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Research Report

p38 MAP kinase mediates transforming-growth factor-β1-induced upregulation of matrix metalloproteinase-9 but not -2 in human brain pericytes

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ABSTRACT

Pericytes are vascular mural cells embedded within the basal lamina of blood microvessels. Within the neurovascular unit, pericytes play important roles in regulating neurovascular homeostasis by secreting soluble factors, such as matrix metalloproteinases (MMPs). However, little is known about the regulatory signaling pathways in brain pericytes. Here we show that transforming growth factor-β1 (TGF-β1) induces MMP-9 upregulation in pericytes via p38 mitogen-activated protein (MAP) kinase signaling. Cultured human brain vascular pericytes were used in this study. When the brain pericytes were treated with purified human TGF-β1 (0.1–10 ng/mL for 24 h), the levels of MMP-2 and MMP-9 in culture media were significantly increased in a concentration dependent manner as measured by gelatin zymography. WST assay confirmed that TGF-β1 did not affect cell survival of the brain pericytes. A TGF-β-receptor inhibitor SB431542 (0.5–5 μM) decreased the TGF-β1-induced upregulation of MMP-2 and MMP-9. To assess the underlying intracellular mechanisms, we focused on p38 MAP kinase signaling, which is one of the major downstream kinases for TGF-β1. A well-validated p38 MAP kinase inhibitor SB203580 (0.5–5 μM) cancelled the effect of TGF-β1 in upregulation of MMP-9 but not MMP-2. Western blotting confirmed that TGF-β1 treatment increased the level of p38 MAP kinase phosphorylation in pericytes, and again, the TGF-β-receptor inhibitor SB431542 (0.5–5 μM) blocked the TGF-β1-induced phosphorylation of p38 MAP kinase. Both TGF-β1 and MMP-9 are major neurovascular mediators, and therefore, our current finding may suggest a novel mechanism for how pericytes regulate neurovascular homeostasis.

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1. Introduction

The neurovascular unit is now relatively well accepted as a conceptual model to understand mechanisms of physiology and pathophysiology of CNS diseases. Fundamentally, this concept shows that all compartments of the neurovascular unit cooperate with each other to maintain normal brain function. Within the neurovascular unit, pericytes attach to cerebral endothelial cells via basal lamina (Diaz-Flores et al., 2009; Stratman et al., 2009), and play critical roles in regulating neurovascular homeostasis (Bell et al., 2010; Sa-Pereira et al., 2012; Winkler et al., 2011). For example, pericytes secrete multiple soluble factors to modulate blood vessel structure and enhance blood–brain barrier (BBB) tightness (Armulik et al., 2010; Winkler et al., 2011). However, mechanisms on how pericytes produce neurovascular mediators are still mostly unknown.

Matrix metalloproteinases (MMPs) are one of the major mediators for cell–cell or cell–matrix interaction in the neurovascular unit. MMPs comprise a family of zinc endopeptidases, and play an important role in regulating extracellular matrix signaling (Nagase et al., 2006). Since MMPs can degrade almost all extracellular matrix molecules, MMPs may contribute to neurovascular homeostasis through modulating axonal growth/regeneration, myelin formation, and vascularization (Verslegers et al., 2013; Yong, 2005). On the other hand, uncontrolled expression/activation of MMPs may result in neurovascular damage such as BBB dysfunction (Lo, 2008; Maki et al., 2013; Moskowitz et al., 2010). Among the MMP superfamily, gelatinases (MMP-2 and MMP-9) have been extensively studied as a therapeutic target for several neurological diseases including stroke, multiple sclerosis, Alzheimer's diseases, and cerebral hemorrhage (Avolio et al., 2003; Davis and Senger, 2005; Kook et al., 2013; Nagase et al., 2006; Rosell et al., 2008).

Although MMP-2 and MMP-9 are secreted from all the cells that contribute to the neurovascular unit, little is known about their regulatory signaling pathways in brain pericytes. Since it has been shown previously that p38 mitogen-activated protein (MAP) kinase signaling contribute to cytokine-induced MMP-9 upregulation in astrocytes (Arai et al., 2003; Wu et al., 2004), we tested if the p38 MAP kinase signaling also mediates MMP-2 and MMP-9 upregulation in brain pericytes. In this study, upregulation of MMP-2 and MMP-9 was induced with transforming-growth factor-β1 (TGF-β1), as TGF-β1 regulates MMP-2 and MMP-9 expression in several types of cells such as astrocytes, meningeal cells, and smooth muscle cells (Hsieh et al., 2010; Okamoto et al., 2009; Zhang et al., 2013). Then p38 MAP kinase phosphorylation and pericyte secretion of MMP-2 and MMP-9 were measured to determine whether p38 MAP kinase plays a critical role in the secretion of these metalloproteinases from pericytes.

2. Results

Primary cell culture systems may get contaminated with other kinds of cells. Therefore, we first confirmed that the cultured human brain vascular pericytes were all positive for pericyte markers PDGF-R-β and α-SMA, assessed by immunocytochemical staining (Fig. 1). This was important because many types of brain cells are known to be potent sources of MMP-9. Next, we examined whether the brain pericytes secreted MMP-2 and MMP-9 in vitro. Gelatin zymography showed that baseline MMP-2 and MMP-9 were both detectable in conditioned media from human pericytes under normal conditions as previously reported (Fig. 2A) (Xing et al., 2010). Treatment of TGF-β1 (0.1–10 ng/mL for 24 h) resulted in an increase of MMP-2 and MMP-9 secretion in a concentration dependent manner (Fig. 2B and C). The WST assay showed that the TGF-β1 treatment induced neither cell proliferation nor cell death in pericyte cultures (Fig. 2D), suggesting that the TGF-β1-induced increase of MMP-2/9 level in the culture media was not due to an increase of cell numbers or to non-specific release from damaged cellular membranes.

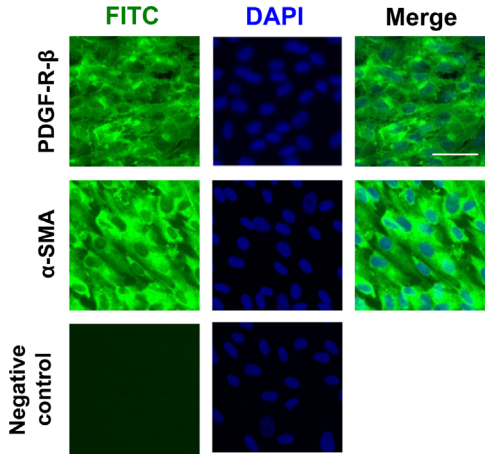

Fig. 1 – Fluorescent-stained cells of cultured human pericytes. Cells were stained for the pericyte markers PDGF-R-β (FITC; green) and α-SMA (FITC; green). Nuclei were stained by DAPI (blue). Negative control indicates a representative image obtained from immunostaining of secondary antibody only. Scale bar indicates 50 μm. These data demonstrate that our cultured pericytes express pericyte marker proteins.

We then examined the intracellular signaling pathway in TGF-β1-induced MMP-2/9 upregulation in pericytes. TGF-β1 activates the TGF-β type1 receptor, which then signals to downstream pathways (Massague, 2000). Therefore, we first confirmed that brain pericytes indeed expressed TGF-β1 receptor. Immunocytochemistry studies showed that the TGF-β type 1 receptor is strongly expressed in our brain pericyte cultures (Fig. 3A). Next, we used SB431542, a selective inhibitor of TGF-β type1 receptor, to further confirm that TGF-β1-induced MMP-2/9 upregulation was in fact mediated by the TGF-β type 1 receptor. As expected, co-treatment of pericytes with SB431542 (0.5–5 μM) reduced the TGF-β1-mediated MMP-2/9 upregulation (Fig. 3B and D) without affecting cell survival (Fig. 3E).

Next, we examined whether p38 MAP kinase was upregulated in pericytes after TGF-β1 treatment, as p38 MAP kinase