EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Extracellular Lactate Dehydrogenase A Release From Damaged Neurons Drives Central Nervous System Angiogenesis

Hsiaoyun Lin [a], Rieko Muramatsu [a,b,*], Noriko Maedera [a], Hiroto Tsunematsu [a], Machika Hamaguchi [a], Yoshihisa Koyama [a], Mariko Kuroda [a], Kenji Ono [c], Makoto Sawada [c], Toshihide Yamashita [a,b,d]

[a] Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan
[b] WPI Immunology Frontier Research Center, Osaka University, Suita, Osaka 565-0871, Japan
[c] Research Institute of Environmental Medicine Nagoya University, Nagoya, Aichi 464-8601, Japan
[d] Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form 24 October 2017
Accepted 30 October 2017
Available online xxxx

Keywords:
Multiple sclerosis
Cell proliferation
Cancer
Inflammation

ABSTRACT

Angiogenesis, a prominent feature of pathology, is known to be guided by factors secreted by living cells around a lesion. Although many cells are disrupted in a response to injury, the relevance of degenerating cells in pathological angiogenesis is unclear. Here, we show that the release of lactate dehydrogenase A (LDHA) from degenerating neurons drives central nervous system (CNS) angiogenesis. Silencing neuronal LDHA expression suppressed angiogenesis around experimental autoimmune encephalomyelitis (EAE)- and controlled cortical impact-induced lesions. Extracellular LDHA-mediated angiogenesis was dependent on surface vimentin expression and vascular endothelial growth factor receptor (VEGFR) phosphorylation in vascular endothelial cells. Silencing vimentin expression in vascular endothelial cells prevented angiogenesis around EAE lesions and improved survival in a mouse model of glioblastoma. These results elucidate novel mechanisms that may mediate pathologic angiogenesis and identify a potential molecular target for the treatment of CNS diseases involving angiogenesis.

1. Introduction

Angiogenesis, the formation of new capillaries from a pre-existing capillary network without the involvement of endothelial precursor cells, is a characteristic of many pathological conditions (Jin and Greenberg, 2005). Because angiogenesis is considered to regulate both pathological progression and wound healing, its modulation is thought to control disease progression (Costa et al., 2007). While most vessels in healthy adults are in a quiescent state, vascular endothelial cells in pathological conditions are actively proliferating (Chung and Ferrara, 2011) through a process that is thought to be affected by environmental stimuli. For example, in the central nervous system (CNS), vascular endothelial cell growth is promoted by vascular endothelial cell growth factor (VEGF) and fibroblast growth factor (FGF), both of which are upregulated in astrocytes, macrophages, and neurons after injury (Vallon et al., 2014). Microglia-derived factors are known to stimulate angiogenesis (Vallon et al., 2014). Most of the research into the molecular dynamics that govern vascular endothelial cell proliferation has been guided by the concept that angiogenic factors are expressed by living cells around lesions under disease conditions (Vallon et al., 2014). In contrast, although cell damage is a key feature of many pathological states, including CNS diseases, the effect of damaged cells around lesions on angiogenesis has not been investigated.

CNS damage causes functional impairment and/or structural dysfunction in nerve cells during disease progression. Neural cell dysfunction in part leads to the disruption of cell membrane, thereby causing the release of intracellular factors into the extracellular space. Recent findings have demonstrated that intracellular factors released from damaged or dying cells, which are recognized as damage-associated molecular patterns (DAMPs), trigger sterile inflammation (Piccinini and Midwood, 2010) and some pro-inflammatory DAMPs have been identified in the CNS (Kigerl et al., 2014; Shichita et al., 2012). Because inflammation and angiogenesis occur simultaneously under pathological conditions (Folkman and Brem, 1992), DAMPs may also influence angiogenesis, although evidence is lacking. It has, however, been reported that molecules identified as proinflammatory DAMP receptors (e.g., toll-like receptors) are also expressed by non-immune cells, including vascular endothelial cells (Stewart et al., 2015). In addition, some pro-inflammatory factors, such as interferon (IFN)-γ and stromal derived factor (SDF)-1, also stimulate vascular endothelial cells, resulting in the regulation of pathological angiogenesis (Belperio et al., 2000). Therefore, we hypothesized that factors released from damaged cells also directly regulate angiogenesis. If this is true, then elucidation of

* Corresponding author at: Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail address: muramatsu@molneu.med.osaka-u.ac.jp (R. Muramatsu).

https://doi.org/10.1016/j.ebiom.2017.10.033

the underlying molecular mechanisms may provide novel concepts and help to promote new therapeutic measures for treating angiogenesis-related diseases.

In the present study, we show that extracellular lactate dehydrogenase A (LDHA), previously known only as a cell damage marker in the circulation, has a pro-angiogenic effect in the adult CNS. LDHA released from degenerating axons drives angiogenesis around the lesions in experimental autoimmune encephalomyelitis (EAE)- and controlled cortical impact (CCI)-induced lesions. Extracellular LDHA interacts with vascular endothelial cells, in a manner dependent on vimentin on the cell surface. Silencing vimentin expression in vascular endothelial cells prevented LDHA-mediated VEGFR2 phosphorylation. In addition, the inhibition of vimentin prevents angiogenesis in response to EAE and prolongs the survival in mouse models of glioblastoma.

2. Results

2.1. Axonal Degeneration Is Synchronized with Angiogenesis in EAE

We used a localized model of EAE (Muramatsu et al., 2012) to generate single lesions, characterized by lymphocyte infiltration and angiogenesis. The time course of axonal degeneration and angiogenesis was examined in the spinal cord after EAE induction. Immunohistochemical analyses revealed a reduced intensity of protein kinase C (PKC)γ^+ corticospinal tract (CST) labeling around the lesion 7 days after EAE induction (Fig. 1a and b). Here, we also observed perilesional expression of the amyloid precursor protein (APP), which is a marker of axonal damage (Fig. 1a and b). Because we previously reported robust angiogenesis around lesions 1 week after EAE induction (Muramatsu et al.,

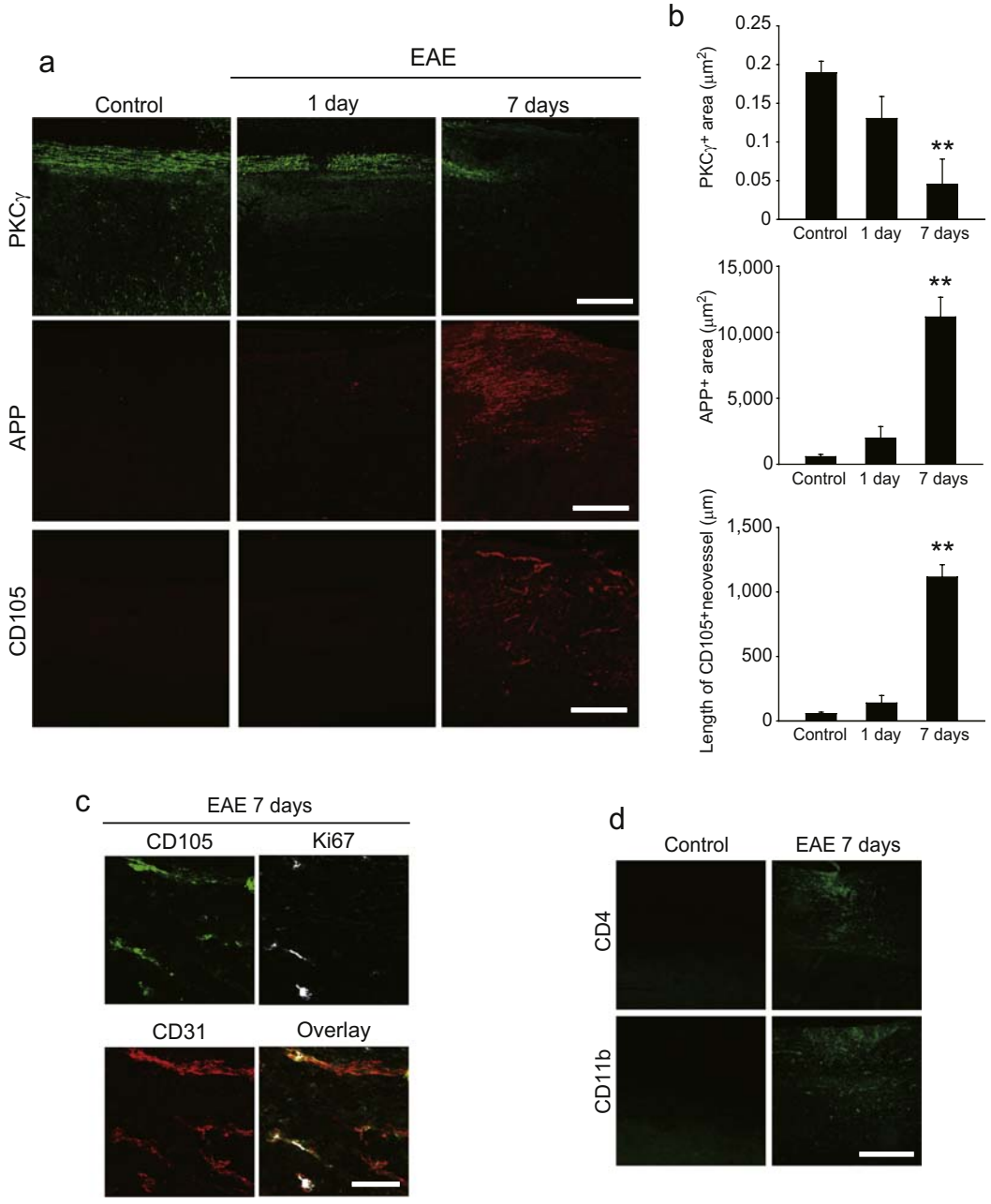

Fig. 1. Neovascularisation synchronises neurodegeneration in EAE. (a) Representative images of spinal cord sections labeled with PKCγ, APP, or CD105. (b) Quantitative analysis of the fluorescent area of PKCγ or APP around EAE lesions. CD105$^+$ neovessel length around EAE lesions; $n = 4$–6 for all experiments; error bars represent the s.e.m. $^{**}P < 0.01$, ANOVA with Tukey's multiple comparison tests. (c) Representative images of spinal cord sections labeled with CD31, Ki67, and CD105. (d) CD4$^+$ and CD11b$^+$ cell accumulation around EAE lesions 7 days after EAE induction. Scale bars for a, d, 200 μm; c, 50 μm.

Please cite this article as: Lin, H., et al., Extracellular Lactate Dehydrogenase A Release From Damaged Neurons Drives Central Nervous System Angiogenesis, EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.10.033