Primary culture of astrocytes

The newborn Wistar rats within 24 hours were supplied by the Center for Experiment Animals of China Medical University (license number: SCXK-2013-0001). The cerebral cortex was stripped out after the newborn rats were sterilized with 75% alcohol. The cerebral cortex was made into single cell suspension with Dulbecco's modified eagle's medium (DMEM, Hyclone, Life sciences, USA) supplemented 20% horse serum (Gibcol, Life technologies, USA). The cell density was adjusted for 1×10^6/ml. Culture cells were maintained at 37 ℃ in a humidified 5% CO_2 incubator. Subculture cells to the third generation for the further experiment. All experiments and surgical procedures complied with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Identification of astrocyte-Immunocytochemistry

The third generation astrocytes grown on a glass coverslip pretreated with Poly-L-Lysine (15-30) (Sigma, USA). Cells grown on the glass coverslip were rinsed with warm 0.1M PBS three times and fixed in 4% paraformaldehyde for 30 min. After three rinses with PBS, cells were incubated for 15 min in 0.5% Triton X-100, and then done according to the manufactures' instructions (Ultrasensitive S-P kit, MXB, China). Cells were incubated with glial fibrillary acidic protein (GFAP) antibody (astrocyte marker, 1:100, Abcam, USA) overnight at 4 ℃. DAB chromogenic (DAB chromogenic reagent kit, MXB, China), gradient alcohol dehydration, transparent in xylene twice in turn. Finally the coverslips were sealed with neutral resin and observed by the optical microscope (Nikon 80i, Japan).

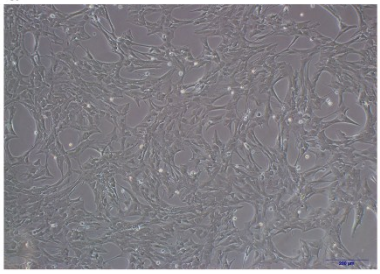
Results

Primary cultured and identified rat astrocytes

Figure.1 The primary rat astrocytes culture and identification. a representative for the primary astrocytes culture, b representative for the identification of astrocytes. The scale bar presented at the bottom of each photomicrograph indicated 200 μm and 20 μm.

Inverted phase contrast microscope was used to observe the primary rat astrocytes. As the photograph showed that there were a large number of astrocytes presented irregular shape or star. Astrocytes owned cell integrity including the smooth membrane, homogeneous cytoplasm, clearly visible nucleus and the rich cellular protrusion. Many astrocytes formed an inseparable network structure from each other. Glial fibrillary acidic protein (GFAP) was the unique in astrocytes that was used to identify the astrocytes through immunocytochemistry. Astrocytes cytoplasm was dyed brownish yellow due to glial fibrillary acidic protein combined with GFAP antibody. The result showed that the rate of GFAP positive cells was more than 95%. Therefore, the primary cultured astrocytes were used to form the model of Wistar rat astrocytes in vitro, and to perform the subsequent experiment.

a



b