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Adult rat hippocampus soluble factors: A novel transplantation model mimicking intracranial microenvironment for tracing the induction and differentiation of adipose-derived stromal cells *in vitro*

Peng Li[a], Haitao Sun[a], Mouxuan Du[a], Zhiqiang Fa[a], Kun Qin[b], Weiwei Xu[c], Run Zhang[a], Lei Chen[a], Chen Yao[d], Zongyu Xiao[e], Shizhong Zhang[a], Yiquan Ke[a], Xiaodan Jiang[a,*]

[a] *The National Key Clinic Specialty, The Neurosurgery Institute of Guangdong Province, Guangdong Provincial Key Laboratory on Brain Function Repair and Regeneration, Department of Neurosurgery, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China*
[b] *The Neurosurgery of Guangdong General Hospital, China*
[c] *The Neurosurgery of Guangdong Overseas Chinese Hospital, China*
[d] *Department of Neurosurgery, Affiliated Hospital of Qinghai University, Xining, China*
[e] *Neurosurgery Depatment of the Affiliated Yuebei People's Hospotal, Shantou University medical College, Shaoguan, Guangdong, China*

HIGHLIGHTS

- We constructed a novel model to trace ADSCs development *in vitro*.
- The formation of NSCs from ADSCs was found in hippocampus soluble factors firstly.
- We explored the effect between ADSCs and microenvironment alone.
- Chromosomes were analyzed of ADSCs undergoing passage.

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ABSTRACT

Intracranial transplantation of ADSCs induces recovery of CNS diseases, but how they develop in host is poorly understood. The aim of this study is to observe induction and differentiation of ADSCs in the presence of hippocampus soluble factors (HiSF) extracted from the hippocampus of adult Wistar rats to mimic an intracranial microenvironment. To determine the optimal microenvironment, five conditions were tested: 0 μg/ml (as control), 50 μg/ml, 100 μg/ml, 200 μg/ml, and 400 μg/ml of HiSF. The number of neurospheres was significantly higher in 200 μg/ml group than in other groups on the sixth day. Immunofluorescence demonstrated that the neurospheres induced from ADSCs in 200 μg/ml group expressed both nestin and CD133, which are more highly expressed in neurospheres than in ADSCs. This result was confirmed by Western blot analysis. Quantitative PCR revealed that the mRNA levels of nestin and CD133 in the neurospheres were 145- and 220-fold higher, respectively, than those in ADSCs. In the presence of 200 μg/ml HiSF and 1% FBS, the neurospheres can further differentiate into Schwann-like cells which expressing characteristic markers GFAP, S100 and P75 NGFR. These data indicated that HiSF, mimicking a destination of ADSCs transplanted model *in vitro*, could effectively induce and differentiate neurospheres, representing a new method to obtain NSCs and Schwann-like cells from ADSCs.

1. Introduction

NSCs, with their characteristics of proliferation, self-renewal and multipotentiality [1], bring great promise to cure central nervous system (CNS) diseases [2,4,8,18]. These potential abilities make NSCs an optimal tool for the treatment of CNS diseases that need a regenerative approach involving, for example, the replacement of injured cells in a structure or the secretion of neuronal factors or functional proteins [20].

At present, mesenchymal stem cells (MSCs) have been isolated from many tissues, including adipose tissue, bone marrow, umbilical cord blood [8], and placenta [15]. Because ADSCs from adipose tissue are easier to obtain, relatively less invasive to harvest [19] and available in low seeding density at primary passage [17]. Thus, ADSCs represent a practical, appealing and promising source of MSCs [7].

The induction of NSCs derived from ADSCs and in turn which can differentiate into Schwann-like cells requires an appropriate microenvironment. A microenvironment, includes intercellular

* Corresponding author at: Department of Neurosurgery, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China. Tel.: +86 20 61643268.
E-mail address: jiangxiao_dan@163.com (X. Jiang).

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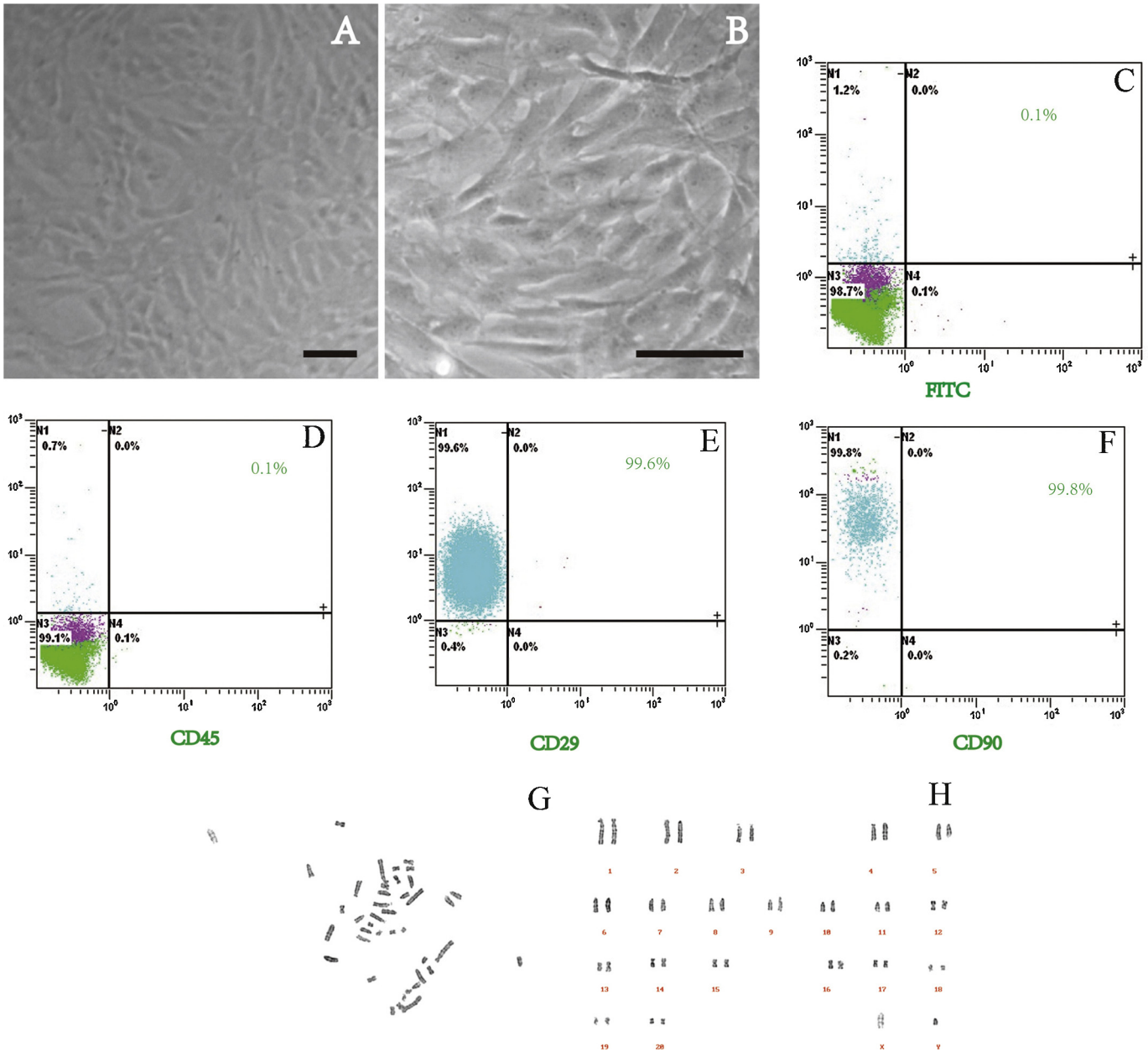

Fig. 1. Characterization of rat ADSCs. Morphology of rat ADSCs (A and B) at passage 2 under light micrograph. Bar: 100 μm. Flow cytometric analysis of ADSCs. ADSCs were negative for FITC (C) and CD45 (D). In contrast, CD29 (E) and CD90 (F) were expressed in 99.6% and 99.8% of the ADSCs, respectively. The karyotype consists of 20 pairs of autosomes and one pair of sex chromosomes (C and D). Abbreviation: ADSCs, adipose-derived stromal cells.

interaction and the extracellular matrix, both of which aid the proliferation, self-renewal, and multipotentiality of NSCs [12]. Here, we focused on the development and destination of ADSCs in the optimal HiSF from the extracellular matrix of hippocampus. This hippocampus factors can be considered as *in vitro* model of transplantation except the effect from intercellular interactions. ADSCs from neonate Wistar rats were identified by flow cytometry, and after analyzing their chromosomes at passage 2, their ability to generate neurospheres was tested. First, the number of neurospheres that were developed in the presence of different concentrations of HiSF was examined. Then, the expression levels of nestin and CD133 at the mRNA and protein levels were detected between the ADSCs and neurospheres after treatment with the HiSF. The differentiation of NSCs was also processed in this microenvironment with the extra addition of 1% FBS and identified by immunofluorescence.

2. Materials and methods

2.1. Preparation and culture of ADSCs

All animal protocols used have been approved by the National Institutes of Health Guidelines. Briefly, the adipose tissue was obtained from the subcutaneous tissue of a neonate Wistar rat. It was digested with collagenase I (Sigma–Aldrich, US) at 37 °C for 30 min and neutralized by the addition of complete cell