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BRAIN RESEARCH

Research Report

Inhibition of matrix metalloproteinase-9 attenuated neural progenitor cell migration after photothrombotic ischemia

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ABSTRACT

Recent studies have shown that neuroblasts migrate from the subventricular zone (SVZ) into the injured area after ischemic brain insults. However, it is not well understood which mechanism mediates this ectopic migration and which types of cells migrate into the damaged region from the SVZ. The present study was designed to investigate the characteristics of the migration of nestin-positive neural stem cells toward the region of ischemic injury after focal cortical ischemia. Nestin-eGFP transgenic mice were used to effectively model the migration of SVZ cells. Photothrombotic ischemia was induced by injection of rose bengal (30 mg/kg) and exposure to cold light. Migration of nestin-positive cells was examined using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and bromodeoxyuridine (BrdU) labeling. The number of nestin-positive cells was increased significantly in the peri-infarct area at 5 and 7 days after photothrombosis. A subset of nestin-positive cells was co-labeled with DiI or BrdU. Some of the nestin-positive cells co-expressed doublecortin (DCX) and only a few nestin-positive cells co-labeled with anti-epidermal growth factor receptor (EGFr) antibody. However, no nestin-positive cells were immunoreactive for glial fibrillary acidic protein (GFAP). The inhibition of matrix metalloproteinases (MMPs) using the MMP inhibitor, FN-439, decreased nestin-positive cells in the peri-infarct region at 7 days after photothrombosis. Although MMP-9 was not co-expressed in the nestin-positive cells in the peri-infarct cortex, MMP-9 did co-localize with GFAP-positive astrocytes. These results suggest that nestin-positive neural progenitor cells migrate into the peri-infarct cortex after photothrombotic ischemia and that MMP-9 is involved in the migration.

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Abbreviations: SVZ, subventricular zone; BrdU, 5-bromodeoxyuridine; DiI, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; DCX, doublecortin; GFAP, glial fibrillary acidic protein; EGFr, epidermal growth factor receptor; PDGFrα, platelet derived growth factor receptor α; MMP, matrix metalloproteinase; RMS, rostral migratory stream; OB, olfactory bulb; DG, dentate gyrus; MCAO, middle cerebral artery occlusion; SDF-1α, stromal cell-derived factor-1α; DAPI, 4',6-diamidino-2-phenylindole; FACS, fluorescence-activated cell sorting

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1. Introduction

Neurogenesis in the adult mammalian brain is restricted to the subventricular zone (SVZ) (Alvarez-Buylla and Garcia-Verdugo, 2002) and the hippocampal dentate gyrus (DG) (Gage, 2000). In particular, the SVZ contains many neuroblasts that migrate tangentially along the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into interneurons (Lois and Alvarez-Buylla, 1993; Luskin, 1993). Focal ischemia enhances neurogenesis in the SVZ of rodent brain (Jin et al., 2001), and the newly generated neurons in the SVZ migrate to the region of ischemic injury (Arvidsson et al., 2002; Parent et al., 2002; Jin et al., 2003). Recent reports have shown that the neuroblasts increased by middle cerebral artery occlusion (MCAO) not only migrate from SVZ into striatum, but also differentiate into mature neurons (Thored et al., 2006; Yamashita et al., 2006). Many studies of cortical injury models, such as cortical ischemic injury (Jin et al., 2003; Tsai et al., 2006; Ohab et al., 2006), cortical aspiration (Goings et al., 2004) and traumatic brain injury (Ramaswamy et al., 2005; Salman et al., 2004), have demonstrated that neuroblasts migrate from the SVZ into the cortical injury region. These findings have increased the possibility that endogenous neural stem cells can be used to replace injured neurons in the treatment of brain ischemia. However, the precise mechanism that mediates the migration of neural stem cells and the molecules that induce migration into the ischemic regions have not been thoroughly investigated.

Recent studies have shown that stromal cell-derived factor-1α (SDF-1α) and matrix metalloproteinase-9 (MMP-9) are molecules that attract neural stem cells toward ischemic regions after MCAO (Thored et al., 2006; Imitola et al., 2004; Robin et al., 2006; Lee et al., 2006; Wang et al., 2006). In particular, MMP-9 was linked to cerebellar granule cell migration and axonal outgrowth during postnatal development (Vaillant et al., 2003). The expression of MMP-9 was increased in ischemic cortex and striatum after MCAO (Asahi et al., 2001). MMP-9 also cleaved laminin on the neuronal surface in brain, as well as collagen and fibronectin (Gu et al., 2005). Therefore, MMP-9 may be an ideal candidate to promote neural stem cell migration into the region of ischemic injury.

Nestin, an intermediate filament protein, is expressed in neural stem and progenitor cells during development and in the adult (Lendahl et al., 1990). The expression of nestin-GFP transgenes correlated with stem and progenitor cells in vivo, and nestin-GFP-positive cells behaved like stem cells in an in vitro study using fluorescence-activated cell sorting (FACS) (Mignone et al., 2004). Nam et al. have previously reported that many nestin-positive neural progenitor cells from the SVZ migrate through RMS to OB in a study using nestin-eGFP transgenic mice (Nam et al., 2007). Thus, we used the same nestin-eGFP transgenic mouse line to characterize the migrating cells and to investigate the mechanism of migration.

The aim of this study was to test the hypothesis that neural progenitor cells migrate into ischemic cortex via a mechanism mediated by MMP-9. For this purpose, focal cortical ischemia was induced by photothrombosis, and the migration of nestin-positive cells into the ischemic cortex and the effect of MMP inhibition on the migration were investigated.

2. Results

Nestin-positive cells were localized in SVZ, RMS, OB, and hippocampal DG, but few nestin-positive cells were observed in cortex. However, after photothrombotic ischemia, many nestin-positive cells were found in the peri-infarct cortex (Fig. 1B). The nuclei of these nestin-positive cells were confirmed by 4',6-diamidino-2-phenylindole (DAPI) staining (Figs. 1C, D). Four areas were photographed using a CCD camera in order to count the nestin-positive cells and are illustrated in Fig. 1A. The number of nestin-positive cells counted in these areas gradually increased after ischemia and was significantly increased at 5 and 7 days (Fig. 1E). DiI was injected into the ventricle to identify the ectopic migration of nestin-positive cells into the injured area. Some nestin-positive cells were double-labeled with DiI fluorescence (Figs. 2A–C). In the BrdU labeling study, some nestin-positive cells also co-localized with BrdU-positive cells (Figs. 2D–F). Nestin-positive cells in the peri-infarct cortex were examined using several cell markers to identify their cell types. DCX-positive

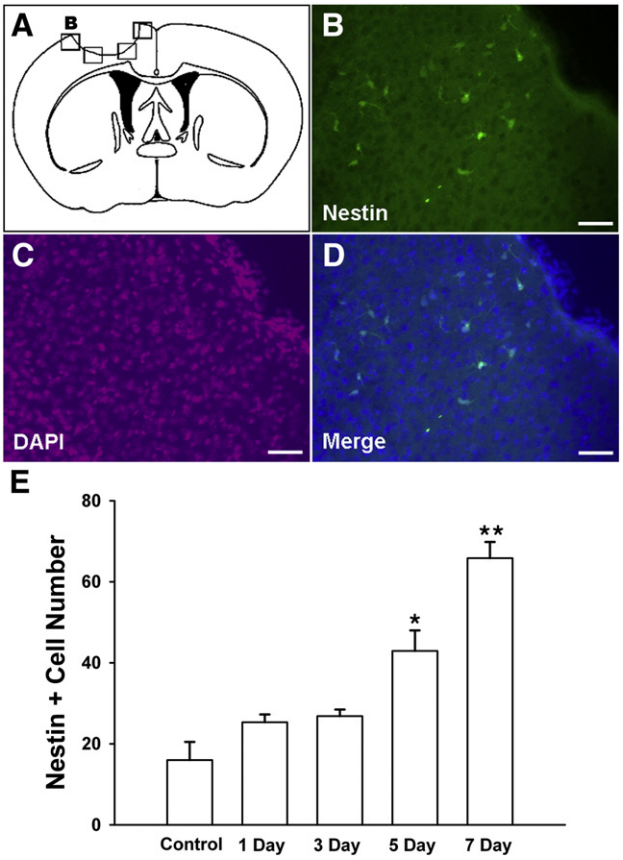

Fig. 1 – Nestin-positive cells in the nestin-eGFP adult transgenic mouse. Four regions pictured with 20× magnification objective lens were used to count nestin-positive cells (A). Nestin-positive cells (B), DAPI-positive nuclei (C), and merged image (D) in the injured cortex of photothrombotic mouse (after 7 days). Scale bars in B–D represent 50 μm. The increase of Nestin-positive cells in the peri-infarct cortex (E). Each column represents mean ± SEM of 4 experiments. *$p<0.05$, **$p<0.01$ compared with control.