Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Overexpression of suppressors of cytokine signaling 1 regulate the proliferation and differentiation of rat-derived neural stem cells

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ARTICLE INFO

Keywords:
SOCS1
NSCs
Proliferation
Differentiation

ABSTRACT

Neural stem cells are a reliable resource in various neural tissue repair and neurodegenerative diseases. Increasing evidence has demonstrated that Suppressor of cytokine signaling proteins (SOCS) was involved in the nervous system development. The universality and diversity of SOCS also suggested their important roles in neurogenesis and nerve regeneration. In this study, we employed a lentiviral vector to investigate the impacts of overexpression SOCS1 on the proliferation and differentiation of rat-derived NSCs. Cells infected with LV-EGFP-SOCS1 showed a prominent increased cell number, diameter, and metabolic activity compared with other groups. Immunofluorescence analysis revealed the proportion of cells positive for microtubule associated protein-2 (MAP2) or myelin basic protein (MBP) was significantly increased in LV-EGFP-SOCS1 group while the proportion of glial fibrillary acidic protein (GFAP)-positive cells in LV-EGFP-SOCS1 group was significantly decreased compare to LV-EGFP and PBS group. Moreover, Western blot results were consistent with immunofluorescence results which indicated that overexpression of SOCS1 could promote neuronal and oligodendrocyte differentiations of NSCs but inhibit astrocyte differentiation of NSCs. In conclusion, our findings provided evidence that SOCS1 could promote the proliferation of NSCs and affect the differentiation of NSCs, providing a potential target for NSCs transplantation strategies.

1. Introduction

Proliferation and differentiation plasticity in Neural stem cells (NSCs) is a complex process which is regulated by intrinsic mechanisms in collaboration with environmental cues. Although many studies have focused on NSCs properties for clinical applications, the accurate mechanism of NSCs differentiation which was important for practical cell therapy remains unknown. It was extensively explored that cytokines, growth factors, and transcription factors were perhaps greatly involved in the regulation of proliferation and differentiation of NSCs. The characteristics of cytokines make them a reliable system in the cell to respond to normal developmental cues or environmental stimuli via specific targeting the appropriate receptor on cells surface. Receptor binding initiates a range of intracellular signaling cascades relating to cell differentiation, proliferation, survival and functional activation (Robb, 2007; Lemmon and Schlessinger, 2010). The Suppressor of cytokine signaling (SOCS) proteins were found as key factors, especially with regard to neuronal differentiation, throughout the development of nervous system (Wormald and Hilton, 2003; O'Sullivan et al., 2007). The SOCS family was divided into three groups, eight mammalian SOCS family members, SOCS1-7, and Cytokine-inducible SH2-containing protein (CISH) (Hilton et al.,1998). Previous studies provided evidence that SOCS proteins are involved in the regulation of a range of receptors. For example, it was shown that CISH and SOCS1-3 are closely associated with regulation of cytokine receptor signaling through the JAK-STAT pathway, whereas SOCS4-7 predominantly contributed to the regulation of growth factor receptor signaling (Kario et al., 2005; Krebs et al., 2002; Banks et al., 2005).

The universality and diversity of SOCS proteins intracellularly suggested that they play important roles in neurogenesis and nerve regeneration. In recent years, increasing studies have demonstrated that SOCS protein participated in the nervous system development. Müller have ever investigated the function of SOCS protein in the proliferation of NSCs and found knocking down of SOCS3 could enhance CNTF effects on neurospheres formation and the expression of stem cell marker (Müller et al., 2009). Overexpression of SOCS3 in NSCs elevated the expression of Nestin protein compared to the control (Cao et al., 2006). Moreover, overexpression of SOCS6 in NSCs promotes neurite outgrowth via the JAK2/STAT5-mediated signaling pathway, which involves negative feedback inhibition (Gupta et al., 2011). SOCS2 was also demonstrated to promote neurite outgrowth, regulate neuronal morphology, induce neurogenesis, and inhibit astrogliogenesis in NSCs

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http://dx.doi.org/10.1016/j.acthis.2017.08.003
Received 13 March 2017; Received in revised form 21 June 2017; Accepted 10 August 2017

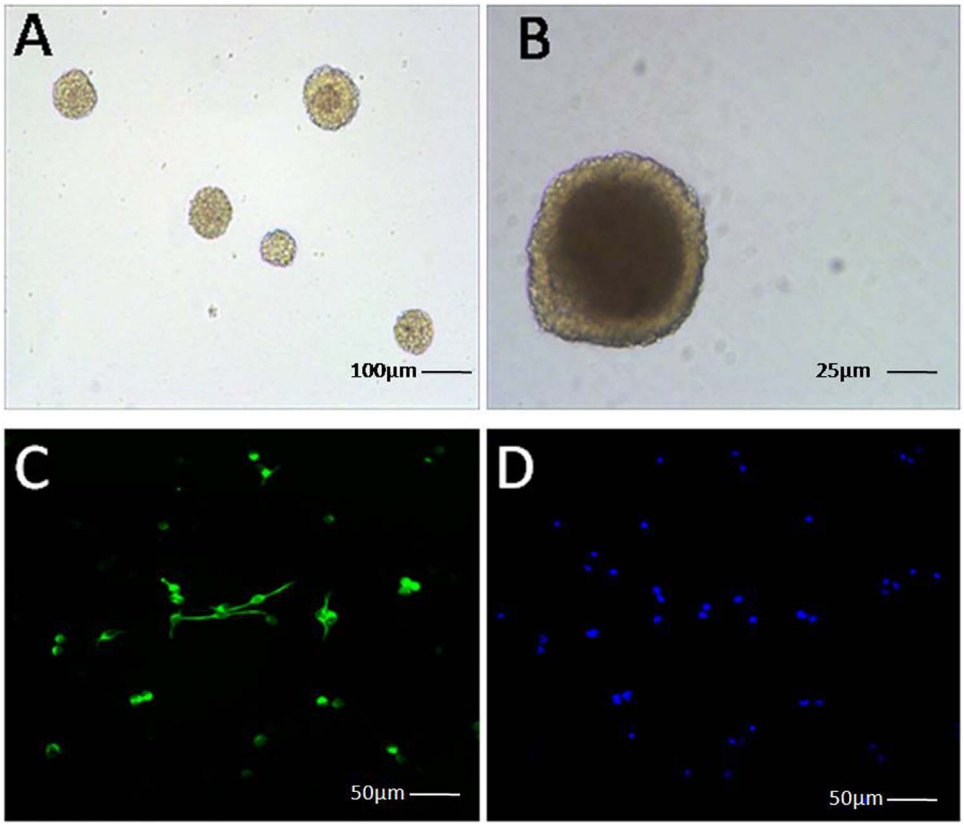

Fig. 1. Photomicrographs of normal NSCs. Neurospheres from the original generation of cultured NSCs in vitro under microscope (A, 100 times; B 400 times); C: Most of NSCs are Nestin positive (green fluorescence); D: Nuclear of NSCs is DAPI staining positive. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

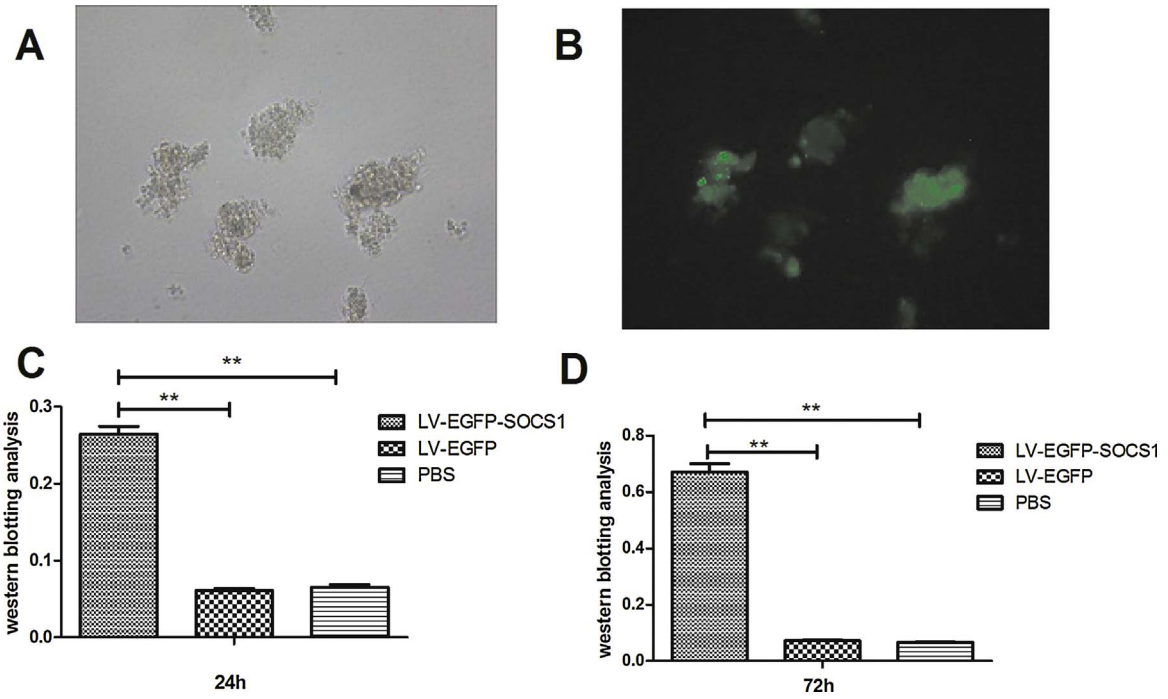

Fig. 2. Photomicrographs of NSCs transfected with lentiviral vectors. A: NSCs are transfected with lentiviral in light field; B: NSCs are transfected with lentiviral for green fluorescence in fluorescence field; C: The expression of SOCS1 24 h after infection; D: The expression of SOCS1 72 h after infection. There were three groupsin theexperimentand each experimentwasin triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which indicated that SOCS maybe indirectly regulate the differentiation of NSCs (Scott et al., 2006). Our previous study indicated that overexpression of SOCS1 in C17.2 NSCs promotes the generation of neurons, this effect probably was mediated by negative feedback inhibition of JAK2 and STAT3 (Cui et al., 2014; Snyder et al., 1992). Taken together, these results suggested that SOCS proteins could affect the maintenance of the NSCs directly or indirectly. Hence, we wanted to know whether SOCS1 was involved in the proliferation and differentiation of neural stem cells.

In this study, we employed the lentiviral vector to obtain the