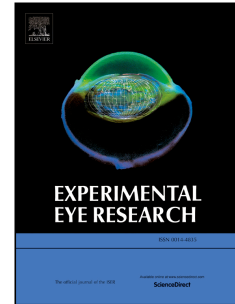
Accepted Manuscript



Establishment of a conditionally immortalized mouse optic nerve astrocyte line

Yang Liu, Gaurang C. Patel, Weiming Mao, Abbot F. Clark

PII: S0014-4835(18)30161-1

DOI: 10.1016/j.exer.2018.07.011

Reference: YEXER 7433

To appear in: *Experimental Eye Research*

Received Date: 2 March 2018

Revised Date: 6 July 2018

Accepted Date: 8 July 2018

Please cite this article as: Liu, Y., Patel, G.C., Mao, W., Clark, A.F., Establishment of a conditionally immortalized mouse optic nerve astrocyte line, *Experimental Eye Research* (2018), doi: 10.1016/j.exer.2018.07.011.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

1 **Establishment of a conditionally immortalized mouse optic nerve astrocyte line**

2 **Abstract**

3 Optic nerve astrocytes play a major role in axonal degeneration and regeneration. Astrocyte lines are an
4 important tool to elucidate the responsible cellular mechanisms. In this study, we established a
5 conditionally immortalized mouse optic nerve astrocyte line. Astrocytes were cultured from explants
6 derived from postnatal day 5-7 *H-2k*^{b}-tsA58 transgenic mouse optic nerves. Cells were cultured in
7 defined astrocyte culture medium under permissive (33°C) or non-permissive (38.5°C) temperature with
8 or without interferon-ɣ (IFN-ɣ). Astrocytes were characterized by immunocytochemistry staining using
9 antibodies against glial fibrillary acidic protein (GFAP) and neural cell adhesion molecule (NCAM). Cell
10 proliferation rates were determined by cell growth curves and percentage of Ki67 positive cells.
11 Karyotyping was performed to validate the mouse origin of established cell line. Conditional
12 immortalization was assessed by western blot-determined expression levels of SV40 large T antigen
13 (TAg), p53, GFAP and NCAM in non-permissive culture conditions. In addition, phagocytic activity of
14 immortalized cells was determined by flow cytometry-based pHrodo florescence analysis. After 5 days in
15 culture, cells migrated out from optic nerve explants. Immunocytochemistry staining showed that
16 migrating cells expressed astrocyte makers, GFAP and NCAM. In permissive conditions, astrocytes had
17 increased expression levels of TAg and p53, exhibited a greater cell proliferation rate as well as a higher
18 percentage of Ki67 positive cells (n=3, $p<0.05$) compared to cells cultured in non-permissive conditions.
19 One cell line (ImB1ON) was further maintained through 60 generations. Karyotyping showed that
20 ImB1ON was of mouse origin. Flow cytometry-based pHrodo florescence analysis demonstrated
21 phagocytic activity of ImB1ON cells. Quantitative PCR showed mRNA expression of trophic factors. Non-
22 permissive culture conditions decreased expression of TAg and p53 in ImB1ON, and increased the
23 expression of NCAM. A conditionally immortalized mouse optic nerve astrocyte line was established.
24 This cell line provides an important tool to study astrocyte biological processes.

25 Key words: optic nerve astrocyte, cell line, conditional immortalization

Download English Version:

https://daneshyari.com/en/article/8791887

Download Persian Version:

https://daneshyari.com/article/8791887

Daneshyari.com