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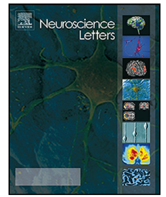

Research article

The condition medium of mesenchymal stem cells promotes proliferation, adhesion and neuronal differentiation of retinal progenitor cells

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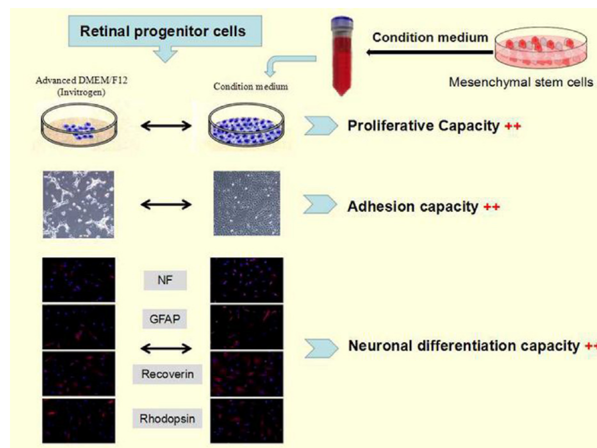
HIGHLIGHTS

- The condition medium of human mesenchymal stem cells can enhance the proliferation capacity of human retinal progenitor cells.
- The condition medium of human mesenchymal stem cells can promote human retinal progenitor cells adherence.
- The condition medium of human mesenchymal stem cells favours human retinal progenitor cells differentiation towards retinal neurons.

GRAPHICAL ABSTRACT



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ABSTRACT

Retinal progenitor cell is a promising candidate in the treatment of retinal pigmentosa diseases. The limiting factors of stem cell transplantation are the proliferation and differentiation capacities of hRPCs, which may be governed by culture conditions. Previous studies have proved that the secretome of human Umbilical Cord Mesenchymal stem cells (hUCMSCs) and human Adipose derived stem cells (hADSCs), including more active cytokines and neurotrophic factors, have the paracrine potential of enhancing proliferation and differentiation in several cell types. The aim of this study was to investigate whether hRPCs could effectively proliferate, adhere and differentiate towards specific retinal cell types by treating with the condition medium (CM) of hUCMSCs (hUCMSCCM) or hADSCs (hADSCCM). Here, we show that hUCMSCCM or hADSCCM enhances the proliferation rate of the S and G2 phase cells, with an upregulation of Ki67 expression. Moreover, the upregulation expression of NF, Recoverin and Rhodopsin indicates that specialized retinal cells including ganglion cells and photoreceptors are favored over hRPCs differentiation due to hUCMSCCM or hADSCCM. Under FBS induced differentiation conditions, hRPCs treated with

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hUCMSCCM or hADSCCM increase the expression of retinal neuron and photoreceptor specific markers. These results suggest that hUCMSCCM and hADSCCM can stimulate the hRPC proliferation, promote its adherence and support hRPC neuronal and photoreceptor differentiation. These findings may provide a new strategy to improve the viability of hRPCs and photoreceptor differentiation capacities.

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1. Introduction

Retinal degeneration, including retinitis pigmentosa (RP) and age related macular degeneration (AMD) result in vision malfunction. The death of retinal photoreceptors is the main cause of retinal degeneration, while no effective treatment is currently available. Cell replacement has been demonstrated as a potentially useful way of treating retinal degeneration diseases [1]. Previous works have shown that hPRCs can differentiate into mature retinal cell types, migrate into the injured retina tissue, and secrete a large array of neurotrophic factors, thereby improving the vision function [2,3]. However, the issues of cell expansion and cell predisposed to photoreceptor cell fate remain a major challenge for the use of hRPCs [4].

Mesenchymal stem cells (MSCs) have the potential of supporting the expansion and function of other cells, like hematopoietic stem cells, embryonic stem cells, natural killer cells and neuron cells [5–8]. Previous works have demonstrated that bone marrow MSCs, dental pulp derived stem cells and adipose-derived stem cells can promote proliferation and differentiation of neural stem cells in vitro and in animal tests through the production of neurotrophic factors [9]. In addition, Umbilical Cord MSCs have been shown to support the viability and proliferation of neural cells via releasing neuroregulatory factors, as well as to promote the expression of MAP2 in neurons cultures [10]. On top of that, the efficacy of MSCs-based applications in improving of cell properties has been attributed to the paracrine secretion of trophic cytokines. A few studies have demonstrated that the secretome from hUCMSCs and hADSCs have represented an important extension of the stem cells function which is associated with cell differentiation and proliferation [11,12], while not much is known about culturing hRPCs in the existence of secretome of hUCMSCs or hADSCs.

In the present study, we investigated whether hRPCs could effectively proliferate, adhere and differentiate towards retinal neuronal cells by treating with hUCMSCCM or hADSCCM, which enriched secretomes.

2. Materials and methods

2.1. hRPCs culture and characterization

hRPCs were isolated from a donated neural retina (obtained from the eye bank of He eye hospital, Shenyang, China) with informed consent and IRB approval. The culture method was performed and improved following a previously developed protocol [13]. Briefly, human retina tissue was harvested, minced and digested with tryple express (Invitrogen) for 2 min. The cell pellet achieved after centrifuge was plated on human fibronectin (Invitrogen) –coated flasks in Advanced medium (Gibco), supplemented with 1% N2 neural supplement (Invitrogen), 2 mM L-glutamine (Invitrogen), 100 U/ml penicillin-streptomycin (Invitrogen), and 20 ng/ml epidermal growth factor (recombinant human EGF, Invitrogen), 20 ng/ml basic fibroblast growth factor (bFGF; Invitrogen) and incubated at 37 °C in the presence of 5% CO_2. Cells were re-fed on alternating days. Cultures were split 1:2 every 3–4 days. All cells were performed at passage 6 in this study. hRPCs at passage 6 were stained with antibodies to Nestin, Ki67, Pax6(BD Biosciences) and Map2, 200 kD Neurofilament Heavy, Rhodopsin, Recoverin, GFAP and Chx 10(Abcam), and characterized by fluorescence-activated cell sorting(FACS) analyses. FAC analyses are detailed in Supplemental Methods Section.

2.2. hUCMSCs/hADSCs culture and characterization

hUCMSCs and hADSCs were isolated from human umbilical cords and adipose tissue, respectively. These tissues were incubated with 0.5% collagenase II (Invitrogen) at 37 °C for 2 h, and then washed with DMEM/10%FBS medium to stop the reaction. Finally, cells were seeded in flasks with OriCellTM Human Umbilical Cord or Adipose-derived Mesenchymal Stem Cell Growth Medium (Cyagen Biosciencses). All cells at passage 6 were used in this study. hUCMSCs and hADSCs at passage 6 were stained with antibodies to CD31, CD34, CD45, HLA-DR, CD29, CD44, CD73, CD90 and CD105(BD Biosciences) and characterized by fluorescence-activated cell sorting(FACS) analyses. FACS analyses are detailed in Supplemental Methods Section. Detailed in vitro differentiation of hUCMSCs and hADSCs into adipocytes, osteoblasts, and chrodrocytes is described in Supplemental Methods Section.

2.3. Preparation of hUCMSCCM and hADSCCM

The hUCMSCs and hADSCs were seeded at a cell density of 1.5×10^4 cells/cm^2. They were cultured in MSC medium and were maintained for 1 day. The attached cells were washed three times with DPBS, and the medium was replaced with advanced DMEM/F12, 1% N2 neural supplement and 2 mM L-glutamine.The cells were then maintained for an additional 24 h.The medium was collected and centrifuged at 300*g* for 5 min, sterile-filtered through a 0.2 μm membrane and store at-20 °C.

2.4. Proliferation, adhesion and differentiantion studies of hRPCs

For the proliferation assay, 6×10^5 hRPCs were cultured in each well of a 6-well plate with either advanced medium, hUCMSCCM or hADSCCM. Cell amount was recorded daily till the 10th day. In addition, through MTT staining, hRPCs were characterized to be seeded at a density of 3×10^4 cells per well in 96-well plates separately, and incubated in 200 μl advanced medium or conditioned medium for 12 h,24 h and 48 h, respectively. After the incubation, 20 μl MTT (0.5 mg/ml) was added into each well for four-hour incubation at 37 °C. Supernatant was then removed and 150 μl DMSO was added in. The optical density (OD) was measured with a microplate reader (Molecular Devices, USA) at 450 nm.

After a treatment of conditioned medium for 48 h, cells (5×10^6) were harvested and fixed with 500 μl of 70% cold ethanol for 2 h 100 μl of RNase was added to cells and incubated at 37 °C for 30 min. Then, 400 μl of PI was added, and the cells were incubated at 4 °C for 30 min protected from light, followed by an immediately flow cytometer (FAC Scan, Becton Dickinson) to detect the cell cycle. The results were analyzed using CELL Quest 3.0 software (BD, USA).

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