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Nestin-positive cells in the spinal cord: a potential source of neural stem cells

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ABSTRACT

Some literatures have reported neural precursor cells (NPCs) exist in spinal cord of adult mammal, however, the NPCs distribution feature in spinal cord of adult mice so far is not described in detail. In order to observe and compare the distribution feature of NPCs in various spinal cord regions of adult mice, to research a potential source of neural stem cells (NSCs), we obtained NPCs distribution feature by analyzing the distribution of the nestin-containing cells (NCCs) in spinal cord of adult nestin second-intron enhancer controlled LacZ reporter transgenic mice (pNes-Tg) with LacZ staining and positive cell quantification. The results showed that: NCCs were observed in various regions of spinal cord of adult mice, but amount of NCCs was different in distinct region, the rank order of NCCs amount in various spinal cord regions was dorsal horn region greater than central canal greater than the ventral and lateral horn. NCCs in dorsal horn region mainly distributed in substantia gelatinosa, NCCs in central canal mainly distributed in ependymal zone, on the contrary, NCCs in ventral, lateral horn, medullae, nucleus regions of spinal cord were comparatively less. The rank order of NCCs amount in various spinal cord segments was cervical segment greater than lumbar sacral segment greater than thoracic segment. There was no significantly difference between NCCs amount in the left and right sides, and within cervical 1–7, thoracic 1–12, lumbar 1–5, sacral segment of spinal cord in adult mice. These data collectively indicate that NPCs extensively distribute in various regions of spinal cord of adult mice, especially in substantia gelatinosa and ependymal zone. NPCs in cervical segment are abundant, NPCs in thoracic segment are the least while compared the different spinal cord segment, the NPCs in various regions of spinal cord of adult mice are a potential source of NSCs.

1. Introduction

The earlier conventional idea was thought that there was not neural precursor cells (NPCs) in mature central nervous system (CNS), neural cells growth immediately stopped at postnatal, consequently, neural tissues could not renovate after damage, but recently, researchers proposed question for this idea, firstly, researchers found that NPCs existed in the subventricular zone (SVZ) or subependymal zone (SEZ) of adult mammalian brain, and confirmed that these cells completely met NPCs feature through cell culture and molecular cloning technology. These cells were "blast cell" of all types of neural cells in mammalian CNS, they might differentiate three types of neural cells, *i.e.* neuron, astrocyte and oligodendrocyte. Secondly, in the 1990s researchers isolated the cell groups which could generate unceasing division growth and possessed multiple differentiation potency, and proposed NPCs concept. Now more and more investigated information has provided proof that NPCs exist in adult animal CNS (Kornack and Rakic, 2001; Shan et al., 2006; Ke et al., 2006; Chi et al., 2006).

In the past decades, several regions have been identified in the adult mammalian CNS that harbor a population of multipotent and self-renewing cells, *i.e.* neural stem cells (NSCs)/neural progenitor cells. As early as in the 1960s, two regions were detected where neurogenesis took place even in the adult rodent CNS: (1) subgranular layer within dentate gyrus of hippocampal formation and (2) rostral migratory stream where neural progenitor cells migrate from the SVZ of lateral ventricles towards olfactory bulb. In the meantime, adult neural progenitor cells have also been detected in other regions of rodent brain, such as rat neocortex (Palmer et al., 1999), rat septum and striatum (Palmer et al., 1995), rat substantia nigra (Lie et al., 2002), rat and mouse spinal cord (Horner et al., 2000; Wei et al., 2002). These cells fulfill at least *in vitro* two main characteristics of stem/progenitor cells as proposed by Lajtha (1979): (1) self-renewal, *i.e.* the ability to maintain a steady population by continuous proliferation and (2) multi-

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potency, *i.e.* the ability to differentiate into one of the three major cell types of the adult CNS: neurons, astroglia and oligodendroglia.

The identification of a population of cells with regenerative potency within the adult mammalian CNS not only has changed our understanding of its histoarchitecture and function, but also has held the possibilities in therapeutic challenges, such as neurodegenerative disorders, multiple sclerosis, stroke, neurooncology or neurotraumatology. The present theories prompt that NPCs exist in adult CNS after embryonic development period stopping, these NPCs can recover proliferation and differentiation potency, can be induced to differentiate certain special neural cells, and can substitute damaged neural cells in special pathological conditions (Shan et al., 2006; Ke et al., 2006; Chi et al., 2006), which brings wishes for neural functional reconstruction and regeneration. At recent years NPCs research was extensively revoked, especially induced endogenous NPCs to proliferate, differentiate and migrate to damaged regions to repair the damaged neural cells, because by the time exogenous NPCs transplantation has not acquired an effective result yet. Liu et al. determined that endogenous NPCs could proliferate, differentiate into neural cells, and migrate to damaged regions of brain and spinal cord in pathological conditions like injury and neuron degenerative disorder, but quantity of *de novo* neural cells was not enough to repair damaged neural cells (Shan et al., 2006; Ke et al., 2006; Chi et al., 2006). Currently, immense interest is focused on finding ways to induce endogenous NPCs to proliferate, differentiate into specific neural cell, and migrate to damaged area, which would be candidates for cell replacement therapies for a variety of neurologic diseases. However, the very quality that makes endogenous NPCs so appealing is the Achilles heel of NPCs research. Firstly, it needs to be defined the endogenous NPCs distribution feature and property in CNS, which will allow for endogenous NPCs replacement therapy to become a reality. Concerning whether or not NPCs exist in adult mammalian CNS *in vivo*, a number of papers have been published over the past few years (Palmer et al., 1995, 1999; Lie et al., 2002; Horner et al., 2000; Wei et al., 2002), however, these NPCs different distribution regions in CNS have not yet been compared with respect to the NPCs amount in various regions. So far, it is the paucity of description about NPCs distribution feature of adult mice spinal cord.

NPCs distribution in spinal cord is an important primary work to study the repairing of spinal cord damage by endogenous NPCs. The prospect of inducing endogenous NPCs to repair the damaged spinal cord has heightened our interest in delineating NPCs distributed feature in adult animal spinal cord and searching the possible NSCs source in spinal cord. Therefore, this study is aimed to search a potential source of NSCs in adult animal spinal cord by observing distribution of NCCs in spinal cord of adult mice with nestin promoter (enhancer) driven LacZ reporter transgenic mice (pNes-Tg).

It is well documented that the expression of nestin protein in the prenatal and postnatal developing CNS of mammals reflects the differentiation or proliferative state of NPCs. Embryonic nestin belongs to class III intermediate filament, which also includes vimentin and glial fibrillary acidic protein (GFAP), and, structurally, it is closely related to class IV intermediate filaments, such as neurofilaments and α-internexin. At the embryonic neurulation stage, multipotential NPCs temporarily express nestin protein. Substitution of nestin protein by vimentin and GFAP or neurofilaments takes place sequentially during the maturation or differentiation of NPCs. Nestin is down-regulated either at the onset of GFAP or neurofilament expression or during subsequent differentiation of multipotential NPCs into astrocytes or neurons, thus, the transient abundant expression of nestin is extensively recognized as a marker for multipotential NPCs in developing CNS of mammals. Previous evidence has shown that nestin protein is abundantly expressed in proliferative regions of embryonic or developing CNS, and is recognized as a sensitive marker for NPCs in the CNS. It implies that, to certain extent, these neural cells expressing nestin reflect their reiterating or sustaining active state, and may implicate in the neurogenesis, remodeling and repairing processes of developing and adult CNS (Dahlstrand et al., 1995; Wei et al., 2002).

pNes-Tg mouse carrying LacZ reporter gene under the control of the nestin second-intron enhancer, is the well established animal model for identification and characterization of NPCs in the CNS (Ke et al., 2006; Chi et al., 2006; Aoki et al., 2000; Mitsuhashi et al., 2001), it is evolved as a transgenic experimental model which is ideally suited to studying NSCs potential source of CNS (Shan et al., 2006; Ke et al., 2006; Chi et al., 2006).

In this report, we described distribution feature of NPCs in spinal cord of adult mice. The data showed that NPCs largely distributed in dorsal horn and central canal, NPCs in the cervical segment were greater than lumbar sacral segment greater than thoracic segment. We provided more evidence that NPCs extensively existed throughout spinal cord of adult mice.

2. Experimental procedures

2.1. Transgenic mice lines

Adult C57BL/6J (70–80 days of age) nestin promoter (enhancer) driven LacZ reporter transgenic mice (pNes-Tg) (Jackson Laboratory, Bar Harbor, ME, http://www.jax.org) were used in our experiment. Transgenic progeny were identified by regular polymerase chain reaction amplification of tail DNA using specific primers. The experimental protocols for mouse studies were approved by the Institutional Animal Use and Care Committee and were in close agreement with the National Institutes of Health Guidelines for the care and use of laboratory animals of America and China.

2.2. LacZ staining

The adult transgenic and control mice were anesthetized and perfused with 20 ml of 0.9% saline buffer followed by 40 ml 4% PFA in 1× PBS (pH 7.5) at room temperature. Mice spinal cord was excised and placed in fixative buffer (4% PFA) overnight, followed by incubation in 20% sucrose in 1× PBS (pH 7.5). After embed in OCT, the spinal cord was coronally successively sectioned 12 μm section on a Leica cryostat (Model 3050S) at 120 μm interval between two sections from rostral to caudal, and collected on Superfrost Plus slides. For LacZ staining, the sections were washed twice with 1× PBS, then incubated in β-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranosid) staining solution (4 mM potassium ferrocyanide, 4 mM potassium ferricyanide, 2 mM $MgCl_2$, 400 μg/ml β-gal) at room temperature for 8 h. Afterward, the sections were washed six times with 1× PBS again, postfixed in 70% ethanol.

2.3. Image collection and analysis

All images were collected with a Nikon fluorescent microscope E800 with digital camera (Nikon Corporation, Tokyo, http://www.nikon.com) and analyzed with image analyze software. Quantification of NPCs distribution was performed by counting amount of LacZ positive cells of 10 sections of each region at 20× magnification and calculating the LacZ positive cells sum of all sections of each region, then the sum of LacZ positive cells were divided into section numbers, *i.e.* a total of 10 sections (every 10th section) amongst per segment of the mouse spinal cord stereotaxic coordinates were counted, five mice per group were used, the averaged amount was used for qualifications analyze.

2.4. Statistics

Statistical analysis of LacZ-positive cells in distinct spinal cord regions of adult mice was performed using the paired Student's *t* test. All data were expressed as average ± S.D., $p < 0.05$ was considered statistically significant.

3. Results

In this study we located the position of various spinal cord regions collating to C57BL/6J mice spinal cord atlas (http://