NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION
RESEARCH ARTICLE
S. H. Chung et al. / Neuroscience xxx (2018) xxx–xxx

The p38α MAPK Deletion in Oligodendroglia does not Attenuate Myelination Defects in a Mouse Model of Periventricular Leukomalacia

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Abstract—Periventricular leukomalacia (PVL) is a severe type of white matter damage in premature infants and the most common cause of cerebral palsy. It is generally known to be caused by hypoxia and inflammation. Currently there is no effective treatment available, in part due to that the pathogenesis of the disease has not been well understood. The p38α mitogen-activated protein kinase (MAPK) is the serine/threonine kinase and several *in vitro* studies demonstrated that p38 MAPK is essential for oligodendroglial differentiation and myelination. Indeed, our nerve/glial antigen 2 (NG2)-specific oligodendroglial p38α MAPK conditional knockout (CKO) mice revealed its complex roles in myelination and remyelination. To identify the specific *in vivo* roles of oligodendroglial p38α MAPK in PVL, we generated a mouse PVL model by combination of LPS-mediated inflammation and hypoxia–ischemia in NG2-p38α MAPK CKO mice. Our results demonstrate that a selective deletion of p38α MAPK in oligodendrocyte did not attenuate myelination defects in the mouse model of PVL. Myelination phenotype revealed by MBP immunostaining was not significantly affected in the p38α MAPK CKO mice compared to the wildtype after PVL induction. The electron microscopic images demonstrated that the microstructure of myelin structures was not significantly different between the wild-type and p38α MAPK CKO mice. In addition, oligodendrocyte degeneration in the corpus callosum white matter area was unaffected in the p38α MAPK CKO during and after the PVL induction. These data indicate that p38α MAPK in oligodendrocyte has minimal effect on myelination and oligodendrocyte survival in the mouse PVL model.

Key words: p38alpha MAPK, periventricular leukomalacia, oligodendrocyte, myelination.

INTRODUCTION

Periventricular Leukomalacia (PVL) is the most common form of white matter injury underlying cerebral palsy in premature infants from maternal-fetal infection and oxygen-deprivation (Volpe, 2001, 2003; Deng et al., 2008; Deng, 2010). The disease phenotype is characterized by necrosis, mostly due to the death of pre-myelinating oligodendrocytes and oligodendrocyte precursor cells (OPC) of white matter region near the lateral ventricle. The OPCs, which differentiate into myelin-forming oligodendrocytes, are known to be especially vulnerable to PVL injury (Haynes et al., 2003). The proper structure of oligodendrocyte and myelination is crucial for maintaining the efficient transmission of electrical nerve potential.

Several useful animal models of PVL have been reported including rabbit, dog and sheep by hypoxia and inflammation induction (Hagberg et al., 2002), though they do not mimic all facets of human PVL pathology. A hypoxic-ischemic rat PVL model was previously described using postnatal day (P) 7 rat pups (Follet et al., 2000, 2004). This rodent model mimics majority

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Abbreviations: CKO, conditional knockout; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; GFP, green fluorescent protein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; NG2, neuron-glial antigen 2; OPC, oligodendrocyte precursor cell; P, postnatal day; PVL, periventricular leukomalacia; UCL, underwent unilateral carotid ligation.

https://doi.org/10.1016/j.neuroscience.2018.06.037
0306-4522/

Please cite this article in press as: Chung SH et al. The p38α MAPK Deletion in Oligodendroglia does not Attenuate Myelination Defects in a Mouse Model of Periventricular Leukomalacia. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.06.037

of the PVL pathology observed in human infants. The major hallmark of this rat PVL model is selective white matter injury, compared to other major stroke models that are characterized by considerable amount of gray matter infarction. Given the cost effectiveness and easy access/handling, we have developed an efficient mouse PVL model and carefully analyzed its phenotype and relevance to human PVL phenotype (Deng et al., 2008; Shen et al., 2010, 2012; Liu et al., 2011). The CNS developmental age in mice that matches with the human developmental stage of major phenotype for PVL lesions is P6–7. Indeed, our careful ischemia/LPS injection time point analysis confirmed that co-induction of LPS injection with hypoxia–ischemia at P6–7 in the mice induce a periventricular white matter lesion very similar to that seen in pediatric PVL (Deng et al., 2008).

The p38 mitogen-activated protein kinases (MAPKs) are essential mediators of stress responses and their physiological roles during oligodendrocyte development and myelination have been reported (Fragoso et al., 2003, 2007; Bhat et al., 2007; Hamanoue et al., 2007; Hossain et al., 2012). Using a variety of general p38 inhibitors, previous studies have demonstrated that p38α MAPK is important for inducing myelination in *in vitro* Schwann cells (Fragoso et al., 2003) and OPCs (Fragoso et al., 2007). Hossain et al., 2012 showed that p38α MAPK regulates Krox-20 to regulate Schwann cell differentiation and peripheral nerve myelination.

In addition, for the first time, we have reported the *in vivo* role of the p38α MAPK in normal peri/postnatal myelination process, as well as during remyelination after demyelination injury, by generating oligodendrocyte-specific p38α MAPK conditional knockout (CKO) mice (Chung et al., 2015). Our study revealed a complex dual role of p38α MAPK: (1) as a positive regulator in normal oligodendrocyte development and differentiation, and (2) as a negative regulator in a white matter injury demyelination model. The p38α MAPK negatively controlled remyelination in cuprizone-induced demyelination mouse model of p38α MAPK CKO by enhancing the remyelination ability (Chung et al., 2015). We now further investigate the possibility of white matter injury protection by p38α MAPK inhibition using p38α MAPK CKO mice model of PVL.

EXPERIMENTAL PROCEDURES

Generation of NG2^{cre} p38α MAPK$^{-/-}$ mice

Generation of NG2cre p38α MAPK$^{-/-}$ (p38α MAPK CKO) mice with p38α MAPK [B6.129-Mapk14 < tm1.2Otsu >] has been described previously (For details, refer to Chung et al., 2015). Briefly, Cre/loxP recombination system is used by breeding NG2/Plp-Cre mice and p38α-floxed (p38α MAPK fl/fl) mice. All mice that were used in this study were carefully maintained in accordance to the NIH guidelines for the Care and Use of Laboratory Animals. Experimental protocols used for this study were approved by the Institutional Animal Care and Use Committee at the University of California, Davis and University of Illinois at Chicago.

PVL mouse model

Newborn mice pups were administered with LPS (lipopolysaccharide, Sigma), a potent inflammatory agent, by intraperitoneal injections at a dose of 0.12 mg/kg body weight, twice a day, from P4 to P7. Then, at P6 or P7 the pups underwent unilateral carotid ligation (UCL) surgery to induce ischemia followed with hypoxia (H/I). This procedure caused selective white matter injury near the periventricular regions. Mice were anesthetized under ice and then underwent UCL followed by a 1-h recovery interval during which the pups were housed with the dam and kept on a thermal blanket to maintain body temperature at 33–34 °C. For a detailed description of the protocol, please refer to (Shen et al., 2010, 2012; Liu et al., 2011).

Electron microscopy

Mice were transcardially perfused with Karnovsky's solution (4% PFA in PBS with 5% glutaraldehyde) and stayed overnight with post-fixation agent containing 2% osmium tetroxide in 0.1 M cacodylate buffer. The brains were dehydrated and embedded and stained with toluidine blue to locate white matter regions. The samples were cut in 70 nm and placed on Formva-coated copper grids. The sections then stained with uranyl acetate and lead citrate, and observed in a Philips CM120 Electron Microscope at 80 kV. Images were acquired to demonstrate myelion sheath and g-ratio via a high resolution CCD camera (Gatan, Pleasanton, CA, USA).

Immunohistochemistry

Mice were anesthetized with intraperitoneal sodium pentobarbital injection (100 mg/kg, i.p.) and transcardially perfused with 0.9% NaCl in 0.1 M PBS, pH 7.4 followed by 4% PFA in 0.1 M PBS (pH 7.4). The brains were then removed from the skull and post-fixed in 4% PFA at 4 °C for 48 h. Brain tissues were cryoprotected with a series of sucrose solutions and sectioned with 40-μm thickness. The rabbit polyclonal, and mouse monoclonal anti-mouse green fluorescent protein (GFP) antibodies (1:1000, Abcam) to identify EYFP, anti-mouse MBP antibody (1:500; Sternberger and Sternberger), anti-rabbit p38 (1:1000, Abcam) and anti-human Olig2 antibody (1:500; Abcam) were used in our study.

For the Diaminobenzidine (DAB) peroxidase immunohistochemistry, sectioned sections were blocked with 10% normal goat serum and then incubated in 0.1 M PBS containing 0.1% Triton-X and the primary antibody for 16–18 h at 4 °C. Brain sections were then treated with HRP-conjugated goat anti-rabbit or HRP-conjugated goat anti-mouse secondary antibodies (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 2 h at room temperature. DAB (0.5 mg/ml) was used to stain with brown colors. Finally, sections were dehydrated and, cover-slipped with Entellan mounting medium (BDH Chemicals, Toronto, ON, Canada).

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