·Basic Research·

Human optic nerve head astrocytes culture *in vitro*: I. the primary culture and passage

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Abstract

• AIM: To culture astrocytes from human donor eyes in order to understand the function of astrocytes in remodelling events in the glaucomatous optic nerve head (ONH).

• METHODS: Primary cultures were prepared by explantation of human ONH tissue in order to get astrocytes. Laminar criborsa (LC) cells were prepared concurrently for comparison. Astrocyte cultures could be separated from LC cells by selecting medium. Similar procedures were used for LC.

• RESULTS: Primary cells grew from human optic nerve head explants 4-8 weeks after explantation. Astrocytes had different morphologies and growth characteristics from LC cells. Type 1B astrocyte cells could grow in medium without FBS. Purified cultures were obtained by second passage and could be harvested by third to fifth passage, which were prepared to use for further study, including being characterized by positive glial fibrillary acidic protein (GFAP) and neural cell adhesion molecule (NCAM) staining.

• CONCLUSION: Precise dissection of fragment is the most important step to get clear explants for primary culture. Economic and rapid method could be useful to select cells by different mediums, which will help us to get more purified cells for further study.

• KEYWORDS: cell culture; glaucoma, open angle; optic nerve head

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INTRODUCTION

Glaucomatous optic neuropathy is characterized by a progressive loss of retinal ganglion cells associated with visual functional deficits, usually in response to elevated intraocular pressure. As the ganglion cells die in glaucoma, there is a progressive thinning of the nerve fibre layer (NFL). The mechanism of this pathology, however, is not totally clear. So far substantial research has shown that damage to the optic nerve axons occurs at the level of the lamina cribrosa in the optic nerve head [1,2]. Increasingly, glial cells in the CNS have been cited as participants in the pathologic course of neuronal damage after mechanical, ischemic and various other insults. The mechanisms by which glial cells are involved in CNS neuronal damage may vary [3,4], but many of the mechanisms are surprisingly similar to those proposed in glaucoma. Glial cells, especially, play an important role in supporting axons and forming the interface between connective tissue surfaces and surrounding blood vessels [3-5]. Different kinds of astrocytes are located in pre-and post-laminar region, as well as in lamina cribrosa. In glaucoma, there is extensive remodeling of the OHN extracellular matrix (ECM), with increased expression of ECM and proteolytic enzymes, cell adhesion molecules, and neurotoxic mediators, by reactive ONH astrocytes [3]. In order to understand all the functions that astrocytes perform in glaucoma development, we attempted to culture the human optic nerve astrocytes *in vitro*.

MATERIALS AND METHODS

Donors Sixty-five human eyes without history of eye disease (age 14-101 years) were obtained within 24-48 hours of death from Toronto Eye Bank. Eyes were transported to the laboratory in a humidified atmosphere container.

Procedures of Dissection Fresh eyes were rinsed and carefully dissected in sterilized PBS (supplemented with penicillin/streptomycin) under dissecting microscope. The whole anterior segment and posterior part of vitreous, retina pigment and remnant non-neural tissues were removed. The resulting cylinder of tissue included the optic nerve head, lamina cribrosa and post laminar myelinated nerve, as well

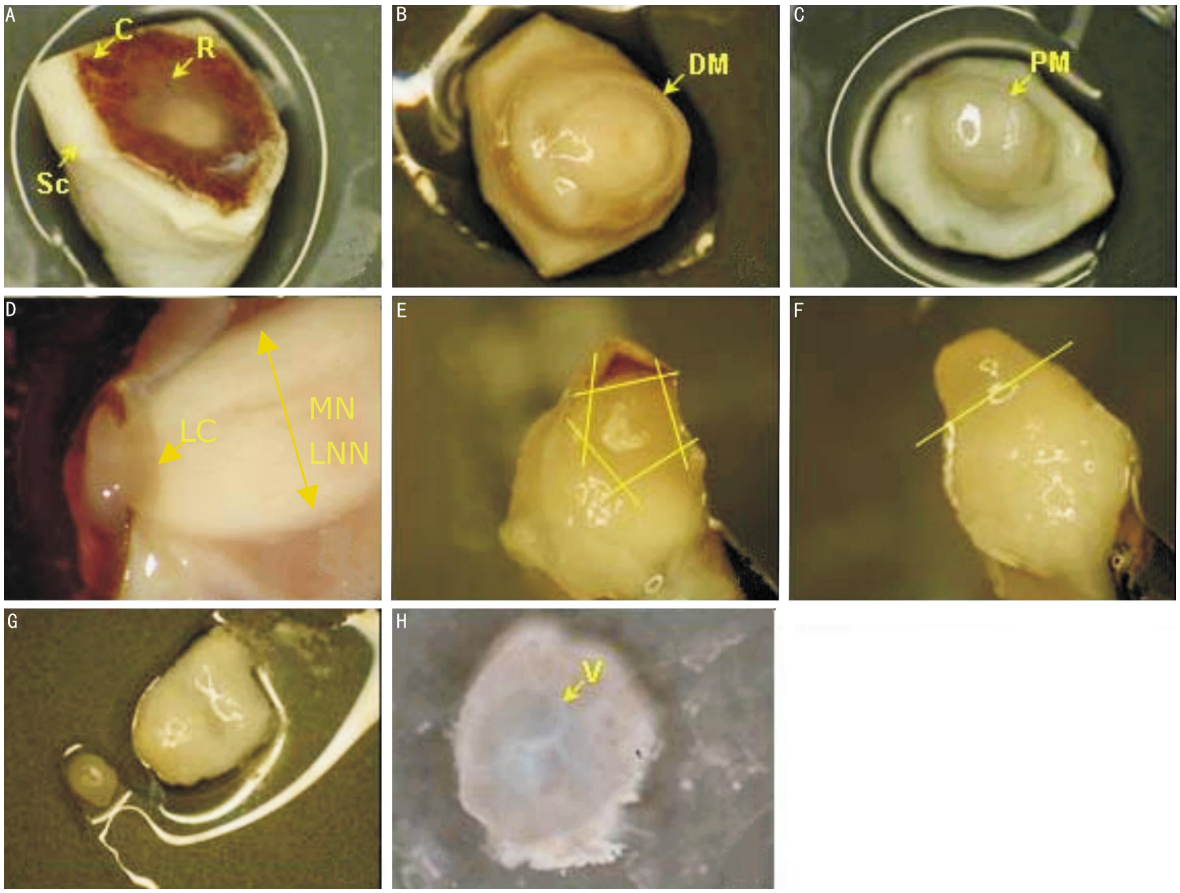

Figure 1 Procedure of dissection A: tissue with sclera (Sc), chloroid (C) and retina (R); B:tissue with dura mater (DM); C: tissue with pia mater; D: side view of picture A (LC: laminar cribrosa; MN: myelinated nerve); E: yellow lines indicate the cuts that will be made in the next steps; F: yellow line indicates the cut of optic nerve head; G: separation part of optic nerve head from lamina tissue; H: vessels in the middle of cutting explant (20×)

as small amounts of sclera and choroid (Figure 1A). Under a dissecting microscope, the post laminar myelinated nerve was identified and excised using fine scissors. The central vessels were carefully teased out of the remaining piece of tissue (Figure 1E-H). The ONH tissue was cut into several smaller pieces and put into a flask with a drop of DMEM (see explants culture).

Explants Culture Tissue culture flasks (25cm^2)were conditioned with 1mL pre-warmed growth medium Dulbecco's modified Eagle's medium (DMEM, Sigma Company)/F-12 (Sigma-Aldrich, Canada), supplemented with 100mL/L FBS, L-Glutamine (2mmol/L),Gentamycin (10mg/L),and Fungizone (250μg/L).

Each explant piece was placed into the culture flask, and kept standing in a humidified incubator (37℃ 50mL/L CO_2, Thermo Forma) for 15-20 minutes allowing the explants to adhere to the flask. After 5 days in the incubator an additional 4mL of media was carefully added to the flask. After another 10 days, the media on the cells was changed biweekly. Three to 8 weeks later, cells were observed growing out from the explants.

Passage When the primary cells from explants were confluent, they were passaged and split in 1:2. For astrocytes, cells from ONH would be selected and cultured in serum-free astrocyte medium (AGM-FBS) (ABM supplemented with growth factors, cytokines, antibiotics and supplements; Cambrex).Once the population looked homogeneous, serum was included in the media at all future media changes. For LC cells, the cells were cultured in low-glucose DMEM (Sigma-Aldrich) supplemented with 100mL/L FBS, L-Glutamine (2mmol/L) and Gentamycin (10mg/L).

RESULTS

The Best Dissecting Method for Cell Culture We were searching a best way to dissecting tissue and got the purified culture explants as best as we can. Follow the procedure we did, a clear anatomic tissue with nicely identified layers could be observed very well (Figure 2) which could help us to get the best explants for our further culture.

Primary Cell Culture from Human Optic Nerve Head Initial outgrowth from the explants appeared within 3-8 weeks of culture and reached confluence in 4-12 weeks.

Cells grew out from the explant and contained a mixed population of cells with two major cell types: astrocyte cells (AST) and laminar cribrosa cells (LC). The former was star-shaped with long processes (Figure 3A) while the latter a large flat polygonal appearance with oval clear nuclei (Figure 3B). Fibroblast-like cells could be easily identified due to their rapid growth rates and multilayering characteristics.

At the first passage, astrocytes could be selected by changing the medium to FBS free medium, in which LC cells could not survive. After this selection process, the cultures appeared to be essentially pure astrocytes. We also found that AST cells could be passaged to 7th passage and grew very well while LC cells could not survive nicely after 4th passage.

DISCUSSION

Glial cells are structurally and functionally linked to neuronal tissues, including the optic nerve and retina. The transition of quiescent mature astrocytes to the activated phenotype represents one of the earliest and most dominant responses of the central nervous system to injury. Pathologically, the activated cells may be characterized by increased size and number, by altered cellular properties, such as upregulation of glial fibrillary acidic protein (GFAP), and by additional cellular changes that may cause or relieve neuronal impairment [4,6,7]. Such astrocytic changes have been found in the glaucomatous optic nerve and retina as well as in experimental glaucoma models [8,9].

In the optic nerve, glial cells include astrocytes, oligodendrocytes and microglia. Astrocytes are the major glial cells type in the nonmyelinated optic nerve head in most mammalian species. In the human optic nerve head, two sub-populations of astocytes are distinguished. Type 1A astroctyes are interspersed in the glial columns and at the edge of cribriform plates. They express GFAP but not neural cell adhesion molecule (NCAM). Type 1B astrocytes express both GFAP and NCAM. They are the major glial cell population in this region and are the primary cells involved in synthesis of the optic nerve head ECM during development and throughout life [10]. At the vitreal surface of the optic disc, which is lined with Type 1B astrocytes, is the source of the cells used for this study (see materials and methods). In our results, Type 1B astrocytes showed a star-shaped morphology, and positive characterization will be shown in our next paper where were GFAP and NCAM positive. The results are consistent with those of Hernandez [11]. The method we used is economical and easy to perform because the astrocytes grow well in AGM without FBS while the LC cells cannot survive in this medium. The precise dissection of explant from ONH is quite important to get the purified cells.

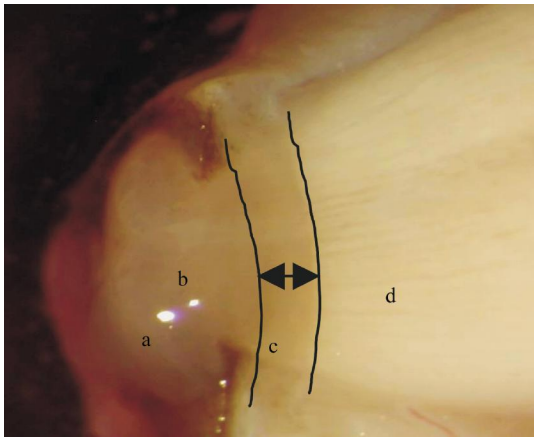

Figure 2 Details in dissection fragment a: retina on the face of optic nerve head; b: optic nerve head; c: lamina tissue including pre-lamina and post-lamina; d: myelinated optic nerve fibers (20×)

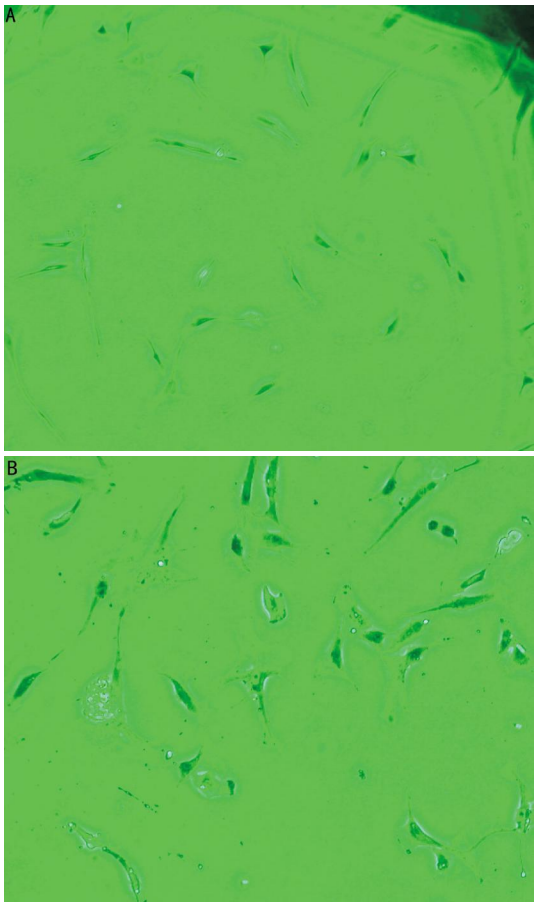

Figure 3 A:2nd passage of AST cells after FBS free selection. Morphological characters (arrows) showed typical star-shaped with long extension processes; B: 1st passage of 2nd day growth LC cells with flat body of polygonal short processes (200×)

The function of Type 1B astrocytes is to form the glial columns, surround blood vessels in the prelaminar region, form the cribriform plates in the lamina cribrosa and separate the sclera from the optic nerve in the insertion area.

Under normal intraocular pressure, the astrocytes remain quiescent. In glaucoma, the lamina cribrosa undergoes significant deformation in response to changes in intraocular pressure (IOP), which generates biomechanical stress on astrocytes and other cell types of LC [12,13], leading to the reactive astrocyte phenotype, optic nerve head remodelling and loss of retinal ganglion cell axons. Histopathologic studies in human donor eyes also demonstrate that astrocytes undergo an activation process in glaucoma [14].

Cultures of adult human ONH astrocytes as a useful *in vitro* model have been reported in several studies. Several studies will be done using the cultured cells to simulate the physical stresses caused by elevated IOP. Such *in vitro* experiments will help us to understand the mechanisms involved in the remodelling of ECM in glaucoma and provide us a model with which to research medications for optic nerve protection in the future [15-17].

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