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Research Report

Nestin overexpression promotes the embryonic development of heart and brain through the regulation of cell proliferation

Jinkai Liu[a,1], Xiaozhen Ji[a,1], Zhenlin Li[b], Hua Zheng[c], Wenhong Zheng[a], Junshuang Jia[a], Hongfen Shen[a], Qianbing Zhang[a], Jing An[a,*]

[a]Cancer Research Institute, Southern Medical University, Guangzhou 510515, China
[b]Department of Embryology and Histology, Southern Medical University, Guangzhou 510515, China
[c]Department of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China

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ABSTRACT

Nestin, an intermediate filament protein, is a key regulator of various extracellular proteins that play important roles in cell growth and differentiation. In recent years, nestin has been widely accepted as a molecular marker for neural stem/progenitor cells. However, its function during embryogenesis remains largely unknown since its depletion is lethal after stage embryonic day 8.5 (E8.5). In order to understand the role of this protein *in vivo*, we compared the heart and brain tissues of control mice with those of mice overexpressing a human *nestin* cDNA transgene under the control of a ROSA26 promoter. In these tissues we examined the general histology and cell size, the presence of apoptotic cells by TUNEL assay, and the presence of progenitor cell markers like SOX2. Compared to controls, mouse embryos overexpressing the human *nestin* transgene have a larger size and display characteristic morphological changes including a larger heart and forebrain. In these tissues we found corresponding increases in the size of cardiomyocytes and brain cells, as well as indications of augmented cell proliferation. In contrast, apoptosis was not significantly altered. Co-staining brain sections with SOX2 and Ki67 showed that most of the proliferating cells in the forebrain were neural stem cells. Moreover, nestin overexpression was responsible for a marked activation of the PI3K/Akt signaling pathway. Taken together, the results of this study indicate that nestin plays an important role in the embryonic development of at least two mouse organs (heart and brain) through the regulation of cell proliferation.

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Abbreviations: IF, intermediate filament; NSCs, neural stem cells; NPCs, neural progenitor cells; E8.5, embryonic day 8.5; WT, wild type; TG, transgenic; PI3K, phosphoinositide 3-kinase

*Corresponding author. Fax: +86 20 61648709.
E-mail address: anjing77@163.com (J. An).

[1]These two authors contributed equally to this work.

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1. Introduction

Nestin, a cytoskeleton-associated class VI intermediate filament (IF) protein, was originally cloned from the central nervous system of rat embryos (Lendahl et al., 1990). Since its identification, it has been used as a marker for neural stem cells (NSCs) in embryonic and adult brain tissue (Gilyarov, 2008; Lendahl et al., 1990). During embryogenesis, nestin is also expressed outside the nervous system in organs such as heart (Kachinsky et al., 1995), muscle (Kachinsky et al., 1994), pancreas (Delacour et al., 2004; Esni et al., 2004), and skin (Li et al., 2003). Interestingly, during early development, the majority of nestin positive cells are stem and progenitor cell populations engaged in active proliferation (Wiese et al., 2004). In contrast, upon cell differentiation, nestin expression is downregulated and gradually replaced by tissue-specific IF proteins such as glial fibrillary acidic protein, neurofilaments, and desmin (Wiese et al., 2004) (in astrocytes, neurons, and muscle cells, respectively).

The functional significance of nestin expression has not been fully elucidated. Recent studies indicate that nestin has an important role in the distribution and organization of cellular factors regulating proliferation, survival, and differentiation (Bieberich et al., 2003; Sahlgren et al., 2003; Shen et al., 2002; Toivola et al., 2005). In adults, nestin expression is upregulated under pathological conditions such as the formation of the glial scar after injury to the central nervous system (Frisen et al., 1995), or during the regeneration of injured muscle tissue (Vaittinen et al., 1999, 2001). In addition, the correlation between nestin expression and cell proliferation can be observed during tumorigenesis. For example, abundant nestin expression was found in gliomas, melanomas, angiosarcomas, and pancreatic adenocarcinomas, where increased levels of expression appear to correlate with malignancy (Dahlstrand et al., 1992; Yang et al., 2008).

Conversely, others studies suggest that the link between nestin expression and cell proliferation is less obvious. For example, the loss of nestin in zebrafish embryos causes increased apoptosis of neural progenitor cells (Chen et al., 2010). In mice, nestin deficiency is embryonically lethal after E8.5 due to extensive apoptosis of neural tube cells. However, no obvious abnormalities are present in other organs where nestin-positive cells are also present (Park et al., 2010). These studies demonstrate that the function of nestin is complex and that additional studies are needed to clarify its function *in vivo*.

The present study is aimed at clarifying the role of nestin during embryonic mouse development. Using mice that overexpress the human version of the gene, we show that nestin participates in heart and forebrain development through the regulation of cell proliferation and not apoptosis. Moreover, we suggest that the regulation of cell proliferation is mediated by the PI3K/Akt signaling pathway.

2. Results

2.1. Nestin overexpression in mouse embryos

In order to study the role of nestin during embryonic development, we generated transgenic mice that express this gene under the control of the ROSA26 promoter by microinjecting the construct *pBroad3-nestin* into pronuclear stage zygotes (see Section 5). The screening of 34 newborn animals by PCR, identified two transgenic mice (5.8% of the population; Fig. 1A) that were bred to yield 25% of transgenic animal in the generation F1 and 75% in the generation F2 (data not show). RT-PCR showed that, compared to wild type (WT) controls, the level of *nestin* mRNA in transgenic mice is significantly higher in most of the major organs including the brain, heart, liver, lung, and skin (Fig. 1B). The protein expression levels of nestin were also significantly increased in all organs as demonstrated by Western blotting (Fig. 1C). Thus, these results indicate that the human nestin transgene is overexpressed in all major organs.

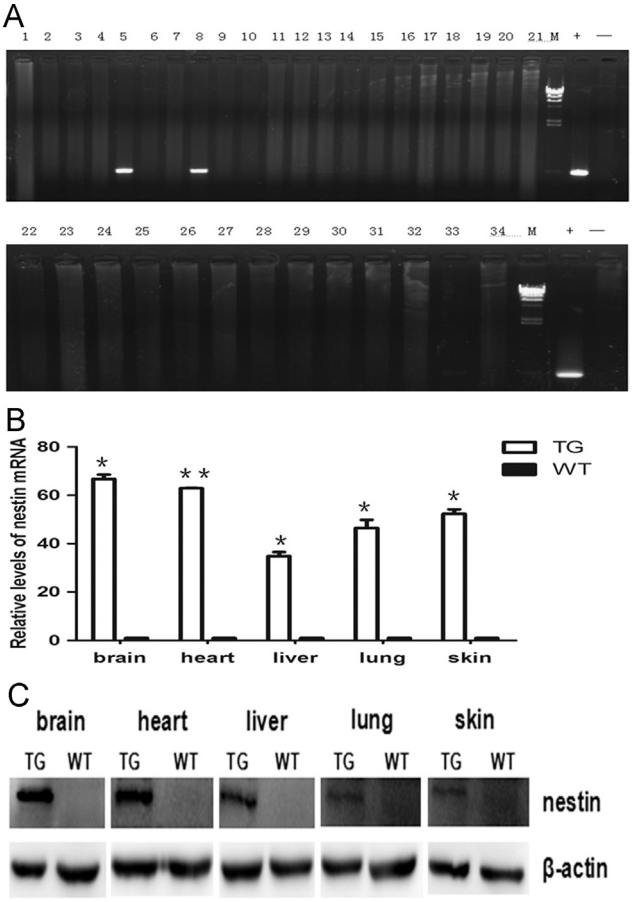

Fig. 1 – Characterization of the transgenic mice. (A) PCR analysis of the *nestin* transgene in 34 mice obtained from the microinjection experiment (lanes 1–34). Lane *M*, Molecular weight marker. Lane +, positive control (*pcDNA3.0-nestin*). Lane −, negative control (no DNA). Lanes 5 and 8, microinjected mice carrying an integrated *nestin* transgene. (B) RT-PCR quantitation of *nestin mRNA* in brain, heart, liver, lung, and skin tissues normalized using *GAPDH* as an internal control. Wild-type (WT) embryos show no detectable levels of *nestin* mRNA, whereas transgenic embryos show high levels of expression. Results are presented as the mean value of three separate experiments with the corresponding standard deviation; [*] $P<0.05$; [**] $P<0.001$. (C) Western blot analysis of nestin expression in brain, heart, liver, lung, and skin tissues (top) compared to an internal control (β-actin, bottom).

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