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Short communication

High purity of human oligodendrocyte progenitor cells obtained from neural stem cells: Suitable for clinical application

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ABSTRACT

Background: Recent studies have suggested that the transplantation of oligodendrocyte progenitor cells (OPCs) may be a promising potential therapeutic strategy for a broad range of diseases affecting myelin, such as multiple sclerosis, periventricular leukomalacia, and spinal cord injury. Clinical interest arose from the potential of human stem cells to be directed to OPCs for the clinical application of treating these diseases since large quantities of high quality OPCs are needed. However, to date, there have been precious few studies about OPC induction from human neural stem cells (NSCs).
New method: Here we successfully directed human fetal NSCs into highly pure OPCs using a cocktail of basic fibroblast growth factor, platelet-derived growth factor, and neurotrophic factor-3.
Results: These cells had typical morphology of OPCs, and 80–90% of them expressed specific OPC markers such as A2B5, O4, Sox10 and PDGF-αR. When exposed to differentiation medium, 90% of the cells differentiated into oligodendrocytes. The OPCs could be amplified in our culture medium and passaged at least 10 times.
Comparison with a existing method: Compared to a recent published method, this protocol had much higher stability and repeatability, and OPCs could be obtained from NSCs from passage 5 to 38. It also obtained more highly pure OPCs (80–90%) via simpler and more convenient manipulation.
Conclusions: This study provided an easy and efficient method to obtain large quantities of high-quality human OPCs to meet clinical demand.

1. Introduction

Demyelination or myelination delay, which leads to severe functional disorders, contributes to the pathological process of a broad range of diseases, including periventricular leukomalacia, pediatric leukodystrophies, multiple sclerosis, and spinal cord injury, for which there is no effective treatment (Deng et al., 2008; Amin-Mansour, 2012). Recent studies have shown that grafted oligodendrocyte progenitor cells (OPCs) can differentiate into oligodendrocytes and develop compact myelin supplies to repair the injured white matter of rodents to an extent, which shines light on the treatment of these diseases (Cavazzin et al., 2006; Windrem et al., 2002, 2004; Webber et al., 2009).

Human OPCs have historically been induced from embryonic stem cells (ESCs) or obtained by immunomagnetic sorting by their specific surface markers from donor brain tissues (Hu et al., 2009; Windrem et al., 2004). However, the former might be contaminated by rudimentary ESCs, which have strong potential tumorigenicity (Ben-David and Benvenisty, 2011), while the latter required large amounts of donor brain tissues to obtain adequate cells. As such, neither could meet clinical demand, which requires both large quantity and high quality. To date, NSCs have safely been used to treat patients with stroke, Parkinson's disease, and cerebral palsy. They also have the capacity to proliferate robustly and differentiate multi-directionally, which may make them an ideal source for human OPCs for clinical use. Studies have shown that, in contrast to their rodent counterparts, human neural stem cells (hNSCs) give rise to small numbers of oligodendrocytes both in vitro and after transplantation in vivo (Wright et al., 2006; Neri et al., 2010); as a result, it is very difficult to obtain OPCs from hNSCs.

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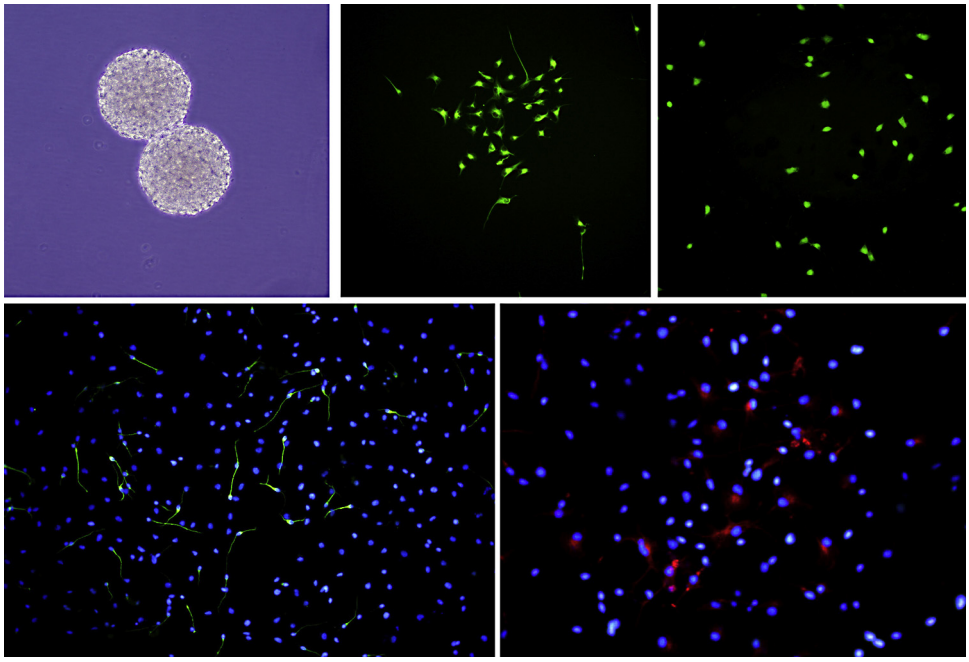

Fig. 1. (A) Human neural stem cells (NSCs) formed neurospheres floating in the medium and tested positive for the NSC markers nestin (B) and Musashi (C). When growth factors were removed from the medium, they differentiated into Tuj1-positive neurons (D) and glial fibrillary acidic protein–positive astrocytes (E). The cells stained green were positive, while the cell nuclei were stained with 4,6-diamidino-2-phenylindle (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Monaco et al. (2012) recently reported that human neural progenitors could be induced to differentiate through many of the stages of oligodendrocyte lineage development; in their culture system, the majority of cells expressed OPC markers. In this study, we established a method that could obtain higher-purity OPCs from hNSCs with much higher stability and repeatability via simpler manipulation that could provide an adequate source of cells for future clinical use.

2. Materials and methods

2.1. Materials and Reagents

The detailed information of the materials and reagents used in this study was provided in supplemental Table 1.

2.2. Culture of hNSCs

We cultured hNSCs as previously described (Jordan et al., 2008). An aborted human fetus aged 11 weeks post-conception was obtained from a woman at the Department of Obstetrics and Gynecology of the Navy General Hospital, Beijing, China, who had requested to terminate gestation and consented to donate the aborted fetus after being fully informed of the study according to the guidelines approved by the hospital's ethics committee. The brain was extracted and placed in cold phosphate-buffered saline (PBS). The brain was mechanically dissociated into a suspension of single cells by repetitive blowing using a 200-μL pipette. The cells were collected by centrifugation (1400 rpm for 5 min), the supernatant was discarded, and the cell pellet was resuspended with Dulbecco's modified Eagle medium (DMEM) mixed with F12 medium (3:1) supplemented with 15 mM HEPES, 0.15% D-glucose, 100 μg/mL transferrin, 20 nM progesterone, 60 μM putrescine, 30 nM sodium selenite, 5 μg/mL insulin, 5 μg/mL heparin, 1% L-glutamine, 20 ng/mL basic fibroblast growth factor (bFGF), 20 ng/mL epidermal growth factor (EGF), 10 ng/mL leukemia inhibitory factor (LIF), and 100 U/mL penicillin and streptomycin. All of the reagents were purchased from Sigma unless stated otherwise.

The cells were counted using a blood cell counting plate and seeded into T25 cell culture bottles in 5 mL of culture media (2×10^6 cells/bottle). Cells were maintained at 37 °C in a humidified atmosphere with 8.5% CO_2 in an incubator. The medium was replenished (half and half) every 4 days. The expanded cells formed colonies and were passaged every 7 days. During passaging, the cells were centrifuged (1400 rpm for 5 min), the supernatant was collected as conditioned medium, and the cell pellet was resuspended in 1 mL of 0.025% trypsin diluted in PBS and incubated at 37 °C until the neurospheres were unconsolidated, after which 100 μL of trypsin inhibitor was added to terminate the digestion. The spheres were gently dissociated into single cells, 15 mL of conditioned medium was added, and the cells were centrifuged, collected, and counted as described above. The cells were then seeded into T25 cell culture bottles in 5 mL of medium containing 2/3 fresh medium and 1/3 conditioned medium. For immunocytochemistry, some cells were plated onto 24-well plates pre-coated with 0.01% poly-D-lysine and 3.3 μg/mL laminin, and incubated for 2 h before being fixed with 4% paraformaldehyde at room temperature for 15 min for immunocytochemistry.

2.3. Induction and amplification of OPCs

The NSCs were cultured for 10 days and the 10-day-old neurospheres were used for this experiment. The neurospheres were dissociated into single cells as described above. The cells were collected by centrifugation, the supernatant was discarded, and the cell pellet was counted and resuspended at a density of 4×10^5/mL in 5 mL of DMEM-F12 medium (3:1) supplemented with 2% B27, 5 μg/mL transferrin, 10 nM progesterone, 30 μM putrescine, 15 nM sodium selenite, 5 μg/mL insulin, 5 μg/mL heparin, 5 mM lactate, 5 ng/mL bFGF, 10 ng/mL platelet-derived growth factor, 10 ng/mL neurotrophic factor-3 (NT3), and 100 U/mL