Semliki Forest Virus 감염은 뉴런의 탈수초를 유발한다

김현주[1] · 사영희[1] · 홍성갑[2]
[1]연세대학교 · [2]목원대학교

Infection of Semliki Forest Virus Induces Demyelination of Neuron

Hyun Joo Kim[1], Young-Hee Sa[1], and Seong-Karp Hong[2]

[1]Department of Anesthesiology and Pain Medicine, Yonsei University College of Medicine, Seoul, South Korea.
[2]Division of Bio and Health Sciences, Mokwon University, Daejeon, South Korea.
E-mail : karp@mokwon.ac.kr

요 약

슈반세포와 뉴런 세포가 쥐의 배아의 척수신경절로 부터 각각 in vitro에서 분리되었다. 배양된 슈반세포와 뉴런 세포는 동일한 평판접시에서 공동배양 되었다. 이들 세포들은 때 수초화가 진행되었다. 이렇게 수초화된 공동 배양은 Semliki forest virus에 의해 감염되었고 그 때 탈수초화 과정을 유발시켰다. 우리는 수초화된 뉴런에 존재하는 peripheral myelin protein 22의 항체를 이용하여 수초화 과정과 탈수초화 과정을 확인하였다.

ABSTRACT

Schwann cells and Neuronal cells were isolated from dorsal root ganglion (DRG) in embryos of rat in vitro respectively. The cultured Schwann cells and cultured neuronal cells, respectively were co-cultured in a same plate. These cells were performed accomplishment of myelination. This myelinated co-culture system was infected by Semliki forest virus and then induced demyelination processing in this myelinated co-culture. We identified myelination and demyelination processing using antibody of peripheral myelin protein 22 (PMP 22) meaning presence of myelinated neuron.

Keyword
demyelination, myelination, peripheral myelin protein 22, Semliki forest virus

Ⅰ. INTRODUCTION

Researchers developed a DRG neuron and schuwann cells co-culture system for myelin research *in vitro* [1]. The study of myelination has been facilitated by the availability to isolate and establish pure population of Schwann cells and Neuronal cells from primary Schwann cells. Mammalian DRG neuron cells can survive and regenerate in culture [2, 3, 4].

Some viruses such as Theiler's virus, mouse hepatitis virus (MHV), corona, measles, and Herpes simplex virus-1 are known as cause of inducing demyelination in nervous system of mice or rats. Virus like Herpes simplex virus-1 infection induces a demyelinating encephalomyelitis in the central nervous system of mice. Mice and rats are used as an important model for the study of myelination and demyelination research *in vitro* and *in vivo*.

In vitro myelination had been established by co-culturing with pure populations of primary Schwann cells and primary neuronal cells. These pure populations of primary Schwann cells and primary neuronal cells were driven from DRG of rat embryos. This method produced highly purified populations of Schwann cells and neuronal cells faster than any other conventional method.

From this research, we constructed a population of myelinated cells with co-culture of neuronal cells and Schwann cells from DRG. After this myelinated cells were infected with Semliki forest virus and processing of demyelination was progressed. We could identify and distinguish myelination and demyelination processing using antibody of PMP22 which represented as myelinated cells.

Ⅱ. MATERIALS AND METHODS

A. preparation 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 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 37℃. 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[5]. 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[2].

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 monoclonal antibody (mAb) against PMP22. 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

This procedure contains following four steps: first step of suspension of the embryonic dorsal root ganglion cells, secondstep of addition of anti-mitoticcocktail, third step of purification of dorsal root cells, and fourth step of addition of Schwann cells to dorsal root ganglion cells.

As a result of this study, for formation of myelination, Schwann cells and neuronal cells, respectively, were prepared and cultured from DRG of rat embryos (E 16 day) (Figure 1).

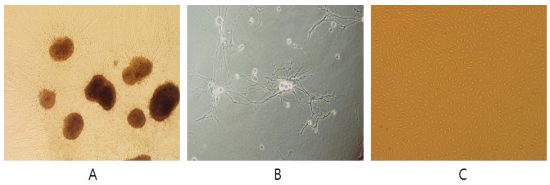

Figure 1. Purfication of populations of Schwann cells and neuronal cells, respectively, from DRG of rat embryo (E 16 day) (A: DRG; B: neuronal cells; Schwann cells).

To identify myelination and demyelination processing, population of cells were labeled with monoclonal antibody against PMP22 and observed by fluorescent microscope. Population of myelinated cells represent fluorescent spots due to monoclonal antibody against PMP22 which binds myelinated proteins. On the other hand, population of demyelinated cells did not because of absence of monoclonal antibody against PMP22 (Figure 2).

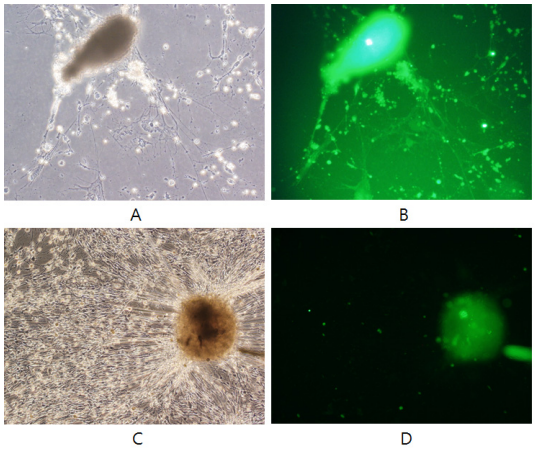

Figure 2. Identification and distinction of myelination and demyelination processing with monoclonal antibody against PMP22 (A: population of myelinated cells, B: anti-PMP22 in myelinated cells C: population of demyelinated cells, D:

anti-PMP22 in demyelinated cells).

In this study, myelination had been constructed by co-culturing with pure populations of primary Schwann cells and primary neuronal cells, respectively. These co-culture populations of primary Schwann cells and primary neuronal cells, respectively, were driven from DRG of rat embryos. When Semliki forest virus infected co-culture populations and broke down myelination processing through acting to nerve cells. Presence of monoclonal antibody (labelled with fluorescent dye) against PMP22 indicates presence of myelinated cells. Moreover, we recognize that infection with Semliki forest virus induces demyelination processing.

ACKNOWLEDGMENTS

This work was supported by a grant from Mid-Career Researcher Program (NRF-2016R1A2B4016552) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (MSIP) and a faculty research grant of Yonsei University College of Medicine for 2016(6-2016-0161).

REFERENCES

[1] R. Liu, G. Lin, and H. Xu, "An efficient method for dorsal root ganglia neurons purification with a one-time anti-mitotic reagent treatment," *PLoS one* vol. 8, pp. 1-7, 2013.

[2] J. Fukuda, "Nerve cells of adult and aged mice grown in a monolayer culture: age-associated changes in morphological and physiological properties of dorsal root ganglion cells in vitro," *Develop. Neurosci.* vol. 7, pp. 374-394, 1985.

[3] J. Fukuda, and M. Kameyama, "Enhancement of Ca spikes in nerve cells of adult mammals during neurite growth in tissue culture," *Nature,* vol. 279, pp. 546-548, 1979.

[4] K. Unsicker, S. D. Skaper, G. E. Davis, M. Manthorpe, S. Varon, "Comparison of the effects of laminin and the polyornithine-binding neurite promoting factor from RN22 Schwannoma cells on neurite regeneration from cultured newborn and adult rat dorsal root ganglion neurons," Developmental Brain Research vol. 7, pp. 304-308, 1985.

[5] H. A. Kim, and P. Maurel, "Protocols for Neural Cell Culture, Springer Protocols Handbooks" Humana Press, a part of Springer Science+Business Media, pp. 253–268, 2001.