Immunocytochemistry: A method for purity assessment of primarily cultured cells

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ABSTRACT

Introduction: Primary culturing outweighs transformed cell culturing with the genetic originality and in-vivo mimicry of samples derived from different organs. However, purity of primarily cultured cells is critical to ensure downstream analysis on the behaviour of the selected cells. During primary culture of cortical astrocytes, neighbouring cells like oligodendrocytes, neurons and epithelial cells could grow together with astrocytes. Therefore, establishing a purity assessment method is necessary to ensure the primary culturing efficacy. **Objective:** This study aimed to determine the purity of primarily cultured mouse cortical astrocytes through immunocytochemistry targeting astrocytes cytoskeletal marker, glial fibrillary acidic protein (GFAP). **Materials and methods:** Cerebral cortices of post-natal-day 2 mouse were mechanically crushed and viable cell counting was performed. Then seeded in plates containing pre-warm medium. Cells were seeded at the concentration of 1 x 10^5 cells per well and grown until confluent. Confluent cells were reseeded in chambered cell culture slides and incubated. Cells were fixed with 4% paraformaldehyde and permeabilized with phosphate buffered saline containing Triton-X. Non-specific binding proteins were blocked with blocking buffer. Then incubated with monoclonal rabbit anti-GFAP antibody followed by Alexa Fluor 647 goat anti-rabbit IgG. Cell nuclei were stained with 4,6-Diamonidino-2-phenylindole (DAPI). Then, immunoreactivity was observed under the fluorescent microscope. The representative photomicrographs were taken for cell counting. The percentage was calculated for the number of GFAP and DAPI positive cells over the total number of DAPI positive cells. **Results and conclusion:** The number of GFAP positive cells obtained 98 ± 0.4% for four independent primary cultures. The immunocytochemistry targeting GFAP is a useful method for assessing the purity of primarily cultured astrocytes. Thus, immunocytochemistry technique could be applicable for purity checking of any primarily cultured cells with known makers.

Keywords: Astrocytes, Cell purity, Immunocytochemistry