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Ectoderm mesenchymal stem cells promote differentiation and maturation of oligodendrocyte precursor cells

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ABSTRACT

Many neurological diseases are closely associated with demyelination caused by pathological changes of oligodendrocytes. Although intrinsic remyelination occurs after injury, the regeneration efficiency of myelinating oligodendrocytes remains to be improved. Herein, we reported an initiative finding of employing a valuable cell source, namely neural crest-derived ectoderm mesenchymal stem cells (EMSCs), for promoting oligodendrocyte differentiation and maturation by co-culturing oligodendrocyte precursor cells (OPCs) with the EMSCs. The results demonstrated that the OPCs/EMSCs co-culture could remarkably increase the number and length of oligodendrocyte processes in comparison with the mono-cultured OPCs and non-contact OPCs/EMSCs transwell culture. Furthermore, the inhibition experiments revealed that the EMSCs-produced soluble factor Sonic hedgehog, gap junction protein connexin 43 and extracellular matrix molecule laminin accounted for the promoted OPC differentiation since inhibiting the function of anyone of the three proteins led to substantial retraction of processes and detachment of oligodendrocytes. Altogether, OPCs/EMSCs co-culture system could be a paradigmatic approach for promoting differentiation and maturation of oligodendrocytes, and EMSCs will be a promising cell source for the treatment of neurological diseases caused by oligodendrocyte death and demyelination.

1. Introduction

Oligodendrocytes, responsible for ensheathing axons in the central nervous system (CNS), have played a pivotal role in maximizing the conduction velocity of nerve impulses, maintaining the axon integrity [1–3] and providing metabolic support directly to long axons [4]. Through the neuronal-oligodendroglial interactions, a multilamellar, lipid-rich structure called myelin sheath gradually forms concentric wrappings which envelop the CNS axons, allowing for rapid communication between neurons [5]. Failure of myelin sheath formation (*i.e.* myelination) or demyelination caused by injuries or diseases interrupts the rapid propagation of action potentials throughout the vertebrate nervous system, resulting in neurodegenerative diseases such as multiple sclerosis (MS), highlighting the great significance of oligodendrocytes in maintaining CNS functions [6]. Therefore, research on oligodendrocytes has been extensively performed over the years.

It is well recognized that differentiation of OPCs is a prerequisite for myelination. Although oligodendrocyte regeneration and spontaneous remyelination is possible following acute CNS demyelination due to the abundance of OPCs in the adult CNS, myelin sheath regeneration is often inadequate and ultimately fails with the progression of diseases such as MS [7]. The possible reason could be the oligodendrocyte maturation arrest at the lesion sites [8]. In the case of spinal cord injury (SCI), even though intrinsic repair process is triggered following the injury, the prolonged secondary damage, especially the massive demyelination and the glial scar formation, would eventually lead to the failure of remyelination [9,10]. Hence, it is imperative to search for effective strategies that could promote oligodendrocyte differentiation and

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maturation.

Ectoderm mesenchymal stem cells (EMSCs), a type of multipotent adult stem cell that derive from the cranial neural crest, have shown strong propensity to differentiate into neurons and Schwann cells [11] in addition to the typical mesenchymal tissues (bone, cartilage, fat, etc) [12,13]. Our previous study demonstrated that EMSCs could significantly promote histological reconstruction, remyelination, and behavioral recovery in a rat model of SCI [14]. However, due to the complex microenvironments in vivo after SCI, the direct effects of the transplanted EMSCs on the recovery of nerve functions remain unknown.

In this study, we conducted an initiative investigation about the direct effects of EMSCs on the OPCs in a contact co-culture system to assess whether EMSCs could promote oligodendrocyte differentiation and maturation, a major contributor to the repair of SCI. We first examined whether the EMSCs were able to express certain growth factors, adhesion molecules or extracellular matrix molecules that could improve oligodendrocyte differentiation by immunofluorescence staining. Next, a OPCs/EMSCs co-culture system was established for the first time to assess its ability to enhance OPC differentiation. Finally, the inhibition experiments were performed to explore the underlying mechanisms contributing to the improved oligodendrocyte differentiation and maturation.

Taken together, this is an innovative study that demonstrated the direct effects of the multifunctional EMSCs on oligodendrocyte differentiation and maturation in the OPCs/EMSCs co-culture system in vitro. It would provide a paradigmatic example of uncovering extracellular cues that influence oligodendrocyte differentiation, enriching the clinical treatments of neurological diseases associated with the loss of oligodendrocytes.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium/F12 (DMEM/F12, Gibco), B27 supplement (Gibco), fetal bovine serum (FBS, Gibco), penicillin/streptomycin, trypsin, L-glutamine were obtained from Invitrogen Corporation (Carlsbad, CA, USA). The recombinant basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and epidermal growth factor (EGF) were purchased from PeproTech (Rocky Hill, NJ, USA). Heparin, ethylenediaminetetraacetic acid (EDTA) disodium salt dehydrate, carbenoxolone (CBX) and Hoechst33342 were provided by Sigma-Aldrich (St. Louis, MO, USA). Arg-Gly-Asp (RGD) oligopeptide was purchased from Aladdin Industrial Corporation (Shanghai, China). Cell strainers (40 μm, 352340) and 24-well transwell plates (pore size: 0.4 μm) were obtained from BD Falcon (BD Biosciences, San Jose, CA, USA). Primary antibodies including mouse monoclonal anti-Sox10, anti-Vimentin, anti-Laminin, anti-Integrin-β1 and anti-(myelin basic protein) MBP were purchased from Abcam (Cambridge, UK); rabbit polyclonal anti-nestin was purchased from Sigma-Aldrich (St. Louis, MO, USA); goat polyclonal anti-Shh was obtained from R&D Systems (Minneapolis, MN, USA); mouse monoclonal Shh-neutralizing antibody 5E1 (5E1) was purchased from Developmental Studies Hybridoma Bank (Iowa City, Iowa, USA); mouse monoclonal anti-galaetocerebroside (anti-GalC) was provided by Millipore (Billerica, MA, USA); rabbit polyclonal anti-Connexin43 (anti-Cx43) was provided by Boster (Wuhan, Hubei, China). Secondary antibodies, including Cy3-labeled goat-anti-mouse/rabbit IgG, Alexa Fluor 488-conjugated goat-anti-mouse IgG, and Alexa Fluor 555-conjugated donkey-anti-goat IgG, were provided by Sigma-Aldrich (St. Louis, MO, USA). Goat-anti-mouse IgG-HRP, goat-anti-rabbit IgG-HRP, and donkey-anti-goat IgG-HRP were purchased from Boster (Wuhan, Hubei, China).

The animal experimental protocols were approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals.

2.2. Isolation and identification of EMSCs

Sprague-Dawley rats (weight: 120–140 g, male, provided by the animal center of Jiangsu University) were deeply anaesthetized by an intraperitoneal injection of pentobarbital sodium (0.05 g/kg). The EMSCs were isolated following the protocol described in a previous study [11]. The isolated EMSCs were cultured in the DMEM/F12 medium containing 10% FBS, 2 mM L-glutamine, and 100 U/mL penicillin-streptomycin, with the medium changed every 3 days. When reached a confluence of 80–90%, the primary EMSCs were passaged using 0.25% trypsin/EDTA at a split ratio of 1:2. The EMSCs (passage 3) were identified by detecting the expression of Sox10, Nestin, Vimentin, Shh, Connexin 43 and Laminin with immunofluorescence staining according to the protocols outlined in the following section (**Immunofluorescence microscopy**).

2.3. Preparation of OPCs

Oligodendrocyte precursor cells (OPCs) were prepared according to previously described procedures [15,16] with some modifications. In brief, spinal cords were dissected out from 1–2-day old Sprague-Dawley rats. The tissue was rinsed with PBS to remove the blood cells, minced with surgical scissors, and digested with 0.1% type II collagenase. After being forced through a 40-μm cell strainer, the dissociated cells were plated in poly-D-lysine-coated 75-cm^2 flasks, and maintained in DMEM/F12 medium containing 20% FBS and 100 U/mL penicillin/streptomycin. The medium was replaced every three days. When the cells were confluent (~10 days), the flasks were shaken for 1 h on an orbital shaker at 220 rpm, 37 °C to remove microglia. After that, the medium was changed, followed by gentle shaking overnight (~20 h). The medium was then collected and plated on non-coated tissue culture dishes for 1 h at 37 °C to remove contaminating astrocytes and microglia. The non-adherent cells were collected and re-plated in Neurobasal media containing L-glutamine, 100 U penicillin/streptomycin, 10 ng/mL PDGF, 10 ng/mL FGF, and 2% B27 supplement onto poly-D-lysine-coated plates. After 4–5 days of incubation, the OPCs were used for the subsequent experiments.

2.4. Co-culturing OPCs with EMSCs

To establish the OPCs/EMSCs co-culture system, the EMSCs were plated in the 6-well plates in advance. Specifically, the EMSCs (passage 3) were dissociated with 0.25% trypsin/EDTA to form single cell suspensions which were then seeded on the 24-well plates at a cell density of 5×10^4 cells/well. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2, and the medium was changed every two days. After the cells reach a confluence of 80–90%, the OPCs were collected with trypsin digestion and then seeded on the EMSCs with a concentration of 5×10^4 cells/well. The OPCs/EMSCs contact co-culture system was incubated in the DMEM/F12 medium containing 10% FBS, 2 mM L-glutamine, and 100 U/mL penicillin-streptomycin. The medium was changed every three days. The co-culture system was preceded with immunofluorescence staining for detecting the expression of MBP and GalC at day 3, 7 and 11.

To quantify the morphological complexity of the processes, the number and length of oligodendrocyte processes were measured at day 11 from more than 100 GalC-positive cells in 15 randomly selected 20 × microscopic fields with AxioObserver (Zeiss, Germany). Images were scanned into Axiovision (Zeiss, Oberkochen,