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Characterization of neuroprogenitor cells expressing the PDGF β-receptor within the subventricular zone of postnatal mice

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We report a considerable number of cells in the ventricular and the subventricular zones (SVZ) of newborn mice to stain positive for the PDGF β-receptor (PDGFRB). Many of them also stained for nestin and/or GFAP but less frequently for the neuroblast marker double-cortin and for the mitotic marker Ki-67. The SVZ of mice with nestin-Cre conditional deletion of PDGFRB expressed the receptor only on blood vessels and was devoid of any morphological abnormality. PDGFRB$^{-/-}$ neurospheres showed a higher rate of apoptosis without any significant decrease in proliferation. They demonstrated reduced capacities of migration and neuronal differentiation in response to not only PDGF-BB but also bFGF. Furthermore, the PDGFR kinase inhibitor STI571 blocked the effects of bFGF in control neurosphere cultures. bFGF increased the activity of the PDGFRB promoter as well as the expression and phosphorylation of PDGFRB. These results suggest the presence of the signaling convergence between PDGF and FGF. PDGFRB is needed for survival, and the effects of bFGF in migration and neural differentiation of the cells may be potentiated by induction of PDGFRB.

Keywords: PDGF-receptor; FGF; Neural stem cells; Neurosphere

Introduction

Neural stem cells have been shown to play important roles for repair and regeneration following ischemic injuries, and also in experimental neurodegenerative diseases (Park et al., 2006; Snyder and Macklis, 1995). The expression of growth factors, such as PDGF and its receptors, increases in such conditions (Funa et al., 1996; Hermanson et al., 1995; Sjoborg et al., 1998). PDGF has been shown to stimulate proliferation (Erlandsson et al., 2001), neuritogenesis (Richards et al., 2006), and survival (Lobsiger et al., 2000) of various types of neuroprogenitors. Furthermore, activation of the PDGF signals has been frequently shown to be associated with tumorigenesis of malignant glioma (Hermanson et al., 1996; Lokker et al., 2002; Ma et al., 2005). Adult neuroprogenitors have been considered to be the cells that could give rise to glioma upon acquiring genetic and epigenetic alterations. These somatic gene alterations have been hypothesized to occur upon cellular stress and injuries, as well as during the repair processes when progenitors are stimulated for proliferation (Manuelidis, 1994). A recent report on the presence of PDGFRA-expressing adult neural stem cells has demonstrated the importance of PDGF in neurogenesis, and also suggesting the identity of such cells as glioma progenitors (Fomchenko and Holland, 2007; Jackson et al., 2006). However, the knowledge about the role of PDGFRB in postnatal neurogenesis still remains to be clarified.

PDGFRA knockout is lethal to mice, exhibiting incomplete cephalic closure similar to that observed in a subset of Patch mutants (Soriano, 1997). In these mice, increased apoptosis was observed on the pathway of migrating neural crest cells. In contrast, the conventional PDGFRB knockout mice have not shown any obvious abnormality in the nervous system (NS) (Betsholtz, 1995). However, since PDGFRB knockout mice die at birth, it is difficult to study dysfunctions in the postnatal NS. Recently, neural cell-specific PDGFRB knockout mice were produced by crossing PDGFRB$^{FL/FL}$ mice with mice expressing Cre recombinase under regulation of the nestin promoter (Ishii et al., 2006). These mice showed no gross anatomical abnormalities or functional deficits in the NS. However, the depletion of PDGFRB resulted in markedly reduced neuronal cell survival after cryogenic and NMDA injuries to the adult mice. Since neural stem cells are known to migrate to the site of damage (Felling and Levison, 2003), such cells lacking PDGFRB might explain the reason of the poor cell survival and ineffective healing in PDGFRB$^{-/-}$ mice.

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In order to test these hypotheses, we have examined the SVZ of nestin promoter PDGFRB$^{-/-}$ mice to see the presence of neural stem cells and their phenotypes in comparison with the PDGFRB$^{FL/FL}$ control mice. We further attempted to isolate and characterize neuroprogenitors from mice with the conditional depletion of PDGFRB. However, depletion of the nestin-specific PDGFRB does not occur in 100% of neuroprogenitors. Consequently, a minute fraction of PDGFRB possessing cells will be enriched after serial passages of such cultures. Therefore, we explanted postnatal day 1 (P1) SVZ cells from the PDGFRB$^{FL/FL}$ mice and stably transfected

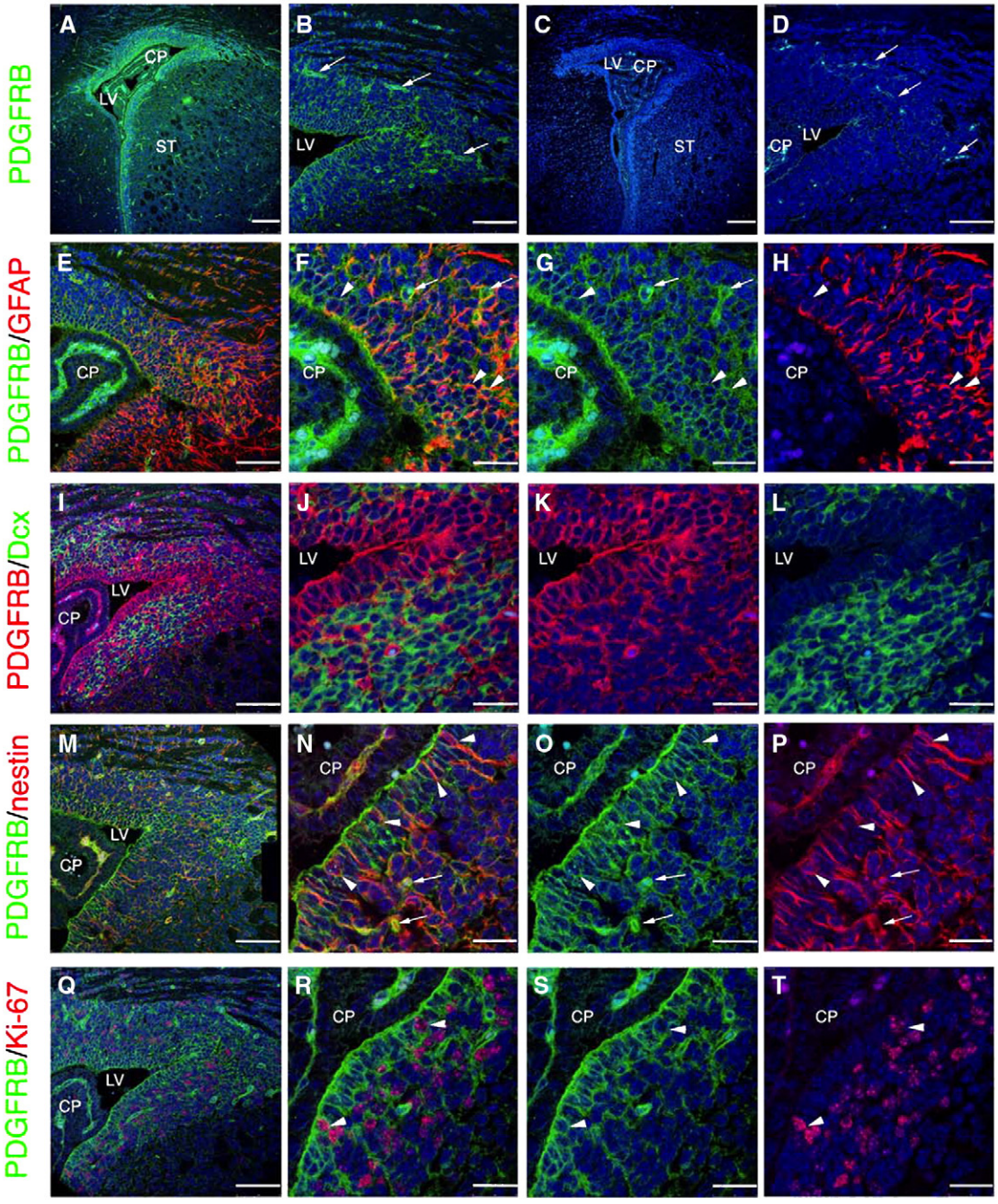

Fig. 1. Coronal sections of the P1 SVZ in control mouse (PDGFRB$^{FL/FL}$; A, B, E–T) and nestin-Cre conditional PDGFRB$^{-/-}$ mouse (C, D) stained for PDGFRB (green except for I–L where PDGFRB is red) identified with the confocal microscope. Note that many PDGFRB-expressing cells are observed in the SVZ of PDGFRB$^{FL/FL}$ mouse (A, B), whereas the immunoreactivity is completely abolished in cells in the SVZ except for the blood vessels (arrows in panels B, D) in conditional nestin-Cre PDGFRB$^{-/-}$ mouse (C, D). (E, F) Co-immunostaining for PDGFRB and GFAP (red). Arrows for PDGFRB-expressing blood vessels and arrowheads for co-immunostained SVZ cells. (I–L) Only a few cells express both PDGFRB (red) and Dcx (green). (M–P) PDGFRB and nestin (red) are coexpressed by a majority of SVZ cells (arrowheads) and blood vessels (arrows). (Q–T) PDGFRB and Ki-67 (red). Arrowheads point to co-expressing cells. Counterstained with DAPI (blue). CP, choroid plexus; LV, lateral ventricle; ST, striatum. Scale bars = 200 μm (A and C), 75 μm (B, D, E, I, M, and Q), and 25 μm (F–H, J–L, N–P, R–T).