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BRAIN RESEARCH

Research Report

The modulatory effects of bHLH transcription factors with the Wnt/β-catenin pathway on differentiation of neural progenitor cells derived from neonatal mouse anterior subventricular zone

ChunQing Zhang[1], *ZhiYuan Zhang*[1], *HaiFeng Shu, ShiYong Liu, YeChun Song, KeJun Qiu, Hui Yang**

Department of Neurosurgery, Xinqiao Hospital, Third Military Medical University, 2-V Xinqiao Street, Chongqing 400037, China

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ABSTRACT

The subventricular zone (SVZ) located adjacent to the lateral ventricles is the major site where neural progenitor cells (NPCs) are concentrated in the adult brain. NPCs in the anterior subventricular zone (SVZa) generate neuronal precursors and migrate along a highly localized pathway—the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into interneurons. To investigate the modulatory effects of basic helix–loop–helix (bHLH) transcription factors on differentiation from SVZa NPCs, we firstly examined the distribution of bHLH family members (Mash1, Id2, and Hes1) in cultured mouse SVZa NPCs and evaluated their regulatory effects on differentiation by transfection with Mash1, Id2, or Hes1 eukaryotic expression plasmid. Furthermore, we assessed the effects of bHLH transcription factors on the expression of downstream molecules of the Wnt/β-catenin pathway, β-catenin and (Glycogen synthase kinase-3β). Our results demonstrated that Mash1, Id2, Hes1 were all widely expressed in *in vitro* progenies from mouse SVZa NPCs. Analyses of SVZa NPCs transfected with eukaryotic expression plasmids showed that Mash1 promoted neuronal differentiation from SVZa NPCs, while Id2 and Hes1 repressed neuronal differentiation. In addition, we found that Id2 and Hes1 simulated expression of β-catenin and GSK-3β, while Mash1 inhibited their expression. Our results suggest that the classic bHLH transcription factors, Mash1, Id2 and Hes1, play important roles in the regulation of differentiation from SVZa NPCs. This modulation is possibly mediated by a coordination of bHLH and Wnt/β-catenin signaling.

* *Corresponding author*. Fax: +86 23 65218204.
E-mail address: huiyang64@yahoo.com (H. Yang).

Abbreviations: SVZ, subventricular zone; NPCs, neural progenitor cells; RMS, rostral migratory stream; OB, olfactory bulb; bHLH, basic helix-loop-helix; Mash, mammalian achaete–scute homolog; Id, Inhibitor of DNA binding; Hes, Hairy Enhancer of Split; NSA, neurosphere assay

[1] The authors contributed equally to this paper.

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1. Introduction

It is generally accepted that the anterior subventricular zone (SVZa) is the major site in which neural progenitor cells (NPCs) reside in the adult mammalian nervous system. It renews the postnatal olfactory bulb interneurons throughout life (Alvarez-Buylla and Garcia-Verdugo, 2002; Gould and Gross, 2002). Different from the NPCs in other regions, the SVZa-derived progenitors are generated with phenotype markers characteristic for the neuronal lineage, and maintain proliferative and neuronal characteristics without further differentiation during migration until they reach their destination and differentiate into dopaminergic and GABAergic neurons *in vivo* (Luskin, 1993; Luskin et al., 1997a; Menezes et al., 1995; Temple, 2001). In addition, the SVZa appears to be comprised exclusively pure population of neural progenitor cells (Luskin et al., 1997b). These features make the SVZa NPCs a reliable model of neural proliferation and differentiation (Liu et al., 2004).

bHLH (basic helix–loop–helix) transcription factors are critical for the neural development, especially the neuronal differentiation of NPCs (Powell and Jarman, 2008). The bHLH family are composed of multiple transcription factors, including Mash (mammalian achaete–scute homolog), Id (Inhibitor of DNA binding) (Ross et al., 2003), and Hes (Hairy Enhancer of Split). The regulation effects of these bHLH transcription factors on neuronal differentiation and proliferation has been well studied in several NPCs lines. Mash1 is the most studied bHLH family member. It participates in the differentiation of NPCs into mature neurons (Kim et al., 2008). Id2 is an important member of the Ids family. It regulates neural cell proliferation, differentiation, apoptosis, and fate with cell type-specific functions during neural development and cell proliferation (Havrda et al., 2008). Hes1 represses neuronal differentiation and is essential for preserving the NPCs' self-renewal capability and suppressing NPCs differentiation from multipotent stem cells into neuronal precursors (Kageyama et al., 2008). Nonetheless, it is uncertain whether bHLH transcription factors have the same

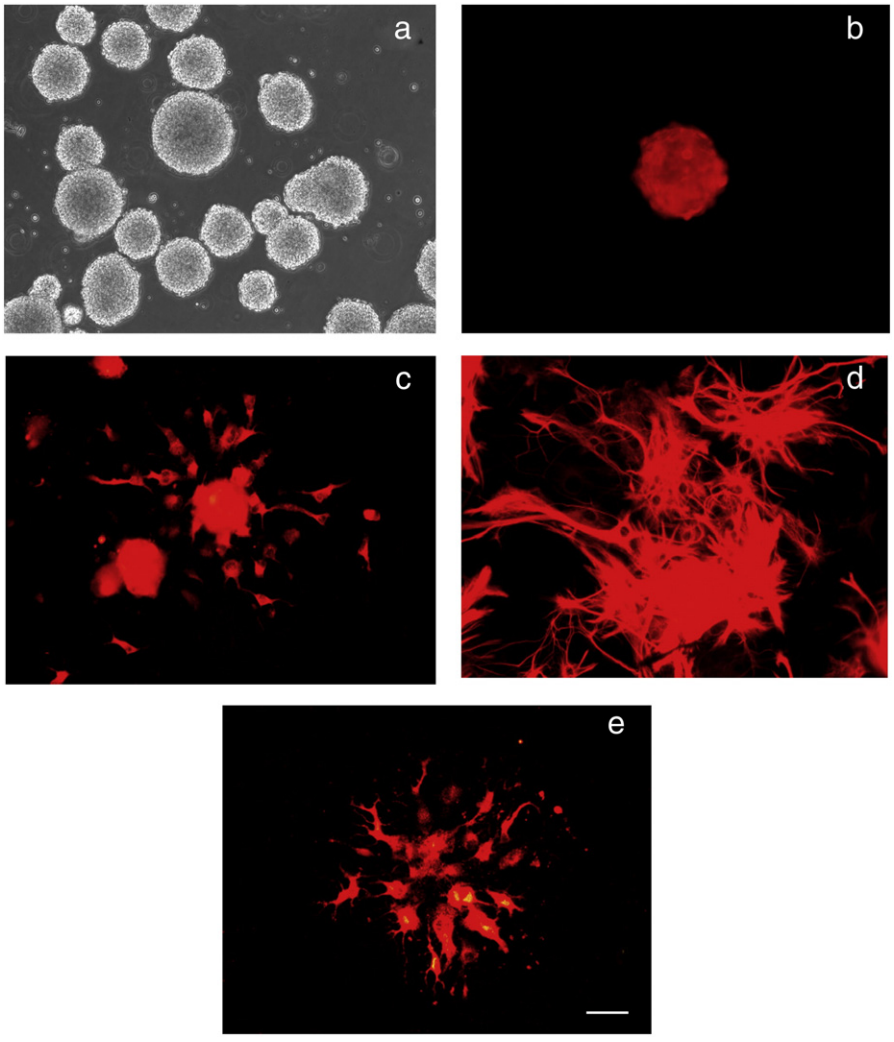

Fig. 1 – Culture and identification of SVZa NPCs. (a) Subcultured SVZa NPCs grew in suspensions as neurospheres that increased in number and size with cell proliferation. (b) The third generation of SVZa NPCs was grown in a serum-free medium for 3 days and fixed for nestin immunohistochemistry staining. (c–e) The SVZa NPCs were seeded onto coverslips pre-coated with poly-L-lysine hydrochloride in DMEM/F12 containing 10% FBS. After 3 days, the cells were immunostained for neurons (NSE), astrocytes (GFAP), and oligodendrocytes (CNPase). Scale bar: 50 μm.