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Construction of a novel inducing system with multi-layered alginate microcapsules to regulate differentiation of neural precursor cells from bone mesenchymal stem cells

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ABSTRACT

Neural precursor cells (NPCs) are a promising cell source for the treatment of nervous system diseases; however, they are limited in their applications due to source-related ethical considerations or legislations. Therefore, a novel approach is necessary to obtain sufficient NPCs. Recently, the usage of bone marrow-derived mesenchymal stem cells (BMSCs) differentiated into neural cells has become a potential method to obtain NPCs. Moreover, growth factors (GFs) are emerging as inducers to evoke the differentiation of BMSCs into NPCs. For example, GFs may activate various signaling pathways related to neural differentiation, such as phosphatidylinositol 3 kinase/protein kinase B, cyclic adenosine monophosphate/protein kinase A, and Janus kinase/signal transducer activator of transcription. However, the utilization of growth factors still has some limitations such as high costs and low rates of neural differentiation. Neuroblastoma cells have been characterized as a potential pool for growth factors. Additionally, basic fibroblast growth factor (bFGF), a type of growth factor, has been demonstrated to be able to increase the differentiation and survival rate of NPCs. For better use of neuroblastoma cells and bFGF, we established a novel system involving multi-layered alginate-polylysine-alginate (APA) microcapsules to encapsulate neuroblastoma cells and bFGF, which may not only provide sufficient growth factors in a sustained manner but also avoid the carcinogenicity caused by neuroblastoma cells. Above all, we hypothesized that neuroblastoma cells and bFGF encapsulated in multilayered alginate microcapsules may efficiently induce the differentiation of BMSCs into NPCs.

Introduction

Neurodegenerative diseases and central nervous system (CNS) trauma have been reported as one of the leading causes of disability and mortality [1]. The limited potential of the CNS in neural regeneration and functional reconstruction is attributed to the difficulty in the treatment of these diseases. Increasing studies have identified that neural precursor cells (NPCs), or neural stem cells (NSCs), facilitate CNS regeneration due to their differential ability and capability to secrete neuroprotective factors. Therefore, it is believed that NPCs can be applied as a promising therapeutic strategy for neural repair [2–6]. However, the source of NPCs is one of the most challenging technical issues. Bone marrow-derived mesenchymal stem cells (BMSCs) that are capable of self-renewal [7–8] have been widely demonstrated as an important source of NPCs/NSCs [9–10]. Nevertheless, the differentiation rate of NPCs derived from BMSCs is relatively low *in vitro* [9]. To date, efficient methods to induce BMSCs to differentiate into NPCs are still unavailable.

Background

The effect of growth factors (GFs) on differentiation of BMSCs

GFs are a well-documented member of the neurotrophin family, which is known to promote neuroprotection and neurite outgrowth [11]. Research studies tend to utilize platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or vascular endothelial growth factor (VEGF) as inducers to evoke BMSCs for neural differentiation [12–14]. It has been demonstrated that these GFs are capable of evoking several intracellular signal transduction pathways such as phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT), cyclic adenosine monophosphate/protein kinase A (cAMP/PKA), and Janus kinase/signal transducer activator of transcription

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(JAK/STAT3) [15–17]. These signaling pathways significantly contribute to promoting the growth and neural differentiation of BMSCs via upregulation of some proteins such as mTOR, p70S6K, and 4E-BP1, which mediate neurite development and neuronal survival [18–20]. These findings suggest that one of the mechanisms involved in the effect of GFs on neuronal differentiation of mesenchymal stem cells (MSCs) is the activation of subsequent induction of intracellular signal transduction pathways.

To date, the application of GFs to induce neuronal differentiation of MSCs still has issues. First, the cost of GFs is high. Second, most research has demonstrated that the function of a single GF in MSC differentiation into NPCs is insufficient [10]. Third, although some reports have testified the combined action of multiple GFs in NPC/NSC differentiation from MSCs [14,21], different combinations of GFs for the optimal differentiation of MSCs and the interaction mechanisms underlying different GFs have not yet been clarified.

GFs secreted by neuroblastoma cells (NBCs) are involved in the regulation of MSC differentiation

Neuroendocrine tumor lines such as neuroblastoma cells (NBCs) are an ideal inducer because they constitutively secrete growth factors and cytokines into the micro-environment. NBCs have been demonstrated as a potential GF pool. Various GFs such as VEGF-B, VEGF-C, bFGF, and PDGF are highly expressed in the conditioned medium (CM) of NBCs (NB-CM) [22–23]. Hu et al. [24] have reported that CM obtained from B104 NBCs (B104-CM) can stimulate the proliferation of oligodendrocyte precursor cells (OPCs) *in vitro* and that bFGF and PDGF-AA in B104-CM are two critical factors that can expand OPCs. Additionally, Lo Furno et al. [25] have shown that B104-CM is capable of inducing the differentiation of adipose tissue-derived MSCs (AT-MSCs) to a neuronal phenotype. These findings indicate that NBCs are an ideal supplier of GFs for neural proliferation and differentiation.

Nevertheless, some issues such as the utilization of NBCs still need to be addressed. First, direct incubation of MSCs with NBCs may lead to canceration of differentiated cells due to the tumorous features of NBCs [26]. Second, the heterogeneity of tumor cells may make this induction unstable [27]. Third, it is difficult to make GFs delivered by NBCs in a sustained manner in growth medium.

Tissue engineering technology of cellular microencapsulation

Cellular microencapsulation as a promising strategy has been applied to experimental studies and clinical treatment. The concept of cellular microencapsulation was initially proposed by Chang et al. in 1964 [28]. Lim et al. [29] first encapsulated islet cells with alginate-polylysine-polymine and transplanted the microencapsulated islets into the enterocelia of diabetic rats, which effectively kept the blood sugar of rats at a normal level for 3 weeks. Since then, extensive attention has been paid to the application of microencapsulated cells. The advantages of this technique are that it achieves the continuous delivery of cytokines secreted by the encapsulated cells [30–32] and that it also provides the unique cultivation of the 3-dimentional environment to protect the cells from damage caused by mechanical stress and the host's immune system [33]. The applications of microencapsulated cells *in vivo* for the treatment of CNS diseases have been reported by many researchers. Yoshida et al. have transplanted microencapsulated dopamine into the subdural space of rats for the treatment of Parkinson's disease [34]. In addition, Borlongan et al. have shown that encapsulated rat choroid plexus cell transplantation could exert neuroprotection in a rodent model of Huntington's disease [35]. Moreover, Wang et al. [36] demonstrated that the hepatocarcinoma cells in alginate-polylysine-alginate (APA) microcapsules could maintain hepaticarcinoma cells with high stability and viability *in vitro*, forming a multicellular spheroid similar to the cytoarchitecture of tumor cells *in vivo*. Furthermore, Wu et al. [37] have shown that the intrathecal implant of microencapsulated rat pheochromocytoma cells (PC12) in rats with neuropathic pain decreased cold allodynia via producing catecholamines, especially dopamine. They also found that neoplasia did not occur in the spinal cords of the rats in the cell-microencapsulated group compared with the rats without cell microencapsulation. Therefore, the cell microencapsulation technique may resolve the problems of NBC application as mentioned above.

Strategy of microencapsulated NBCs combined with bFGF supplementation for NPC/NSC differentiation derived from MSCs

The results of several experiments have shown that NPCs/NSCs are unstable in the early stage of neuronal differentiation from MSCs and that they are prone to further differentiate into mature neural cells (MNCs) [38]. However, bFGF, one type of GF, is able to postpone the transformation of NPCs into MNCs [39] and induce nerve cells to dedifferentiate [40]. In addition, bFGF enhances the survival rate of NPCs and facilitates cell proliferation at the early growth stage, boosting NPC production [41]. Moreover, bFGF also has been widely used as a major constituent of the CM for NPCs and increases the expression of retinoic acid receptor in NPCs [42]. Although NBCs can secrete bFGF, it is insufficient for the differentiation of NPCs. Therefore, the addition of more bFGF is necessary to promote the differentiation of NPCs whenever it is needed.

Based on the advantages of the cellular microencapsulation technique and the features of bFGF, we have used multilayered alginate-polylysine-alginate (MAPA) as an induction system [43]. In our system, the inner layer is used to encapsulate the NBCs, and the outer layer is used to envelop bFGF (Fig. 1). It has been reported that the alginate core coated with a poly-L-ornithine (PLO) layer can prevent the free expansion of macromolecules such as immune antibodies or tumor factors but not nutrients [44]. The outer alginate layer made with 1.25% low-viscosity ultrapure sodium alginate with high guluronic acid (LVG) provided a daily release of GFs greater than 0.5 ng/day and could sustain the release level for 26 days [43]. In addition, heparin could also prolong the release of GFs [45], even increasing the half-life of GFs by 100-fold [46]. Therefore, the PLO coat was made for the inner alginate layer, and heparin and 1.25% LVG were used for the synthesis of the outer alginate layer (Fig. 2).

Hypothesis

We hypothesized that a novel system of encapsulated NBCs and bFGF in MAPA may elicit its induction effect for the differentiation of MSCs into NPCs. One mechanism underlying the differentiation of MSCs into neurons induced by GFs may be mediated by its subsequent induction of intracellular signaling pathways.

Evaluation of the hypothesis

Additional experimental studies are required to determine the biological activities of the GFs released from the microcapsules and the efficiency of BMSC differentiation into NPCs. In these experiments, the concentrations of GFs secreted by microencapsuled NBCs will be determined by an enzyme-linked immunosorbent assay after the completion of the microcapsule preparation. In the process of induction, the expression of tyrosine-phosphorylated PI3K/AKT, cAMP/PKA, and JAK/STAT3 during the differentiation of BMSCs will be detected by western blot analysis. After the induction, the expression of the specific markers of NPCs/