슈반세포와 뉴런세포을 이용한 수초화의 확인

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Identification of Myelination using Schwann Cells and Neuron Cells

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요 약

쥐에서 슈반세포와 뉴런세포를 이용한 수초화가 수행되었다. 슈반세포와 뉴런 세포는 쥐의 배아(임신 16일)의 척수신경절로 부터 각각 분리되어 배양되었다. 배아의 척수신경절이 배양되었고 항 유사분열제가 첨가되었다. 분리 정제된 배아의 슈반세포가 배양되었고 이것은 분리 정제된 배아의 척수신경절 세포에 첨가되었다. 실험실상에서 분리 정제된 수초화 군을 완성할 수 있었다. 뉴로필라멘트 단백질의 항체를 이용하여 수초화의 형성되었음을 확인하였다.

ABSTRACT

Myelination using Schwann cells and neuron cells was performed in rat. Schwann cells and neuron cells from dorsal root ganglion (DRG) of rat embryos (E16) were cultured, respectively. The embryonic DRG cells purified were cultured and anti-mitotic agents were added. Purified the embryonic Schwann cells were cultured and added to the embryonic DRG cells purified. A purified population of myelination in vitro system was accomplished and identified formation of myelination using antibody of neurofilament protein.

Keyword

Schwann cell, myelination, ndeurofilament protein, neuronal cell

Ⅰ. INTRODUCTION

The specialized source of neurons from nonneuronal cells were provided in Dorsal root ganglia [1]. Adult mammalian DRG neuron cells can survive and regenerate in culture [2, 3, 4]. There are several researches on purified populations of these neurons. Coculture of purified DRG neurons and Schwann cells can be used in myelin formation.

The most widely used method for preparing primary Schwann cell culture uses DRG as the primary source of Schwann cells. The procedure is very simple and produces a highly purified population of Schwann cells in a short time. The method has also been used to prepare Schwann cells from mouse embryos.

In this research, we performed a purified population of myelination by coculture of DRG neuronal cells and Schwann cells using this method and identified a myelination using antibody of neurofilament protein.

II. MATERIALS AND METHODS

A. dissection and dissociation of embryonic DRG

Embryos of mouse were decapitated with spring scissors and transfered the head to a pre-labeled 1.5 mL Eppendorf tube prepared. The tips of spring scissors vertically were inserted into the rostral end, just anterior to the vertebral column and begin making 3–4 sequential cuts from rostral to caudal (from head to tail) direction. The ganglia from the dorsal root starting from one end of the spinal cord were pinched off. All the ganglia (approximately 37–42 ganglia/spinal cord/embryo) were removed and repeated with the step for all the spinal cords. The 1.5 mL of 0.25% trypsin were added to each dish and placed the dishes into a 37℃ air incubator for 15 –20 min. It were added with 10 mL of L-15/10% FBS into each tube and centrifuged the ganglia at 50 *g* for 5 min. The pellet was added and resuspended with 10 mL of L-15/10% FBS. After centrifugation for 5 min as above, it was added with 1 mL of DRG plating medium to each tube. Using narrow-bored glass Pasteur pipettes by flaming the tips, the pellet was triturated 10–15 times and added with additional 1 mL of DRG plating medium to the cell suspension. This dissociated cells in an uncoated 35 mm dish were incubated.

B. suspension of the embryonic dorsal root ganglion cells

After 18 hr, plating of the dissociated DRG was replaced the medium with 2 mL of N2/NGF. Under the serum-free condition, fibroblast growth is suppressed while axon-associated Schwann cell precursors continue to proliferate. There is no need to change the medium. After 6-7 days, Schwann cell-neuron network forming axon bundles was transferred into culture medium.

C. purification and expansion of Schwann cells.

After collecting Schwann cell–neuron networks, the cells were pelleted by centrifugation at 200 *g* for 5 min.

The pellet was resuspend in trypsin-collagenase solution and incubated at 37 ℃ for 30 min. The reaction was stopped by adding Schwann cell medium and the cells were pelleted as above. The pellet was resupended in 1 ml of anti-Thy 1.2 antibody solution and incubated 30 min at 37 ℃. After centrifugation, pellet was resupended in 1 ml of rabbit complement solution and incubated 30 min at 37℃. Cells were plated onto a poly-L-lysine coated 100 mm culture dish in Schwann cell medium and incubated 30 min at 3 7℃. Next day, bipolar, spindle-shaped Schwann cells were changed the medium to N2-Schwann cell growth medium. Neuregulin and Forskolin contained in the growth medium start to stimulate Schwann cell proliferation while growth of any contaminating fibroblasts is suppressed under the serum-free condition. Neurons should die off within 2 days in the absence of NGF in the medium. After 6-7 days, the cells were replated onto new poly-L-lysine-coated plates at a density of 1×10^6 cells/100 mm plate.

D. addition of anti-mitotic cocktail

Cultures were incubated at 37 ℃, with 5% CO_2. 24 h later, 150 mL of NGF stock solution (40 mM of 5-fluorodeoxyuridine and uridine, 1 mM Arabinofur-anosyl Cytidine (Ara C, Sigma-Aldich, Saint Louis, MO) in NG medium) was added into each well to make the final concentration of 5-fluorodeoxyuridine/uridine 20 mM. Cultures were incubated at 37 ℃ in a 5% CO_2 incubator. After 72 hours, 2/3 of the NGF stock solution was changed to NG medium and the cultures were re-fed every 2 days with NG medium. After three medium changes, the neurons were ready for the Schwann cell addition.

E. addition of Schwann cells into DRG neuronal cell culture

Schwann cells were digested and washed once with DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, and then resuspended in C medium (MEM, 10% FBS, 2mM l-glutamine, 0.4% glucose, and 50 ng/mL 2.5 S NGF). 100 mL (approximately 200,000 cells) of Schwann cells was added to each of the DRG cultures in C media. After 3 days, the co-cultures were supplemented with 50 mg/mL of ascorbic acid to initiate basal lamina formation and myelination. Myelination was allowed to proceed for up to 21 days. The process of

myelination was observed and recorded under phase contrast microscope.

F. Immunocytochemistry

To observed the formation of the myelin, the DRG neuron/SC co-cultures (14 days after initiation myelin formation) were then labeled with primary antibodies, which included anti-NF-200 kDa (1:2000; clone N52; Sigma) After three washes with PBS, the coverslips were further incubated with Alexa Fluor 488 mouse anti-rabbit IgG and Alexa Fluor 594 goat anti-mouse IgG (Invitrogen, Carlsbad, CA) for 60 min at room temperature. After a final wash in PBS, the slides were mounted with mounting fluid (DAKO Ltd., Carpenteria, CA) and visualized under a fluorescence microscope (Olympus, Tokyo, Japan). The images were digitally recorded and processed with Image-Pro Plus (Media Cybernetics, Atlanta, GA).

III. RESULTS AND CONCLUSIONS

The method using oculture of Schwann cells and neuronal cells for myelination from DRG of rat embryos was described in Fig 1.

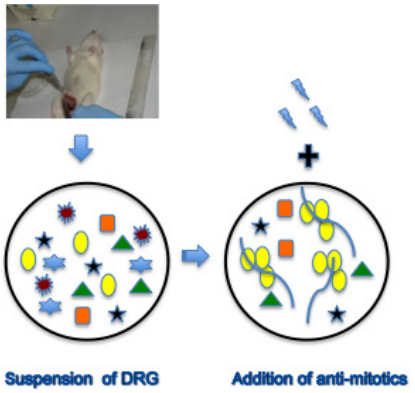

Fig. 1. Procedure for coculture of Schwann cells and neuronal cells for myelination from rat embryos.

This procedure contains following four steps: first step of suspension of the embryonic dorsal root ganglion cells [4], second step of addition of anti-mitotic cocktail [1], third step of purification of dorsal root cells [4], and fourth step of addition of Schwann cells to dorsal root ganglion cells [1].

For formation of myelination, cultured DRG cells and neuronal cells, respectively, were prepared from rat embryo (E 16 day) (Fig. 2).

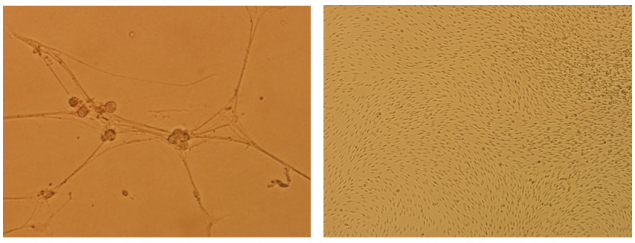

Fig. 2. Preparation of cultured DRG neuronal cells and Schwann cells, respectively, from rat embryo (E 16 day). (A: DRG neuronal cells, B: Schwann cells)

To idenfy the myelin formed by coculture of DRG cells and neuronal cells, cultured cells were labeled with antibody of mouse neurofilament protein and were observed by fluorescent microscope. The green-fluorescent regions represent myelinated cells (Fig. 3).

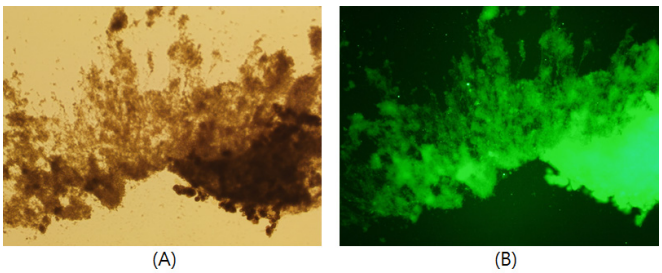

Fig. 3. Identification of myelination which is formed by coculture of DRG neuronal cells and Schwann cells (A: optical microscope, B: fluorescent microscope)

We accomplished a purified population of myelination in a short period through this procedure and identified myelination basic protein using antibody of myelination basic protein.

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