addition to VSMCs, recent studies have shown that pericytes control brain capillary diameter by constricting the vessel wall (Peppiatt et al. 2006), which under ischemic conditions can completely obstructs capillary flow (Yemisci et al. 2009). Astrocytes have also been shown to regulate CBF responses by influencing contractile properties of small penetrating intracerebral arteries (Takano et al. 2007; Kuchibhotla et al. 2009).

Functional hyperemia is the basis for functional magnetic resonance imaging that has revolutionized our understanding of human brain in health and disease (Girouard and Iadecola 2006). Functional neuroimaging studies in AD individuals have shown that neurovascular uncoupling or diminished CBF responses to brain activation may occur prior to neurodegenerative changes (Smith et al. 1999; Bookheimer et al. 2000; Ruitenberg et al. 2005; Knopman and Roberts 2010). In addition, it has been reported that cognitively normal individuals bearing the apolipoprotein E (APOE) ε4 allele, which is known to be the major genetic risk factor for late-onset AD (Bertram et al. 2007; Kim et al. 2009; Verghese et al. 2011), have reduced functional hyperemia response in the absence of brain atrophy or Aβ/amyloid accumulation (Sheline et al. 2010). Diminished resting CBF has been also shown in elderly individuals at risk to develop AD (Iadecola 2004; Knopman and Roberts 2010).

Studies in animal models have indicated that CBF reductions can induce and/or amplify neuronal dysfunction and/or neuropathological changes resembling AD pathology. For example, it has been shown that Aβ constricts cerebral arteries (Thomas et al. 1996), and that endothelium-dependent regulation of cortical microcirculation is diminished in a mouse model of AD before Aβ accumulation (Iadecola et al. 1999). Moreover, in AD mice, mild hypoperfusion increases neuronal Aβ levels and tau phosphorylation at an epitope associated with AD-type paired helical filaments (Koike et al. 2010). Brain ischemia in rodents leads to accumulation of hyperphosphorylated tau in neurons and filament formation similar to that present in human AD tauopathy (Gordon-Kraicer et al. 2007). Arterial carotid occlusion in rats leads to memory impairment, neuronal dysfunction, synaptic changes and accumulation of neurotoxic Aβ oligomers (Wang et al. 2010). Mice expressing APP and transforming growth factor β develop neurovascular uncoupling, cholinergic denervation, accelerated Aβ deposition and age-dependent cognitive decline (Ongali et al. 2010).

It has been demonstrated that moderate CBF reductions, comparable to those as seen in the aging brain, are associated with diminished cerebral protein synthesis (Hossmann 1994; Iadecola 2004). CBF reductions >50% impair ATP synthesis and decrease the ability...
of neurons to fire action potentials. In addition, focal CBF reductions comparable to those as in chronic neurodegenerative disorders such as AD lead to shifts in intracellular pH, water and electrolytes that are attributed to a loss of activity of multiple energy-dependent ion pumps such as sodium/hydrogen exchanger and ATP-dependent sodium pump at the BBB (Zlokovic 2008). CBF reductions may also lead to accumulation of different toxins and glutamate in brain owing to a loss of activity at the BBB of ATP-binding cassette (ABC) efflux transporters (Dutheil et al. 2010; Elali and Hermann 2011) and Na-dependent transporters for the excitatory amino acids (O’Kane et al. 1999; Hardingham 2009), respectively. Severe reductions in CBF (>80%), similar to those found after an ischemic stroke lead to neuronal death. It is of note that changes in the NVU including degeneration of brain capillaries and/or reductions in the resting CBF may be the first sign of the disease process prior to neuronal changes and neurodegeneration.

MICROVASCULAR DEGENERATION

Alzheimer disease individuals and other dementia patients frequently have focal degenerative changes in brain microcirculation including atrophy and reductions in capillary network, a rise in endothelial vacuolization and loss of mitochondria, accumulation of collagen and perlecans in the basement capillary membrane, loss of BBB tight junction proteins (Farkas and Luiten 2001; Bailey et al. 2004; Iadecola 2004; Wu et al. 2005; Zlokovic 2005; Kalaria 2010), and leakage of blood-derived molecules (Paul et al. 2007; Zipser et al. 2007; Kalaria 2010). Aβ accumulation and amyloid deposition in pial and intracerebral arteries lead to CAA, which according to some studies is present in >80% of AD patients (Jellinger 2010; Viswanathan and Greenberg 2011). AD patients with CAA frequently develop atrophy in the VSMC layer of small arteries, causing a rupture of the vessel wall and intracerebral bleeding in about 30% of patients, which in turn aggravates dementia (Ghiso and Frangione 2002; Cordonnier 2011). Patients with hereditary cerebral β-amyloidosis with CAA in leptomeningeal and intracerebral arteries of the Dutch, Iowa, Arctic, Flemish, Italian, and Piedmont L34V type develop massive hemorrhagic strokes and dementia owing to VSMC degeneration in the vessel wall (Fossati et al. 2010). Similar, duplication of the APP gene results in AD dementia with CAA and intracerebral hemorrhage (Rovelet-Lecrux et al. 2006).

BBB DYSFUNCTION IN AD

Changes in the expression of several BBB transporters mediating nutrient transport, ion pumps, ABC transporters, and/or receptors mediating transport of peptides and proteins, including blood-to-brain and brain-to-blood exchanges of Aβ, have been described in AD and AD models (see below). Here we will focus on (1) glucose transporter 1 (GLUT1), which is a BBB-specific transporter that is of special importance because glucose is a key energy source for brain, (2) the receptor for advanced glycation end products (RAGE) that mediates Aβ reentry into the brain from circulation and the neurovascular inflammatory response, and (3) lipoprotein receptor-related protein 1 (LRP), which is a major Aβ clearance receptor at the BBB mediating Aβ efflux from brain and its systemic clearance.

GLUT1

GLUT1 expression at the BBB is diminished in AD individuals (Mooradian et al. 1997), suggesting a shortage in glucose supply to the brain. Positron emission tomography (PET) studies with 18F-2-fluoro-2-deoxy-D-glucose (FDG), have demonstrated diminished glucose uptake by the brain in individuals at increased risk for dementia (Hunt et al. 2007; Herholz 2010). Several studies have suggested that reduced glucose uptake across the BBB as seen by FDG-PET can precede brain atrophy (Mosconi et al. 2006; Hunt et al. 2007; Samuraki et al. 2007; Mosconi et al. 2008; Herholz 2010) and may be used as a potential biomarker for AD (Miller 2009; Perrin et al. 2009).
RAGE

RAGE is a multiligand receptor of the immunoglobulin superfamily (Neeper et al. 1992). RAGE binds distinct classes of ligands including AGE proteins, S100/calgranulins, Aβ, amphoterin, and the family of crossed β-sheet macromolecules (Yan et al. 2010). RAGE interaction with ligands activates signal transduction pathways, leading to sustained cellular stress as shown in chronic diseases such as diabetes, inflammation, and AD (Bucciarelli et al. 2002; Bierhaus et al. 2005; Schmidt et al. 2009). The extracellular domain of RAGE contains one V-type and two C-type immunoglobulin domains (Yan et al. 2010). Most ligands bind to RAGE’s V-domain. A single, transmembrane spanning domain is followed by a short, charged cytoplasmic domain-mediating signal transduction after ligand binding to RAGE (Yan et al. 2010). A recent crystal structure analysis of RAGE revealed a versatile structure, which explains the ability of RAGE to bind multiple, structurally distinct ligands (Koch et al. 2010; Park et al. 2010).

As a cell surface receptor for Aβ (Yan et al. 1996), RAGE binds monomeric and oligomeric Aβ via its V-domain and aggregated Aβ via its C1 domain (Sturchler et al. 2008; Yan et al. 2010). RAGE mediates Aβ-induced neurotoxicity directly by causing oxidant stress and indirectly by activating microglia (Yan et al. 1996). In addition, intraneuronal Aβ transport via RAGE leads to mitochondrial dysfunction (Takuma et al. 2009). Targeted expression of RAGE in neurons in APP-transgenic mice accelerates cognitive decline and Aβ-induced neuronal perturbation (Arancio et al. 2004).

Expression of RAGE is increased in cerebrovascular endothelial cells under pathological conditions, including those seen in AD models and AD (Yan et al. 1995, 1996; Deane et al. 2003). At the BBB RAGE mediates (1) transport of circulating Aβ into the brain (Mackic et al. 1998; Deane et al. 2003), (2) NF-κB-dependent endothelial cell activation resulting in neuroinflammatory response, and (3) generation of endothelin-1 suppressing the CBF (Fig. 3; Deane et al. 2003). In addition, expression of RAGE in brain endothelium initiates cellular signaling, leading to monocyte trafficking across the BBB (Giri et al. 2000). It is of note that RAGE expression is increased in both neurons and endothelium in an Aβ-rich or AGE-rich environment as in AD (Yan et al. 1995), which amplifies Aβ-mediated pathogenic responses.

The cellular events triggered by RAGE at the BBB, neurons, microglia, and VSMCs may be implicated in the onset and progression of disease in AD models and possibly in AD. Therefore, RAGE is a potential therapeutic target in AD and blocking RAGE might contribute to control of Aβ-mediated brain disorder.

LRP

LRP, a member of the LDL receptor family, has a dual role as a rapid cargo endocytotic cellular transporter and a transmembrane cell signaling receptor (Zlokovic et al. 2010). LRP regulates transport and metabolism of apoE-associated cholesterol (Herz 2001; Herz and Strickland 2001; Herz et al. 2009). Its extracellular heavy α-chain (515 kDa) is noncovalently linked to a transmembrane and cytoplasmic light β-chain domain (85 kDa). The α-chain has four ligand-binding domains (clusters I–IV; Obermoeller-McCormick et al. 2001; Meijer et al. 2007). Domains II and IV bind more than 40 structurally diverse ligands including, to name a few, apoE, α2-macroglobulin (α2M), tissue plasminogen activator (tPA), proteinase-inhibitors, blood coagulation factor VIII, receptor-associated protein (RAP), Aβ, prion protein, and aprotinin (Hussain et al. 1999; Neels et al. 1999; Herz 2001; Herz and Strickland 2001; Croy et al. 2003; Deane et al. 2004; Meijer et al. 2007; Demeule et al. 2008; Lillis et al. 2008; Parkyn et al. 2008; Herz et al. 2009; Zlokovic et al. 2010). LRP’s cytoplasmic tail comprises two NPYX motifs, and one YXXL motif and two di-leucine motifs that both are required for rapid endocytosis of LRP ligands (Li et al. 2001; Deane et al. 2004, 2008). The cytoplasmic tail phosphorylated on serine and/or tyrosine residues (Bu et al. 1998; van der Geer 2002) interacts with different adaptor proteins associated with cell signaling such as disabled-1,
FE65, and postsynaptic density protein 95 (Trommsdorff et al. 1998; Gotthardt et al. 2000; Herz et al. 2009).

Within the NVU, LRP is expressed in brain endothelium, VSMCs, pericytes, astrocytes, and neurons (Herz and Bock 2002; Polavarapu et al. 2007). LRP internalizes its ligands and directs them to lysosomes for proteolytic degradation. Recent studies have demonstrated that LRP also transports its ligands transcellularly across

Figure 3. The role of blood–brain barrier (BBB) transport in homeostasis of brain Aβ. **Influx pathway**: RAGE, the receptor for advanced glycation end products, mediates influx and reentry of circulating Aβ across the BBB. RAGE-mediated Aβ influx is accompanied by generation of reactive oxygen species (ROS) and activation of nuclear factor-κB (NF-κB)-mediated inflammatory response in endothelium, that is, increased production of cytokines including tumor necrosis factor α (TNFα), interleukin (IL) 1β and 6, and monocyte chemotactic protein-1 (MCP1), as well as increased expression of several leukocyte adhesion molecules (not shown). RAGE also mediates transport of Aβ-laden monocytes across the BBB. **Efflux pathway**: LRP, the low-density lipoprotein receptor-related protein-1, mediates Aβ clearance from brain via transport of free Aβ and Aβ-bound to apoE2 and apoE3, but not apoE4, across the BBB. Other Aβ transport proteins in brain interstitial fluid such as apoJ and α-2 macroglobulin (α2M) influence Aβ clearance from brain. Aβ enzymatic clearance, oligomerization, and aggregation also control Aβ levels in brain. Soluble form of LRP (sLRP) in plasma is a major binding protein of plasma Aβ. sLRP is produced by the proteolytic cleavage from LRP mediated by β-secretase (BACE). Liver and kidneys mediate systemic clearance of free Aβ and of sLRP–Aβ complexes. APP, Aβ-precursor protein. TJ, tight junctions. (Modified from Zlokovic 2008.)
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the BBB including Aβ (Shibata et al. 2000; Deane et al. 2004), RAP (Pan et al. 2004), tPA (Benchenane et al. 2005), lipid-free and lipid-dated apoE2 and apoE3, including their respective complexes with Aβ (Deane et al. 2008), and a family of Kunitz domain-derived peptides (Demeule et al. 2008).

Initial studies have suggested that LRP is linked genetically to AD and CAA (Kang et al. 1997; Deane et al. 1998; Wavrant-DeVrieze et al. 1999; Christoforidis et al. 2005; Ballatore et al. 2007), but this has not been confirmed by later studies (Harold et al. 2009; Lambert et al. 2000; Donahue et al. 2006). Several studies have indicated that LRP expression in brain endothelium decreases with normal aging in rodents, nonhuman primates, and humans, as well as in AD models and AD patients (Kang et al. 2000; Shibata et al. 2000; Bading et al. 2002; Deane et al. 2004; Donahue et al. 2006; Bell and Zlokovic 2009). LRP reductions have been reported in cerebral VSMCs associated with Aβ accumulation in the wall of small pial and intracerebral arteries (Bell et al. 2009). Therefore, LRP down-regulation at the BBB and in vascular cells may contribute to cerebrovascular and focal parenchymal Aβ accumulations.

In blood, the circulating form of LRP (i.e., soluble LRP sLRP) provides a key endogenous peripheral “sink” activity for Aβ, as shown in a mouse model of AD (Sagare et al. 2007). In neurologically healthy humans and mice, sLRP binds >70% of circulating Aβ, preventing free Aβ access to the brain (Fig. 3; Sagare et al. 2007). In AD patients and AD transgenic mice, Aβ binding to sLRP is compromised by oxidation, resulting in increased levels of oxidized sLRP, which does not bind Aβ (Sagare et al. 2007). This is associated with elevated levels of free Aβ40 and Aβ42 in plasma that can reenter the brain via RAGE-mediated transport across the BBB (Deane et al. 2003; Ujjie et al. 2003; Donahue et al. 2006; Sagare et al. 2007). Moreover, in the human hippocampus, an increased RAGE expression in brain endothelium of the BBB has been shown in advanced AD compared with early stage AD and/or individuals with mild cognitive impairment (MCI; Miller et al. 2008). This might further contribute to Aβ accumulation in brain via accelerated Aβ influx from blood. In one study,
a diminished sLRP-Aβ peripheral binding has been shown to precede an increase in the tau/Aβ42 CSF ratio and a drop in global cognitive decline in individuals with MCI converting into AD (Sagare et al. 2011a). Importantly, recombinant LRP fragments can effectively replace oxidized sLRP and sequester free Aβ in plasma in AD patients and AD transgenic mice, ultimately reducing Aβ-related pathology in brain (Sagare et al. 2007). Consistent with these findings, it has been suggested that sLRP and anti-RAGE antibodies that are present in Baxter’s intravenous immunoglobulin preparation Gammagard Liquid may contribute to the observed beneficial effects of Gammagard Liquid in AD patients (Relkin et al. 2009; Weber et al. 2009) by improving the peripheral Aβ sequestration and preventing entry of free Aβ into the brain (Dodel et al. 2010).

LRP in the liver mediates rapid peripheral clearance of Aβ (Tamaki et al. 2006, 2007). It is of note that reduced hepatic LRP levels have been shown to be associated with decreased peripheral Aβ clearance in the aged rats (Tamaki et al. 2006, 2007). Regulation of Aβ brain levels by the liver has been recently demonstrated in an independent study (Sagare et al. 2011b; Sutcliffe et al. 2011).

VASCULAR-SPECIFIC GENES

Recent findings suggest that unsuccessful vascular regeneration may lead to degeneration of brain endothelium in AD and AD models. It has been shown that brain endothelial cells in AD express extremely low levels of the mesenchyme homeobox gene 2 (MEOX-2; Wu et al. 2005), a transcription factor that regulates vascular cell differentiation and remodeling, and whose expression in the adult brain is restricted to the vascular system (Gorski and Walsh 2003). Low levels of MEOX-2 expression in AD brain endothelium have been shown to mediate an aberrant angiogenic response to vascular endothelial growth factor (Wu et al. 2005), ultimately resulting in vessel regression associated with reductions in the resting CBF (Fig. 4). Low levels of MEOX-2 also promote proteasomal degradation of LRP in brain endothelium (Wu et al. 2005) that diminishes Aβ clearance at the BBB. On the other hand, accumulation of Aβ on the outer membrane of the blood vessels is anti-angiogenic per se (Paris et al. 2004a,b). Therefore, Aβ may act in concert with low expression of MEOX2 at the BBB to focally reduce brain capillary density in AD models and AD. Importantly, MEOX2 expression is diminished by hypoxia, suggesting that hypoxia may be upstream of MEOX2 depletion seen in AD brain endothelium (Wu et al. 2005).

Interestingly, mice with a single allele of Meox2 develop a primary cerebral endothelial hypoplasia with an intact BBB, but a significant brain perfusion deficit (Wu et al. 2005), which has been shown to lead to secondary neurodegenerative changes prior to Aβ accumulation (Bell et al. 2010). Neurodegenerative changes in Meox2+/− mice were, however, significantly less pronounced than in pericyte-deficient mice, which have a comparable brain hypoperfusion to Meox2+/− mice but also a compromised BBB (Bell et al. 2010). These data indicate that chronic hypoperfusion alone can cause neuronal injury, but not to the same extent as when combined with BBB breakdown.

Recent studies have also shown that AD patients as well as mouse models with high cerebrovascular levels of serum response factor (SRF) and myocardin (MYOCD), the two transcription factors that control VSMC differentiation, develop a hypercontractile cerebral arterial phenotype, resulting in brain hypoperfusion, diminished functional hyperemia, and CAA (Chow et al. 2007; Bell et al. 2009). MYOCD, a SAF-A/B, Acinus, and PIAS domain family nuclear protein, is a VCMC-specific transcriptional co-activator that binds SRF to induce gene expression (Wang et al. 2001). SRF is a ubiquitously expressed transcription factor that binds to a ten-base pair cis element called a CARG box, which is located in the regulatory region of numerous target genes (Sun et al. 2006). MYOCD and SRF constitute a molecular switch for the VSMC differentiation program (Chen et al. 2002; Li et al. 2003). In addition, it has been shown that increased levels of MYOCD and SRF in AD VSMCs may suppress
Aβ clearance and thus exacerbate CAA (Bell et al. 2009). Namely, high levels of MYOCD and SRF in VSMCs lead to directed expression of sterol response element binding protein 2 (SREBP2), which is a major LRP transcriptional suppressor ultimately resulting in LRP depletion, which diminishes LRP-mediated Aβ clearance from the vessel wall (Fig. 4). Hypoxia increases MYOCD levels in VSMCs (Reynolds et al. 2004; Chow et al. 2007; Bell et al. 2009), and it has been shown that it is also upstream of elevated MYOCD/SRF expression in cerebral arterial VSMCs (Bell et al. 2009). More studies are needed, however, to establish the exact role of vascular-specific genes MEOX2 and MYOCD in the development of Alzheimer neurovascular dysfunction.

**CONCLUDING REMARKS**

Recent clinical observations provide strong evidence for the link between cerebrovascular disease and AD and the role of vascular risk factors in AD. In this chapter, we have briefly reviewed literature on dysregulated and diminished CBF, BBB dysfunction, and impaired vascular clearance of Aβ from brain, supporting an essential role of the neurovascular and BBB mechanisms in AD pathogenesis. Several studies in animal models of AD and more recently in AD patients (Mavuenyega et al. 2010) have demonstrated a diminished Aβ clearance from brain. The recognition of Aβ clearance pathways opens exciting new therapeutic opportunities for AD. It is now established that faulty clearance from brain
and across the BBB leads to elevated Aβ levels in brain that in turn have been shown to contribute to the formation of neurotoxic Aβ oligomers (Walsh et al. 2002) and the development of Aβ-mediated brain storage disorder and cerebral β-amyloidosis (Zlokovic 2008).

The activation of neurovascular pathogenic pathways has been shown to compromise synaptic and neuronal functions prior to and/or in parallel with Aβ accumulation and development of intraneuronal tangles, neuronal loss, and dementia. Some early molecular targets within the neurovascular pathway include receptors RAGE and LRP at the BBB and possibly vascular-specific genes MEOX2 and MYOCOD.

Focusing on comorbidity, vascular risk factors associated with AD such as hypoperfusion, hypertension, ministrokes, and/or diabetes might generate useful models of human dementia. The proposed neurovascular model of AD raises a set of new important questions that require further study, as recently discussed (Zlokovic 2011). For example, the molecular basis of the neurovascular link with neurodegenerative disorders is still poorly understood as well as the molecular cues underlying the cross talks between different cell types of the NVU, including vascular and glia cells, and how these cellular interactions influence neuronal activity. Addressing these questions will lead to better understanding of the neurovascular link with neurodegeneration process, which will lead to the development of novel neurovascular-based approaches for AD (Zlokovic 2011).

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