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Effects of Ferric Ammonium Citrate (FAC) on Rab Family in SH-SY5Y Dopaminergic Cells

Zhenlei Zhufu[1]

[1]Department of Physiology, Medical College of Qingdao University, Qingdao 266071, China

Abstract: To examine the effects of ferric ammonium citrate (FAC) on Rab family in SH-SY5Y dopaminergic cells.Western blot was used to investigate Rab7,Rab11,Rab27a and Rab35 protein levels in SH-SY5Y dopaminegic cells with FAC for 4h and 12h.When SH-SY5Y cells were treated with 100 μmol/L or 1 mmol/L FAC for 4h and 12 h, Rab7, Rab11 and Rab27a protein levels was not significantly different from those of the control group.However, Rab35 protein was significantly up-regulated in SH-SY5Y cells with 1 mmol/L FACtreatment.Iron overloadupregulates Rab35 protein expressionin SH-SY5Y dopaminergic cells.

Keywords: Iron, SH-SY5Y Cells, Rab

Introduction

The exosomes are 30-100 nm membranous vesicles, which are released after fusion of the multivesicular and cell membranes, and then re-uptake by surrounding cells.Exosomes are capable of transporting intracellular components such as RNA, DNA, and proteins to the outside of cells. Itact on cells in adjacent or distant distances, and then exchange information between different cells under physiological and disease states. Theycan be found in biological samples such as blood, urine, saliva, and in vitro cells[1].Rab proteins, as well as some intracellular molecules, play an important role in the secretion of exosomes.

In recent years, studies have shown that misfolded proteins (such as α-synuclein in Parkinson's disease(PD), tau and β-amyloid in Alzheimer's disease (AD), etc.) associated with the onset of neurodegenerative diseases can be transported through exosomes. Thus they promote the spread of these proteins between cells and then spread the disease to uninfected areas, accelerating disease progression.

Parkinson's disease(PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD) [2].Although genetic, environmental and aging factors play a role in the pathogenesis of PD, the exact pathogenesis is not clear[3, 4].Two important neuropathological features of PD are characterized by specific iron accumulation in the substantia nigra and residual eosinophilic inclusions in dopamine neurons, which is the Lewy body[5].Because of the cytotoxic effect of iron and its ability to promote the production of oxygen free radicals, the role of iron in PD cannot be ignored[6-8].A large number of studies have confirmed that black matter iron accumulation is involved in the pathogenesis of PD. Iron-selective deposition has been found in the substantia nigra of PD patients and animal models [9]In the present study, SH-SY5Y dopaminergic cells were treated with iron to explore theregulationofRab protein associated with exosomes.

Materials and Methods

Material source

The SH-SY5Y cell line used in this experiment was provided by the Shanghai Cell Bank of the Chinese Academy of Sciences. This cell line was derived from the three-cloned metastasis of metastatic bone tumor cells SK-N-SH from a neuroblastoma patient established in 1970. Line (SK-N-SH→SH-SY→SH-SY5Y), this cell showed moderate levels of dopamine-β-hydroxylase activity. Ferric ammonium citrate (FAC) is a product of American Sigma Company. DMEM medium is a product of Hyclone Corporation of the United States. The β-actin antibody is a product of Boosen. Rab5, Rab7, Rab11, Rab27a antibodies are products of Cell Signalling TECHNOLOGY, USA. The goat anti-rabbit IgG labeled with HRP was a product of Santa Cruze (Dallas, USA). Fetal bovine serum (FBS) and DMEM / F-12 are products of Hyclone (Logan, Utah, USA).Penicillin-streptomycin solution was purchased from Beyotime (Shanghai, China). Other biological agents and materials come from local commercial sources.

The SH-SY5Y cell strain was cultured in DMEM medium (pH 7.4),whichcontains fetal bovine serum at a volume fraction of 0.05, penicillin at a concentration of 100 kU/L, and streptomycin at a concentration of 100 mg/L. Then, the SH-SY5Y cell strain was cultured in a CO2 cell incubator containing a volume fraction of 0.05 at 37 °C. The cells were inoculated into the culture plate and treated with dosing until they were 70% to 80%

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Zhenlei Zhufu (Correspondence)
zhufuzhenlei @ 163.com
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confluent. The control cells were incubated with serum-free DMEM medium, and the treatment group was treated with 100 μmol/ml FAC and 1 mmol/ml FAC for 12 h.

Western Blot detection of Rab protein levels

Control group and FAC-treated cells were washed 3 times with pre-cooled phosphate buffered saline (PBS), 100 μl of lysate was added to each well, lysed on ice for 30 min, and centrifuged at 12 000 r/min for 20 min at 4 °C. The supernatant was transferred to a new EP tube and the protein content was determined by the BCA method. The protein was electrophoresed by SDS-PAGE and electrotransferred to PVDF membrane. After blocking for 2 hours at room temperature with a volume fraction of 0.005 skim milk powder, Rab5 (1:1000), Rab7 (1:1000), Rab11 (1:1000) and Rab27a were added. 1:1000), β-actin primary antibody. It was incubated overnight at 4 °C. The goat anti-rabbit HRG-IgG secondary antibody diluted 1:10 000 was incubated for 1 h at room temperature, then developed with ECL chemiluminescence solution, and the UVP gel imaging system gel imaging system was observed and photographed. Strip grayscale analysis was performed using UVP VisionWorkTM LS Software.

Statistical processing

The experimental results were processed using Prism 5.0 statistical software (GraphPad Software, Inc., San Diego, CA).Data were analyzed statistically using one-way ANOVA.The difference was significant at $P < 0.05$.

Result

Expression of Rab protein in SH-SY5Y cells withFAC treatment

We studied SH-SY5Y cells treated with FAC at concentrations of 100 μmol/L and 1 mmol/L for 4h and 12 h.The protein levels of Rab7, Rab11, Rab27a and Rab35 in SH-SY5Y cells were examined by Western blot.The results showed that there was no significant change in Rab7, Rab11, and Rab27a when the concentration of FAC was 100 μmol/L and 1 mmol/L compared with the control, but the expression of Rab35 was significantly up-regulated when the concentration of FAC was 1 mmol/L.

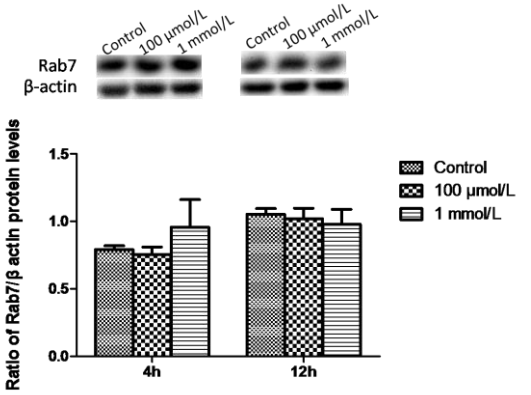

Figure 1. Changes in Rab7 protein expression in SH-SY5Y cells after treatment with 100 μmol/L and 1 mmol/L FAC for 4 h and 12h.

Treatment of SH-SY5Y cells with 100 μmol/L and 1 mmol / L FAC for 4 hours and 24 hours resulted in no significant change in the expression of Rab7 protein in cells. Each bar represents the mean ± S.E.M (one-way ANOVA, compared to the control; n = 4).

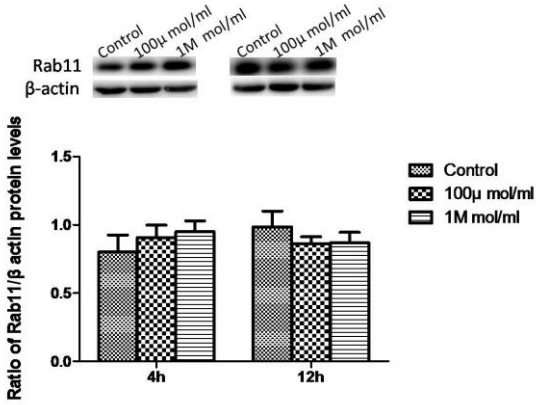

Figure 2. Changes in Rab11 protein expression in SH-SY5Y cells after treatment with 100 μmol/L and 1 mmol/L FAC for 4 h and 12h.

Treatment of SH-SY5Y cells with 100 μmol/L and 1 mmol/L FAC for 4 and 24 hours resulted in no significant change in intracellular Rab11 protein expression. Each bar represents the mean ± S.E.M (one-way ANOVA, compared to the control; n = 4).

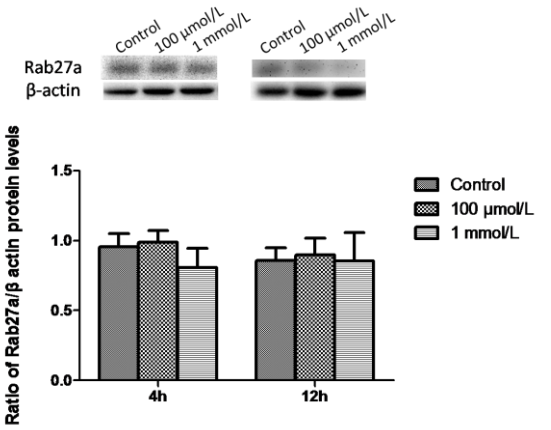

Figure 3. Changes in Rab27a protein expression in SH-SY5Y cells after treatment with 100 μmol/L and 1 mmol/L FAC for 4 h and 12h.
Treatment of SH-SY5Y cells with 100 μmol/L and 1 mmol / L FAC for 4 hours and 24 hours resulted in no significant change in intracellular Rab27a protein expression. Each bar represents the mean ± S.E.M (one-way ANOVA, compared to the control; n = 4).

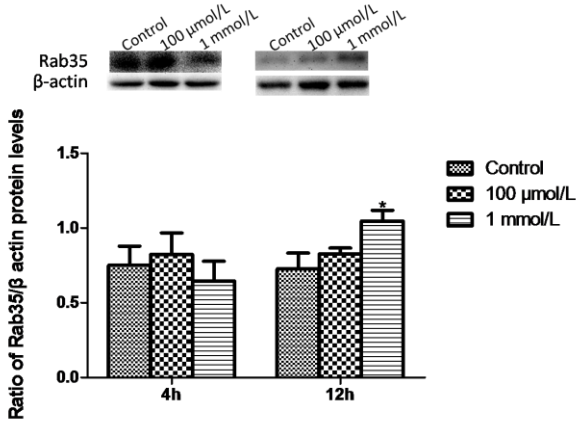

Figure 4. Changes in Rab35 protein expression in SH-SY5Y cells after treatment with 100 μmol/L and 1 mmol/L FAC for 4 h and 12h. (*P<0.05, compared with control)

Treatment of SH-SY5Y cells with 100 μmol/L and 1 mmol/L FAC for 4 and 24 hours resulted in a significant increase in the expression of Rab35 protein in the cells. Each bar represents the mean ± S.E.M (one-way ANOVA, * $P < 0.01$ compared to the control; n = 4).

Discussion

Exosomes have also been found to be inextricably linked to angiogenesis, inflammation, and programmed cell death. [10]。More importantly, exosomes are capable of carrying pathogenic proteins that are transmitted between cell-cells and interconnected structures. They can even transport intracellular components through the blood-brain barrier, which has been linked to neurodegenerative diseases. [11]。

Therefore, it is particularly important to study the Rab protein that promotes the release of exosomes.

Rab protein is a small GTPase that participates in membrane recognition, vesicle germination, vesicle decapsulation, and vesicle trafficking by recruiting effector proteins[12].Rab11, Rab35, Rab7 and Rab27 proteins in the Rab family promote exosome release and can act on different vesicular bodies (MVBs) along the endocytic pathway[12].The first Rab protein that has been shown to be involved in exocrine secretion is Rab11, which promotes the docking and fusion of MVBs with cell membranes in a calcium-dependent manner[13].Rab11 is involved in the transport of vesicles between the recirculating endosomes and the trans-Golgi network[14, 15].Rab7 and Rab27 (including Rab27A and Rab27B) also play a role in the secretion process of exosomes.Rab7 is shown to be associated with late endosomes[16].Inhibition of Rab27A, MVBs volume increased significantly, while inhibition of Rab27B, MVBs will be concentrated around the nucleus, not moving to the cell membrane[17].

When Rab35 is inhibited, the secretion of exosomes containing myelin PLP in oligodendrocytes is significantly reduced[18]. In other words, Rab35 plays an important role in the secretion of exosomes. In addition, Rab35 plays a role in the endosomal circulation of the transferrin receptor (TfR).TfR is a key protein responsible for transferrin-mediated cellular iron uptake[19]. In the present study, there was no significant change in Rab35 when dopamine SH-SY5Y cells were treated with FAC for 4 h. However, at 12 h, the expression level of Rab35 protein was up-regulated. There are two ways of transporting iron in the brain: transferrin (Tf) binding iron (Tf-Fe) and non-transferrin binding iron (NTBI). However, Fancheux et al. studied the Tf/TfRiron transport system in the brain of PD patients and found that the TfR density in the brain of PD patients is no different from that in normal people, suggesting that the increase of iron in the brain of PD patients is not due to Overexpression of TfR.These evidences suggest that in high-iron-treated dopamine SH-SY5Y cells, the up-regulation of Rab35 expression after iron loading may be related to the nuclear endosome of the TfR.

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