

LRP1 mediates bidirectional transcytosis of amyloid- β across the blood-brain barrier

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Abstract

According to the “amyloid hypothesis”, the amyloid- β (A β) peptide is the toxic intermediate driving Alzheimer’s disease (AD) pathogenesis. Recent evidence suggests that the low density lipoprotein receptor-related protein 1 (LRP1) transcytoses A β out of the brain across the blood-brain barrier (BBB). To provide genetic evidence for LRP1-mediated transcytosis of A β across the BBB we analyzed A β transcytosis across primary mouse brain capillary endothelial cells (pMBCECs) derived from wild-type and LRP1 knock-in mice. Here, we show that pMBCECs in vitro express functionally active LRP1. Moreover, we demonstrate that LRP1 mediates transcytosis of [¹²⁵I]-A β _{1–40} across pMBCECs in both directions, whereas no role for LRP1-mediated A β degradation was detected. Analysis of [¹²⁵I]-A β _{1–40} transport across pMBCECs generated from mice harboring a knock-in mutation in the NPxYxxL endocytosis/sorting domain of endogenous LRP1 revealed a reduced A β clearance from brain-to-blood and blood-to-brain compared with wild-type derived pMBCECs. Therefore, for the first time, we present genetic evidence that LRP1 modulates the pathogenic actions of soluble A β in the brain by clearing A β across the BBB.

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1. Introduction

Currently, more than 26 million people worldwide suffer from Alzheimer’s disease (AD) and considering increased life expectancy, the number of AD patients will increase to more than 140 million cases by 2050 (Pahnke et al., 2009). One major hallmark of AD is the generation of hydrophobic amyloid- β (A β) peptides from the amyloid precursor protein (APP), resulting in the formation of amyloid plaques in the brain (Pietrzik and Behl, 2005). A β occurs in various

isoforms between 36 and 46 amino acids in length, whereas A β _{1–40} and A β _{1–42} are the most prevalent isoforms. It has been shown, that the most toxic and aggregation/oligomerization prone A β _{1–42} isoform accumulates in the brain of AD patients and, thus, can elicit pathologic events such as neuronal cell death and blockade of long term potentiation (Yankner and Lu, 2009). However, A β _{1–40} is the main species found under physiologic conditions and its soluble levels strongly correlate with AD etiopathology and synaptic changes (Fonte et al., 2001).

Low density lipoprotein receptor-related protein 1 (LRP1) is a member of the lipoprotein receptor family that has been linked to AD (Jaeger and Pietrzik, 2008). During biosynthesis, the receptor-associated protein (RAP) acts as a chaperone for proper folding of LRP1 (Willnow et al., 1996).

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Furthermore, RAP has been used as an inhibitor of ligand binding to LRP1 in various studies (Martin et al., 2008; Reekmans et al., 2009). LRP1 is synthesized as a 600 kDa full-length precursor molecule in the endoplasmic reticulum, before furin cleavage in the Golgi network generates an 85 kDa transmembrane β -subunit that remains noncovalently linked to the extracellular 515 kDa α -subunit. The α -subunit contains 4 ligand binding domains for more than 30 ligands while the β -subunit contains two intracellular NPxY motifs (Herz and Strickland, 2001). The NPxYxxL motif has been shown to play distinct roles in the functionality of LRP1. Generation of cells from mice carrying a knock-in mutation in the NPxYxxL amino acid sequence revealed a reduced internalization rate for LRP1 (Reekmans et al., 2009; Roebroek et al., 2006). Additionally, the NPxYxxL motif has also been shown to be responsible for basolateral sorting of LRP1 in polarized epithelial cells. LRP1 minireceptors carrying a tyrosine to alanine mutation in the NPxYxxL motif lost their preferential basolateral distribution and appeared randomly on both the apical and basolateral membrane (Marzolo et al., 2003). Furthermore, the cytoplasmic domain of LRP1 has also been shown to play a role in multiple steps of APP processing including modulation of A β generation (Pietrzik et al., 2002, 2004; Waldron et al., 2008). However, the role of LRP1 in AD is not only delineated by its role in APP processing, but also by evidence of LRP1-mediated endocytosis of A β (Kang et al., 2000).

One proof of LRP1-mediated endocytosis of A β was obtained from polarized endothelial cells (ECs) lining the blood vessels (Yamada et al., 2008). Brain ECs are the main cell type involved in the formation of the blood-brain barrier (BBB), especially at the capillaries. In contrast to ECs from other vascular beds, they are characterized by the absence of fenestrations, elevated expression of tight junctions (TJs), and specialized nutrient transporters (Madara, 1988). Therefore, the BBB separates the circulating blood from the central nervous system (CNS), maintaining tissue homeostasis and restricting free exchange of solutes, pathogens, or even cells. Although A β treatment of ECs has been shown to elicit toxic cascades (Vukic et al., 2009), in vivo and in vitro studies have elucidated a role for endothelial LRP1 in transcytosis of A β from the brain into the blood (Deane et al., 2004; Shibata et al., 2000). To date, this mechanism is controversial due to the lack of genetic evidence confirming these findings and due to studies that failed to show a role for LRP1-mediated transcytosis but rather demonstrated a role for LRP1 in endocytosis and degradation of A β (Nazer et al., 2008). In AD, A β deposition can also occur in blood vessel walls of the central nervous system during perivascular drainage, a symptom referred to as cerebral amyloid angiopathy (CAA). Accumulation of A β in the adventitia of small arteries alters the composition of the basement membrane, eventually resulting in intracerebral hemorrhage observed in AD/CAA patients (Scolding et al., 2005). In addition, it has been reported that AD patients have a reduced

density in brain capillaries due to MEOX2 (also known as GAX) downregulation, a regulator of vascular differentiation, and smaller vessel diameter (Bouras et al., 2006; Wu et al., 2005). Accordingly, clearance of soluble A β from the brain into the blood can be facilitated by LRP1-mediated transcytosis across the BBB ECs or via the perivascular drainage route. Both pathways seem to be impaired in AD patients. Deposition of A β in blood vessel walls during CAA, with the concomitant changes in arterial wall architecture, might alter drainage and, therefore, further impede its elimination through this route. Moreover, reduced number of capillaries and smaller diameter observed in AD patients implies that the overall surface for LRP1-mediated transcytosis of A β across the BBB is diminished compared with non-AD individuals, increasing the pool of soluble A β in the brain.

In this study, we analyzed the possible involvement of LRP1 in transcytosis of A β across primary mouse brain capillary ECs (pMBCECs). Using an in vitro BBB model, we set out to investigate LRP1 as a target for therapeutic intervention in AD by modulating the transcytotic capacity of LRP1. For the first time, we provide genetic evidence for the participation of LRP1 in transcytosis of A β across pMBCECs. Furthermore, our experiments show that LRP1 acts as a receptor for bidirectional transcytosis of A β across the BBB, whereas no role for LRP1-mediated degradation of A β was found in our BBB model.

2. Methods

2.1. Isolation and cultivation of primary mouse brain capillary endothelial cells

Primary mouse brain capillary endothelial cells (pMBCECs) were isolated from 6–10-week-old C57Bl6 wild-type (WT) and C57Bl6/129 LRP1 NPxYxxL knock-in mice harboring the mutated NPxYxxL motif as previously described (Roebroek et al., 2006; Weidenfeller et al., 2005). In brief, mice were sacrificed by cervical dislocation, the forebrains were pooled, meninges were removed, and the tissue was mechanically dissociated, followed by a digest with a mixture of 0.75 mg/mL collagenase CLS2 (Worthington, Lakewood, NJ) and 10 U/mL DNase (Sigma, Schnellendorf, Germany) in Dulbecco's modified Eagle medium (DMEM; Gibco, Darmstadt, Germany) carried out at 37 °C on a shaker set at 200 rpm for 1 hour. The pellet was resuspended in 20% BSA-DMEM (w/v) and centrifuged at 1000g for 20 minutes to remove myelin. The pellet was further digested with 1 mg/mL collagenase-dispase (Roche, Mannheim, Germany) and 10 U/mL DNase in DMEM at 37 °C on a shaker for 1 hour. Endothelial capillaries were separated on a 33% continuous Percoll (GE Healthcare, Munich, Germany) gradient, collected, and plated in dishes coated with collagen IV/fibronectin (Sigma). Cultures were maintained in DMEM supplemented with 20% plasma-derived bovine serum (PDS; First Link, Birmingham, UK), 100 U/mL pen-

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