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The relationship between mitochondrial fusion/fission and apoptosis in the process of adipose-derived stromal cells differentiation into astrocytes

Lili Zhang, Xiaodong Yuan*, Shujuan Wang, Ya Ou, Xinyue Zheng, Quanquan Wang

Department of Neurology, Affiliated Kailuan General Hospital of Hebei United University, No. 57, East Xinhua Road, Tangshan 063000, Hebei, China

HIGHLIGHTS

- Mitochondrial fusion decreased with the induction time extending.
- Mitochondrial fission enhanced with the induction time extending.
- Caspase-dependent apoptosis led to cell death in the process of induction.

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ABSTRACT

To research the features of mitochondrial fusion and fission proteins and their relationship with apoptosis in the process of adipose-derived stromal cells (ADSCs) differentiation into astrocytes. Immunocytochemistry and Western-blotting were used to analyze the expression of glial fibrillary acidic protein (GFAP), mitochondria fusion-2 (Mfn2), mitochondrial fission-1 (Fis1) and cysteine aspartate specific protease-3 (Caspase-3). Flow cytometry for quantification of the number of apoptotic cells. Transmission electron microscopy (TEM) for observation of the ultrastructure. After induced for 48 h, 7, 14 and 21 days showed expression of GFAP, reached the peak on the 7th day ($P < 0.05$). The expression of Mfn2 was decreased with the induction time extending, reached the minimum on the 14th day ($P < 0.05$). The expression of Fis1 and Caspase-3 was increased with the induction time extending, reached the peak on the 14th day ($P < 0.05$, respectively). The rates of early apoptosis, late apoptosis or necrosis were increased gradually ($P < 0.05$). Our findings suggest that in the process of ADSCs differentiation into astrocytes, mitochondrial fusion decreased while mitochondrial fission enhanced significantly; caspase-dependent apoptosis was one of the main reasons leading to cell death.

1. Introduction

ADSCs are multi-differentiation potential stem cells [1]. Previous studies showed that ADSCs could be successfully differentiated into neurons and astrocytes [2,3]. However, because of the disadvantages such as few target cells and short cell survival time during differentiation process, the research and application of ADSCs-derived astrocytes were limited [4].

Studies showed that apoptosis was one of the reasons leading to cell death in the process of ADSCs differentiation into astrocytes [5], the morphological and functional changes in mitochondria played an important role in apoptosis [6], which was the important reason of caspase-dependent and caspase-independent apoptosis [7,8]. Mitochondrial fusion/fission protein was a key factor in maintaining and regulating the morphology and function of mitochondria [9]. Mitochondria dynamics was related to apoptosis [10], the integrity of the mitochondrial membrane was destroyed when cells underwent apoptosis stimulation, resulting in lack of fusion or excessive fission of mitochondria and leading to mitochondrial fragmentation [11]. As important members of mitochondrial fusion and fission protein family, Mfn2 and Fis1 involved in the regulation process of cells apoptosis [12]. Our previous studies showed that in the differentiation process, mitochondrial morphology changed a lot such as mitochondrial swelling, cristae fracture, vacuolization etc [13]. However, the role of fusion/fission in mitochondria dynamics and its relationship with apoptosis is unclear in this differentiation process.

* Corresponding author. Tel.: +86 13903380386; fax: +86 3152820331.
E-mail address: yxd68@sohu.com (X. Yuan).

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2. Materials and methods

2.1. The extraction and culture of adult ADSCs

According to the method by Ye et al. [2], fatty tissues, approximately 20 ml, were harvested from healthy adult female volunteers who underwent liposuction surgery, aged 20–35 years old from Cosmetic Plastic Surgery Center of Kailuan General Hospital. Then ADSCs were extracted and cultured. The medical ethics issues involved in this research, in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, had been approved by the Medical Ethics Committee from Hebei United University.

2.2. ADSCs differentiation into astrocytes

After the cells grew to 70–80% confluence, cells were added with an inducer in which IBMX was a main component according to the method by Ou et al. [3] cells were induced for 48 h and 7, 14, and 21 days, respectively.

2.3. The expression of GFAP/Mfn2/Fis1/Caspase-3 detected by immunocytochemistry

Cells that were uninduced and, respectively, induced for 48 h and 7, 14, and 21 days were incubated by primary antibodies: rabbit antihuman GFAP (1:100; Beijing Biosynthesis Biotechnology, China); rabbit Anti-mitifusion-2 (1:200; Beijing Biosynthesis Biotechnology, China); rabbit Anti-Caspase-3 (1:100; Beijing Zhongshan Goldbridge, China); rabbit Anti-Fis1 (1:200; Epitomics, California, USA); goat anti-rabbit IgG antibody horseradish peroxidase (Beijing Zhongshan Goldbridge, China) was added; 3,3′-diaminobenzidine (Beijing Zhongshan Golden Bridge Biotechnology, China) which was colored and stained with hematoxylin. Determination of results: the cells which have brown particles in the cytoplasm were positive cells. The number of positive cells at high magnification was counted. Each sample at different fields was counted for five times, and three samples were observed altogether.

2.4. The expression of GFAP/Mfn2/Fis1/Caspase-3 detected by Western-blotting

Cells that were, respectively, induced for 48 h and 7, 14, and 21 days and the uninduced ADSCs were extracted the whole protein. Protein samples were quantified by BCA, after electrophoresis, the gel were transferred to PVDF membrane, then incubated by primary antibodies: rabbit antihuman GFAP (1:100); rabbit Anti-mitifusion-2 (1:200); rabbit Anti-Caspase-3 (1:100); rabbit Anti-Fis1 (1:1000); rabbit Anti-β-actin (1:5000); AP Affinipure Goat-Anti-Rabbit IgG (EarthOx, San Francisco, USA) was added; and the PVDF membranes were colored by BICP/NBT solution (amResco, USA). Image J software (National Institutes of Health, USA) were used to analyze the optical density.

2.5. Ultrastructural characteristics of astrocytes after induction by TEM

The cells induced for 14 days were digested and centrifuged and then fixed with 3% glutaraldehyde and 1% osmic acid, followed by propionaldehyde dehydration and epoxy resin embedding. The cells were sliced using a microtome and stained using 2% uranyl acetate and lead citrate. The ultrastructure of the cells were observed and photographed by TEM (H7650, Hitachi, Japan).

2.6. Annexin V/propidine iodide double staining assay for survival state of cells

After being induced for 48 h and 7, 14, and 21 days, cells and uninduced ADSCs were digested and collected and 190 μl buffer (Invitrogen, USA) and 10 μl propidine iodide (PI) dyebath (Invitrogen, USA) were added and detected by flow cytometry (BD FACSCalibur, New Jersey, USA). The procedure was repeated for three times. Determination of results: Annexin V−, PI+ represented mechanical damage cells; Annexin V+, PI+ represented late apoptotic or necrotic cells; Annexin V−, PI− represented living cells; Annexin V+, PI− represented early apoptotic cells.

2.7. Statistical analysis

All statistical analyses were performed with SPSS software (version 17.0). Measurement of data was expressed as mean ± SD. Intragroup differences were compared using *one-way analysis of variance*. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. The morphological characters of ADSCs differentiation into astrocytes

Primary ADSCs adhered at the 24 h; cells became triangular and short fusiform (Fig. 1A). Cultured for 7–10 days, there were a large number of long spindle cells that showed a whorled arrangement (Fig. 1B). When induced for 48 h, the cells refractivity was enhanced, parts of the cell bodies stretched out slender processes and multiple branches (Fig. 1C). Having been induced for 7 days, the cell body refractivity was enhanced; cell protrusions were richer, slender branch became increased and reticular (Fig. 1D). When induced for 14 days, about (72.63 ± 2.97)% of the cell morphology became typical astrocytes, there was no significant changes compared with the 7th day ($P < 0.05$) (Fig. 1E). Induced for 21 days, cells were triangular or irregular-shaped, and cell protrusions were shorter (Fig. 1F).

3.2. The expression of GFAP/Mfn2/Fis1/Caspase-3 of ADSCs differentiation into astrocytes by immunocytochemistry

There was no positive expression of GFAP in uninduced ADSCs, but after induction for 48 h and 7, 14 and 21 days GFAP showed positive expression; the positive parts mainly located in cell bodies and protrusions. GFAP positive expression ratio had no significant difference between 7 and 14 days ($P > 0.05$) which reached the peak on the 7th day ($P < 0.05$). Mfn2, Fis1 and Caspase-3 in uninduced and induced cells were positive expression, positive parts mainly located in the cytoplasm surrounding the nucleus. The expression of Mfn2, Fis1 and Caspase-3 between uninduced and induced ADSCs was significant difference ($P < 0.01$, respectively). The expression of Mfn2 decreased gradually with the time extending, reached the minimum on the 14th day ($P < 0.01$). The expression of Fis1 and Caspase-3 increased gradually with the time extending and reached the peak on the 14th day ($P < 0.05$, $P < 0.01$) (Fig. 2, Table 1).

3.3. The expression of GFAP/Mfn2/Fis1/Caspase-3 of ADSCs differentiation into astrocytes by Western-blotting

There was no expression of GFAP in uninduced ADSCs. The expression of GFAP, reached the peak on the 7th day ($P < 0.01$), there was no significant difference between induction for 7 and 14 days ($P > 0.05$). The expression of Mfn2, Fis1 and Caspase-3 between uninduced and induced cells were significant differences