Effect of Rat Schwann Cell Secretion on Proliferation and Differentiation of Human Neural Stem Cells[1]

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Objective To investigate the effect of rat Schwann cell secretion on the proliferation and differentiation of human embryonic neural stem cells (NSCs). **Methods** The samples were divided into three groups. In Group One, NSCs were cultured in DMED/F12 in which Schwann cells had grown for one day. In Group Two, NSCs and Schwann cells were co-cultured. In Group Three, NSCs were cultured in DMEM/F12. The morphology of NSCs was checked and β-tubulin, GalC, hoechst 33342 and GFAP labellings were detected. **Results** In Group One, all neural spheres were attached to the bottom and differentiated. The majority of them were β-tubulin positive while a few of cells were GFAP or GalC positive. In Group Two, neural spheres remained undifferentiatied and their proliferation was inhibited in places where Schwann cells were robust. In places where there were few Schwann cells, NSCs performed in a similar manner as in Group One. In Group Three, the cell growth state deteriorated day after day. On the 7th day, most NSCs died. **Conclusion** The secretion of rat Schwann cells has a growth supportive and differentiation-inducing effect on human NSCs.

Key words: Stem cells; Schwann cells; Nerve growth factors; Coculture; Immunohistochemistry

INTRODUCTION

Recently, it has been found that some cells are capable of self-renewal and multi-differentiation in the central nervous system (CNS)[1-3]. These cells are called neural stem cells (NSCs). The conditions and mechanisms underlying the induced differentiation of NSCs are not yet clearly known, but they are important for NSCs transplantation therapy to various CNS diseases in future. Inhabiting in peripheral nervous system (PNS), Schwann cells exert a strong nutritional and supportive effect on neural cells[4,5]. In this experiment, attempts were made to explore whether Schwann cells had any effect on human NSCs.

MATERIALS AND METHODS

Materials

Sprague-Dawley (SD) rats were purchased from Animal House, Chinese Institute of Medical Science. The materials were from the following sources: L-15 medium, Dulbeco's

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modified Eagle's medium (DMEM)/F12 and B27 supplement (GIBCO/BRL); Recombined human bFGF and recombined human EGF (Promega); Bisbenzimide Hoechst 33342 (SIGMA). The antibodies were from the following sources and were used at the indicated dilutions: monoclonal anti- -tubulin (1 500, ZYMED); polyclonal anti-glial fibrillary acidic protein (GFAP,1 100,CHEMICON); polyclonal anti-galactocerebroside (GalC,1 50, CHEMICON); Phase contrast microscope (Nikon DIAPHOT-TMD) and fluorescent microscope (Nikon ECLIPSE E600).

Methods

Culture of human embryonic NSCs. Brain of abortive human fetus (fetus of the 12th embryonic week, obtained from abortion due to traffic accident) was dissected, the meninges were removed, washed twice with cold Hank's and cut into small segments with scissors. The tissues were passed 120 coppery screen, the filtrated solution was collected and centrifuged. The precipitation was resuspended and allocated averagely into flasks containing DMEM/F12 supplemented with 20 ng/mL EGF, 12.5 ng/mL bFGF and 1×B27, incubated at 37℃ in 5% CO_2.

Culture of neonatal Schwann cells. The method was reported previously[6]. Briefly, sciatic nerves of 1 to 3-day-old SD rat pups were dissociated bilaterally and placed into L-15 medium. The epineurium and connective tissue were removed under microscope, the sciatic nerves were cut into 2 mm^3 fragments and dissociated with 0.25% trypsin and 0.125% collagenase for 15 min at 37℃, centrifuged at 1000 rpm/min for 5 min, supernatant was removed, cells were resuspended in DMEM containing 10% fetal bovine serum, 2 mol/L forskolin and 20 μg/mL bovine pituitary extract. Then cells were placed in dishes coated with poly-lysine and incubated at 37℃ in 5% CO_2 for 24 h. The medium was replaced by serum-free DMEM/F12 and the cells were incubated continuously.

Experimental grouping. Four dishes were used. According to different culture media, NSCs were divided into 3 groups, with each group in one dish, marked as Dish 1, 2, and 3 correspondingly. Schwann cells were seeded in Dish 2 and Dish 4. When they were in exponential growing state, Schwann cells were washed with Hank's solution for 3 times, and then the medium was replaced by DMEM/F12. Hoechst 33342 was added into the medium of NSCs at the final concentration of 5 μg/L. 48 h later, NSCs were washed with Hank's solution and were equally divided into Dish 2 and Dish 3. DMEM/F12 was added into Dish 2 and Dish 3 and half-changed daily. For every 24 h, the medium in Dish 1 was discarded. The old medium in Dish 4 in which Schwann cells had grown for 24 h was totally moved into Dish 1. Fresh DMEM/F12 was re-added into Dish 4.

Immunofluorescence. Dishes 1, 2, and 3 were rinsed with PBS for three times after 7-day co-culture, fixed with 4% paraformaldehyde in PBS for 30 min, permeabilized with 0.1% Triton X-100 in PBS, blocked with 1% goat serum, 1% bovine serum, 0.5% bovine albumin in PBS. Then the cells were incubated overnight at 4℃ with polyclonal or monoclonal antibodies. After being washed with PBS, the cells were incubated with FITC- or TRITC-labeled secondary antibodies at room temperature for 5 h. The labelled cells were visualized using fluorescent optics.

RESULTS

Phenotype change was visualized by phase contrast microscope. NSCs grew in the medium as suspending spheres increased gradually in their volume.

In Dish 1, after growing in the medium containing Schwann cells secretion for several hours, neural spheres began to attach to the bottom. 24 h later, the spheres became flat and protruded radially with numerous thin and long processes. The processes were gradually elongated and connected with those from neighbor spheres, forming a "net-work". NSCs proliferated well in 7 days (Fig. 1). The spheres were labelled blue by hoechst 33342. The majority of cells surrounding spheres were double labelling positive: nucleus was blue while cellular bodies and processes were red (β-tubulin positive), implying that the NSCs proliferated actively and that the β-tubulin positive cells were originated from NSCs (Fig. 2). These differentiated cells presented typical phenotype of neurons under higher-powered optics: each cell had several short and thin dendrites, one long and thick axon, with more tiny branches protruding from dendrites and axon (Fig. 3). A few of GFAP positive astrocytes and GalC positive oligodendrocytes scattered here and there (Fig. 4).

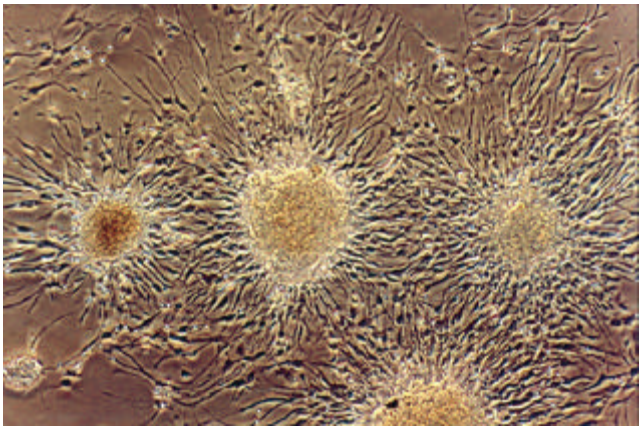

FIG. 1. Growing in the medium containing Schwann cells' secretion, neural spheres protrude radially with processes. The processes are connected with those from neighbor spheres, forming a "net-work". (×56)

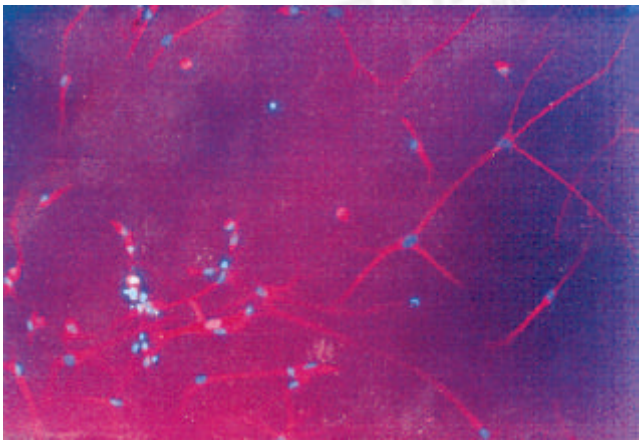

FIG. 2. The spheres are labelled blue by hoechst 33342. The majority of cells surrounding spheres are double labelling positive: nucleus is hoechst labelling positive (blue) while cellular bodies and processes are β-tubulin staining positive (red). (×112)

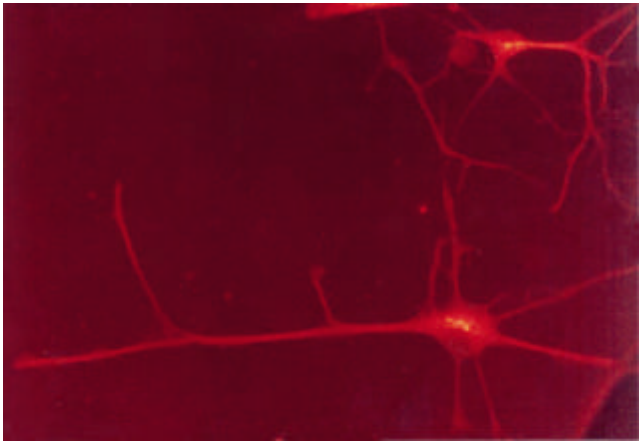

FIG. 3. The β-tubulin staining positive cells present typical phenotype of mature neurons. (×224)

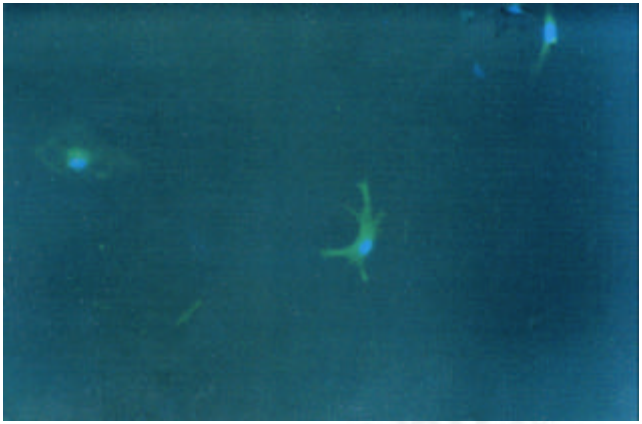

FIG. 4. A few of GalC positive oligodendrocytes scatter around the sphere. (×112)

In Dish 2, NSCs showed two quite different morphologies. Neural spheres kept undifferentiated and their proliferation was inhibited in places where Schwann cells were robust. No cells were β-tubulin, GFAP or GalC staining positive. In places where there were few Schwann cells, NSCs performed like those in Dish 1.

In Dish 3, the cell growing state deteriorated day after day and most NSCs died within 7 days.

DISCUSSION

Both *in vitro* and *in vivo* experiments confirmed that various environmental stimuli induced NSCs to be differentiated into neural cells or glial cells (including astrocytes and oligodendrocytes)[7-9]. Therefore, it is very important to further investigate the exact mechanism underlying the induced differentiation for our future NSCs transplantation therapy.

Previous study showed that some neurotrophic factors as bFGF and EGF were necessary

to maintain NSCs in proliferating and un-differentiated state[10,11]. Inhabiting in PNS, Schwann cells secrete more than 20 kinds of proteins, including several neurotrophic factors such as nerve growth factor, brain derived neurotrophic factor, ciliary neurotrophic factor and fibroblast growth factor and others. They also secrete extracellular matrix and cellular adhesive factors, which are important to the development, regeneration and repair of PNS[12-14]. Therefore, we are eager to know whether Schwann cells have any effect on the proliferation and differentiation of CNS derived NSCs.

Our study showed that the secretion of rat Schwann cells significantly supports the growth of human NSCs and induces differentiation of major cells into neurons. Furthermore, these neurons possess secondary and tertiary processes, which are morphologically similar to mature neurons. A few of NSCs become astrocytes and oligodendrocytes. Without the support of Schwann cells' secretion, NSCs would go to death gradually. Schwann cells themselves are contact-inhibitory to NSCs. We also found that the secretion worked as initialing-signal to NSCs differentiation. After growing in the medium containing Schwann cells' secretion for 24 h, even if the secretion was completely removed and bFGF, EGF were added into the medium, NSCs would continuously differentiate.

Above all, the secretion of Schwann cells strongly promotes the proliferation and differentiation of NSCs. The effect is non-species-specific. Further work should be done to identify and purify these proteins.

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