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Research Paper

Pericyte implantation in the brain enhances cerebral blood flow and reduces amyloid-β pathology in amyloid model mice

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ABSTRACT

Pericytes are a major component of cerebrovasculature playing a key role in maintaining cerebrovascular homeostasis. These cells have also been suggested to regulate brain metabolism of amyloid-β (Aβ), disturbances of which are believed to contribute to the pathogenesis of Alzheimer's disease (AD). To examine the effects of pericytes on brain Aβ metabolism, C3H/10T1/2 mouse mesenchymal stem cells were differentiated into pericytes and stereotaxically injected into the brains of amyloid AD model APP/PS1 mice at the age of 18 to 20 months. Consistent with a role of pericytes in modulating cerebrovascular function, brain microcirculation in the pericyte-injected hemisphere of the mice was increased 3 weeks after implantation compared to the contralateral hemisphere when measured by laser speckle contrast analysis technology. Importantly, enzyme-linked immunosorbent assay revealed that the levels of insoluble Aβ40 and Aβ42 were significantly lower in the hippocampus of the pericyte-injected hemisphere of the APP/PS1 mice than that of the contralateral side. Consistently, immunohistochemical analysis demonstrated that the pericyte implantation reduced Aβ deposition in the hippocampus. When brain slices from the APP/PS1 mice were incubated with C3H/10T1/2 cell-derived pericytes, Aβ42 levels were significantly reduced in a manner that depends on the expression of a major Aβ endocytic receptor, the low-density lipoprotein receptor-related protein 1 (LRP1). While LRP1 mediated the cellular uptake of Aβ in the pericytes, the amounts of major Aβ-degrading enzymes were not affected by LRP1 knockdown. Together, our findings indicate that mesenchymal stem cell–derived pericytes have the capacity to reduce brain Aβ and related pathology, and suggest that cell-based therapy through transplantation of pericytes may be a promising approach to prevent and/or treat AD.

1. Introduction

Pericytes are vascular mural cells originally discovered as capillary adventitial cells (Sims, 1986). Pericytes are likely composed of heterogeneous populations, and sometimes display mesenchymal stem cell (MSC)-like properties (Crisan et al., 2008; Guimaraes-Camboa et al., 2017). In brain capillaries, pericytes helically surround the microvessels with their long processes, while other pericyte subtypes reside on precapillary arterioles (hybrid smooth muscle–pericyte cells and mesh pericytes) and post-capillary venules (mesh pericytes) (Hartmann et al., 2015; Kisler et al., 2017). As pericytes are more abundant in the central nervous system compared to peripheral tissues/organs (Armulik et al., 2011), which cover approximately 37% of the abluminal surface of endothelial tubes in rat brains (Mathiisen et al., 2010), pericytes play a critical role in maintaining cerebrovascular homeostasis. By interacting with endothelial cells, pericytes regulate blood-brain barrier integrity, angiogenesis, and hemodynamic responses in brain microvessels. Furthermore, pericytes have immune cell–like properties, such as responding to inflammatory stimuli, presenting antigen, and regulating fibrosis (Kisler et al., 2017; Rustenhoven et al., 2017; Sweeney et al., 2016; Thomas et al., 2017). Pericytes also have the phagocytic ability to eliminate toxic molecules, including amyloid-β (Aβ) peptides, in perivascular regions (Rustenhoven et al., 2017; Sweeney et al., 2016). Indeed, in an amyloid mouse model, the pericyte deficit caused by *Pdgfrb* haploinsufficiency suppresses Aβ clearance, resulting in exacerbated brain Aβ deposition (Sagare et al., 2013).

Importantly, brain accumulation of Aβ likely triggers the pathogenic cascade of Alzheimer's disease (AD) (Musiek and Holtzman, 2015; Selkoe and Hardy, 2016), which is the most common cause of dementia in the elderly (Alzheimer's Association, 2017). Aβ deposition is predominantly detected as senile plaques in brain parenchyma and as cerebral amyloid angiopathy (CAA) in cerebrovasculature (Kapasi et al., 2017; Thal et al., 2015), where Aβ is less efficiently eliminated from the brain in AD patients compared to healthy individuals (Mawuenyega et al., 2010). While several proteases, including neprilysin (NEP) and insulin-degrading enzyme (IDE), can efficiently degrade Aβ in

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extracellular space (Saido and Leissring, 2012), most brain cells have sufficient capacity to endocytose Aβ for subsequent lysosomal degradation (Kanekiyo et al., 2014; LaFerla et al., 2007; Querfurth and LaFerla, 2010). In addition, Aβ is also cleared through the interstitial fluid drainage pathway and glymphatic system along the cerebrovasculature (Morris et al., 2016). Increasing evidence demonstrates that disturbances of cerebrovascular functions are involved in brain Aβ accumulation and AD development (Kapasi et al., 2017; Kisler et al., 2017). Consistently, diminished pericyte coverages on microvessels have also been demonstrated in AD patients (Sengillo et al., 2013). Because pericytes are critical components with diverse functions in the neurovascular unit, pericytes are predicted to directly or indirectly contribute to Aβ clearance through multiple pathways.

Therefore, to further explore the potential mechanism of pericyte-mediated Aβ clearance and to develop pericyte-targeted therapy for AD, we implanted mouse mesenchymal C3H/10T1/2 cell-derived pericytes (Bose et al., 2013; Darland et al., 2003; Hirschi et al., 1998; Reznikoff et al., 1973) into the brains of amyloid model APP/PS1 mice and investigated their effect on Aβ pathology. In addition, we assessed the contribution of low-density lipoprotein receptor-related protein 1 (LRP1) (Kanekiyo and Bu, 2014), a major Aβ clearance receptor, to the pericyte-mediated Aβ metabolism using *ex vivo* and *in vitro* approaches. Our study demonstrates that C3H/10T1/2 cell-derived pericytes are capable of eliminating brain Aβ deposition with competence.

2. Material and methods

2.1. Cell culture

The C3H/10T1/2 cell line was purchased from ATCC (Manassas, VA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM), with 10% fetal bovine serum (FBS) in normal culture condition, and differentiated into pericytes by culturing with mouse transforming growth factor-β (TGF-β; 1 ng/ml, Invitrogen [Carlsbad, CA]) for at least 3 days before beginning experiments (Bose et al., 2013). In some experiments, LRP1 knockdown and green fluorescent protein (GFP)-transfection were performed in the cells. Single-stranded sense and antisense RNA oligonucleotides for mouse LRP1 were synthesized by Dharmacon (GE Healthcare; Lafayette, CO), and double-stranded RNA molecules were generated according to the manufacturer's instruction. The sequences of the single-stranded RNAs were 5′-GGA GUC ACU UAC AUC AAU AUU-3′ (sense) and 5′- UAU UGA UGU AAG UGA CUC CUU-3′ (antisense). Non-targeting siRNA was also purchased from Dharmacon (GE Healthcare) and used as the control. For GFP transfection, the pmax GFP vector provided in Amaxa Basic Nucleofector Kit (Lonza; Cologne, Germany) was used. LRP1-siRNA (300 nM) or GFP plasmid vector (2 μg) was transfected into C3H/10T1/2 cell-derived pericytes by nucleofection using the Amaxa Basic Nucleofector Kit (Lonza) according to the manufacturer's instructions (program A-033). Cells were cultured for an additional 48 h before being used for experiments.

2.2. Animals and cell injection

All animal procedures were approved by the Institutional Animal Care and Use Committee at Mayo Clinic and in accordance with the regulations of the American Association for the Accreditation of Laboratory Animal Care. APPswe/PSEN1ΔE9 (APP/PS1) mice were purchased from Jackson Laboratory (Jankowsky et al., 2004). Both male and female APP/PS1 mice were subjected to intracranial cell implantation at the age of 18 to 20 months. The mice were subjected to anesthesia, and the skull was exposed by skin incision. Stereotaxically, holes were bored above both hemispheres (lateral 2.0 mm and posterior 2.0 mm from the bregma). C3H/10T1/2 cell-derived pericytes in 2 μl of phosphate-buffered saline (PBS; 1×10^5 cells/μl) were injected into the right hemisphere (depth 2.0 mm from surface of the skull) using a Hamilton Neuros Syringe (Reno, NV) with 33-gauge needle at a rate of 1 μl/min. After injection, the needle was held steady for 2 min, and then gradually withdrawn for 1 min. In the same manner, PBS (2 μl) was injected in the left hemisphere as a control.

2.3. Monitoring of brain microcirculation

To monitor cerebral microcirculation in real time, laser speckle contrast analysis (LASCA) technology was used through PeriCam PSI HR (Perimed AB, Sweden). The mouse was anesthetized with ketamine and xylazine, and placed on a small animal stereotaxic device equipped with a heating blanket to maintain body temperature. The surgical site was prepared by shaving, following transection of the skin along the midline to expose the skull to the anterior-posterior coordinates in the brain, bregma, and lambda. The cerebral microcirculation of the mouse was monitored for 5 to 10 min to ensure that stable perfusion was obtained. The region of interest was selected around the injection site in both hemispheres, and the average cerebral microcirculation rate was quantified.

2.4. Aβ enzyme-linked immunosorbent assay (ELISA)

Mouse hippocampus samples were homogenized using a Polytron homogenizer (KINEMATICA; Lucerne, Switzerland) in 10 times volumes (w/v) of ice cold tris-buffered-saline with 1% triton-X (TBSX) containing cOmplete protease inhibitor cocktail (Roche; Mannheim, Germany) and PhosSTOP phosphatase inhibitor (Roche). After incubation with mild agitation at 4 °C for 1 h, samples were centrifuged at 100,000*g* at 4 °C for 60 min. The residual pellet was further homogenized in 5 M of guanidine hydrochloride (GDN-HCl; pH 7.6) and incubated with mild agitation at room temperature for 12 to 16 h, followed by centrifugation at 16,000*g* for 1 h. Levels of Aβ40 and Aβ42 were determined by ELISA as previously described (Kanekiyo et al., 2013; Liu et al., 2017). Samples were incubated with horseradish peroxidase-linked streptavidin (Vector Laboratories; Burlingame, CA) and developed with 3,3′,5,5′-tetramethylbenzidine substrate (Sigma-Aldrich; St Louis, MO) by a Synergy HT plate reader (BioTek Instruments; Winooski, VT) followed by colorimetric measurement. All measured values were normalized against protein concentration as determined by BCA protein assay kit (Pierce; Rockford, IL).

2.5. Immunohistochemical analysis

Frozen sections (30-μm thickness) were permeabilized in PBS with 0.3% triton X-100, blocked with 3% bovine serum albumin, and incubated with anti-platelet–derived growth factor receptor β (PDGFRβ) antibody (1:50, R & D Systems; Minneapolis, MN) or anti-collagen IV antibody (1:100, EMD Millipore) at 4 °C overnight followed by incubation with Alexa Flour 568 secondary antibody (1:200, Thermo Fisher Scientific; Waltham, MA). Subsequently, sections were incubated with anti-GFP antibody (1:500, Aves Labs; Tigard, OR) at 4 °C overnight, followed by a secondary Alexa Fluor 488 antibody (1:200). Stained sections were captured using a Zeiss LSM 510 META confocal microscope. For the quantification of Aβ plaque burden, frozen brain sections were stained by mouse monoclonal anti pan-Aβ 33.1.1 (human Aβ 1–16 specific) antibody, visualized with 3,3′-diaminobenzidine (DAB), and captured with an Aperio AT2 scanner (Aperio Technologies Inc.; Vista, CA) (Kanekiyo et al., 2013; Kanekiyo et al., 2012; Liu et al., 2017). The immunoreactivity in the hippocampus was measured by ImageJ software in a blinded manner.

2.6. Ex vivo cellular Aβ clearance assay

Using APP/PS1 mouse brain slices, *ex vivo* cellular Aβ clearance assay was performed as previously described (Koistinaho et al., 2004; Wyss-Coray et al., 2003; Xiang et al., 2016), with some modifications.