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BRAIN RESEARCH

Research Report

An *in vitro* comparison of two different subpopulations of retinal progenitor cells for self-renewal and multipotentiality

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ARTICLE INFO

Article history:
Accepted 28 November 2011
Available online 6 December 2011

Keywords:
Retina
Progenitor cell
Proliferation
Differentiation

ABSTRACT

Retinal progenitor cells (RPCs) show enormous potential for the treatment of retinal degenerative diseases. It is well known that *in vitro* cultures of RPCs comprise suspension spheres and adherent cells, but the differences between the two cell populations are not fully understood. In this study, cultured RPCs were sorted into suspension and adherent cells. Analyses of cell morphology, cell growth and retinal progenitor-related expression markers were performed using quantitative polymerase chain reaction (qPCR) and immunocytochemistry to identify the proliferative and multipotent capacity of the cells *in vitro*. The data showed that both the suspension and adherent cells were maintained in an undifferentiated state, although the former exhibited a greater proliferative potential than the latter. Immunocytochemistry analysis indicated that the two subsets of RPCs were able to differentiate into different retinal cells in the presence of fetal bovine serum (FBS); the adherent cells were more likely to differentiate toward the β3-tubulin-, AP2α- and Map2-positive neuronal lineage, while the suspension cells were more effective at differentiating into rod photoreceptors, which was consistent with the qPCR results. These findings suggest that adherent RPCs may be a potential candidate for retinal interneuron or ganglion cell substitution therapies, whereas suspension RPCs may be preferred for photoreceptor cell replacement.

1. Introduction

Many people lose their sight every year due to common retina-related diseases, such as glaucoma, age-related macular degeneration and diabetic retinopathy. These diseases are all characterized by the loss of photoreceptors or other retinal neurons, leading to an irreversible decline in visual function. However, there are no effective treatments currently available to prevent the loss of retinal neurons. Stem cells are widely regarded as cells with long-term self-renewal capabilities and an ability to generate special cell populations in a given tissue. The isolation of different tissue-derived stem cells has attracted considerable attention for its potential as a treatment for various diseases. Fortunately, after further

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Abbreviations: RSCs/RPCs, retinal stem/progenitor cells; mRPCs, mouse RPCs; FBS, fetal bovine serum; EGF, epidermal growth factor; DAPI, 4′, 6-diamidino-2-phenylindole; PKC-a, protein kinase C-alpha; AP2α, activator protein 2 alpha; GFAP, glial fibrillary acid protein; PBS, phosphate buffered saline; qPCR, quantitative polymerase chain reaction

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doi:10.1016/j.brainres.2011.11.054

research on the properties of various types of stem cells, replacing lost neurons with stem cells may become a promising way to treat people with retinal diseases. Recently, several populations of stem cells or progenitor cells, including neural stem cells, bone marrow-derived stem cells and embryonic stem cells, have been used for retinal transplantation (Jagatha et al., 2009; Sakaguchi et al., 2004; Tomita et al., 2006). However, previous studies have demonstrated that progenitor cells that are not derived from the eye have a very limited ability to generate specific cell populations required for the treatment of retinal diseases (Van Hoffelen et al., 2003).

Retinal stem/progenitor cells (RSCs/RPCs) have been identified in the mammalian eye, and retinal stem/progenitor cells of rodents, pigs and humans can be cultured *in vitro* (Coles et al., 2004; Gamm et al., 2005; Gu et al., 2007; Tropepe et al., 2000). In principle, RPCs could provide a source of new retina-specific cells (Livesey and Cepko, 2001). Numerous retinal transplantation studies have demonstrated that mouse RPCs (mRPCs) may be the best cells for the restoration of vision by cell-replacement therapy. For example, previous studies have shown that RPCs are able to attain functional integration into the outer nuclear layer of the retina and express specific markers of photoreceptors, such as recoverin and rhodopsin (Lamba et al., 2009; MacLaren et al., 2006). Moreover, animals with photoreceptor degeneration showed partial preservation of light sensitivity after the transplantation of RPCs (Klassen et al., 2004). Other experiments have also indicated that the different developmental stages and growth states of stem cells may influence transplantation results (Cepko et al., 1996; Gamm et al., 2008; MacLaren et al., 2006; Reh, 2006; Wang et al., 2002; Young, 1985), thus, stem cells or progenitor cells with good self-renewal and a specific differentiation potential are a key step for transplantation therapy. Specifically, pre-selection of RSCs/RPCs that show a highly preferential differentiation into specific retinal neurons is a key factor for treating retinal disorders by cell transplantation.

During RPC expansion, an interesting phenomenon occurs in which some cells grow as floating spheres and others adopt adherent growth; we describe these two types of cells as suspension cells and adherent cells, respectively. These two types of cells display different morphologies. Whether the morphological differences between these two subsets of RPCs indicate different intrinsic characteristics of their proliferative capacity and differentiation potential remains unclear. In this study, we characterized the differences between suspension and adherent RPCs *in vitro*, which may be useful for the treatment of retinal diseases.

2. Results

2.1. *Morphology and expansion potentials of suspension and adherent RPCs*

The treatment of two subsets of RPCs with the same culture conditions resulted in different morphological changes. In the proliferation medium, the number of both suspension and adherent mRPCs increased with time. The suspension cells continued to grow as floating spheres, which became larger in size, and only a few derived cells were found attached to the flasks (Figs. 1-A, C and E). Under the proliferation condition, the majority of the adherent cells remained attached to the surface of the flask with two or more short processes, and only a few floating clusters were detected (Figs. 1-B, D and F).

The RPC proliferative capacity was examined using growth curves. Both suspension and adherent RPCs were able to proliferate *in vitro* for up to one month, and suspension RPCs had slightly more expansion potential than adherent RPCs (Fig. 1-G).

2.2. *Expression of progenitor and proliferation markers of RPCs under proliferation conditions*

To investigate if the self-renewal and expansion potential of the suspension RPCs were different from the adherent RPCs

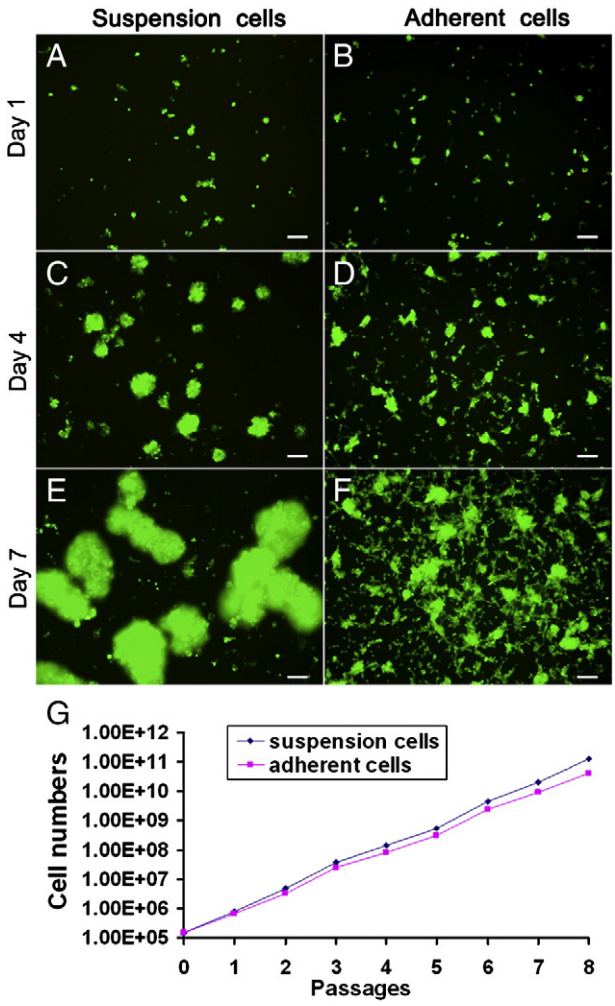

Fig. 1 – Morphology and expansion potential of RPCs. The two cell subgroups were cultured and assessed on days 1 (A, B), 4 (C, D) and 7 (E, F). The majority of the suspension cells proliferated to form spheres, and the size of the spheres became larger over a 7-day period (A, C, E). The majority of the adherent cells attached to the surface of the flask with two or more short processes (B, D, F). The expansion potential of these two subgroups was assessed through long-term culture; the suspension cells exhibited approximately 14.6% more expansion potential than the adherent cells (G). Scale bars: 100 μm.