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Effects of 6-hydroxydopamine on the Expression of Iron Metabolism - Related Proteins in Primary Oligodendrocyte Progenitor Cells

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Abstract: Oligodendrocyte progenitor cells play an important role in the formation of myelin sheath and the protection of neurons. After the action of 6- hydroxy dopamine on primary oligodendrocyte progenitor cells, elevated iron regulatory proteins resulted in an increase in Transferrin Receptor-1 and a decrease in Ferroportin-1 in oligodendrocyte progenitor cells.The extracellular iron entering cells is mediated by TfR1 endocytosis. As the expression of Transferrin Receptor-1 increases, the large number of extracellular iron moves into oligodendrocyte progenitors, but the expression of Ferroportin-1 was decreased, the intracellular iron turnover decreased, increase of iron content in the cell. 6-hydroxydopamine acts on oligodendrocyte progenitor cells leading to changes in intracellular iron metabolism, so that oligodendrocyte progenitor cells increased iron content.

Keywords: 6-hydroxydopamine; primary oligodendrocyte precursor; IRP1; FPN1; TfR1

Introduction

Iron is essential for growth and proliferation of mammalian cells [1]. The central nervous system is particularly vulnerable to altered iron metabolism. Abnormally high brain iron is associated with common neurodegenerative disorders [2, 3] . There have been many reports on the role of 6-OHDA in neurons, microglia and astrocytes[4, 5], But with the glial cells of oligodendrocytes in this area of research is quite scarce. Oligodendrocytes are surrounded by axons in the central nervous system, forming an insulated myelin structure that facilitates the efficient transmission of electrical signals[6], maintaining and protecting the normal functioning of neuronal glial cells. Although most oligodendrocyte precursor cells (OPCs)differentiate into myelinating oligodendrocytes, some portion ofOPCs remains undifferentiated after the completion of myelina-tion[7-10]. In recent years, adult OPCs have generated much interest asa reservoir of cells with the potential to self-renew, differentiateand remyelinate the CNS. Oligodendrocytes are no longerconsidered as non-functional supporting cells of neurones owingto recent findings, which recognise the importance of oligodendro-cytes in playing a dynamic role in neuronal functioning. The OPCs in the striatum and glaucoma of mice treated with MPTP were activated and mature, and the number of oligodendrocytes increased sharply in a short time and then decreased with time[11].It is seen that OPCs play an important role in the early stages of PD. At the same time,the relationship between OPCs and PD is important because the iron that is a factor of maturation of oligodendrocytes and the factors of the dopaminergic neuronal damage is tightly linked significance.

1. Materials and methods

Primary OPCs cultures: 1 to 2-day-old Sprague–Dawley rat(provided by Qingdao Fucheng Animal Husbandry Co., Ltd.) pups were anesthetized and then decapitated. The brain was removed and placed in PBS solution (Gibco). The meninges were surgically removed, and cells were separated by mechanical blowing. The brain homogenate was passed through 200 μm Nitex screens (SEFAR America, Depew, NY, USA) and resuspended in Dulbecco's modified Eagle's medium/10% fetal bovine serum (FBS). Cells were plated in poly-Dlysine (Sigma)-coated flasks. Cells were allowed to grow for 2 weeks, microglia were removed by shaking at 160 rpm for 2 h. Subsequently, the oligodendrocyte-enriched fraction was obtained by shaking the remainder of cells at 240 rpm for 18~20 h. This fraction was plated on poly-D-lysine-coated slides and used for staining, or it was distributed into 6-well plates at a concentration of 1 million cells per well in 2 mL of media.

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Immunocytochemical staining: Cells were stained with 0.01 mol/L PBS (pH 7.4), and 4% Paraformaldehyde was fixed for 20 min. Cell immunofluorescence staining was performed according to the conventional method. The primary antibodies were rabbit anti-rat Oligo2 antibody (1: 1000), Secondary antibody goat anti-rabbit LgG-FITc (1: 500). Blank control were replaced with PBS primary antibody.

Western blot analysis: The protein extracts were isolated by SDS-PAGE and tested with the respective antibodies. The relative level of protein was quantified using Image J software from NIH (Bethesda, MA, USA).

Statistical analysis: All data are expressed as mean ± standard deviation. The t-test was performed between the t-test and the control group. Statistical analysis was conducted using a statistical software package for Social Sciences (SPSS). P <0.05 was considered statistically significant.

2 results

2.1 Morphology and purity of OPCs

The OPCs are extracted and purified by mechanical separation. (a) The cells are small in size, the cells are translucent, round or oval, and most of them have bipolar processes. Branch. (b) Oligo2 labeled oligodamone progenitor cells by immunofluorescence. The red fluorescent marker is the OPCs (d), the blue fluorescently labeled extracts of all the cells of the nucleus (e), The purity of OPCs was calculated to be more than 95% (c).

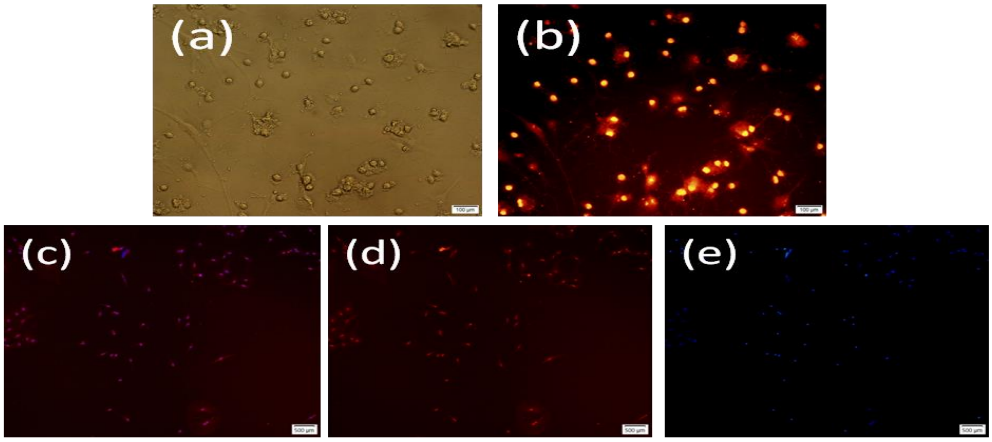

Fig 1 Extraction of purified OPCs. (a) Morphology of OPCs under bright field, (b) Oligo2-labeled OPCs under fluorescence microscopy, in cytoplasmic and nucleus are distributed. Immunofluorescence assay to detect the purity of OPCs, (c) blue fluorescence versus red fluorescence, where blue fluorescence is the nucleus of the entire field of view marked by Hochest33258. The red fluorescence is a FITC-labeled oligo2 protein with a red fluorescent group. (d) oligo2-labeled oligodendrocyte progenitor cell-positive cells. (e) Hochest33258-labeled nucleus positive cells.

2.2 Expression level of IRP1,FPN1 and TfR1 in primary OPCs treated with 10 μM 6-OHDA

Treatment of primary OPCs with 10 μM 6-OHDA treatment for 24 hours compared with the experimental group observed that the expression of IRP1 and TfR1 was significantly increased and the expression of FPN1 decreased.

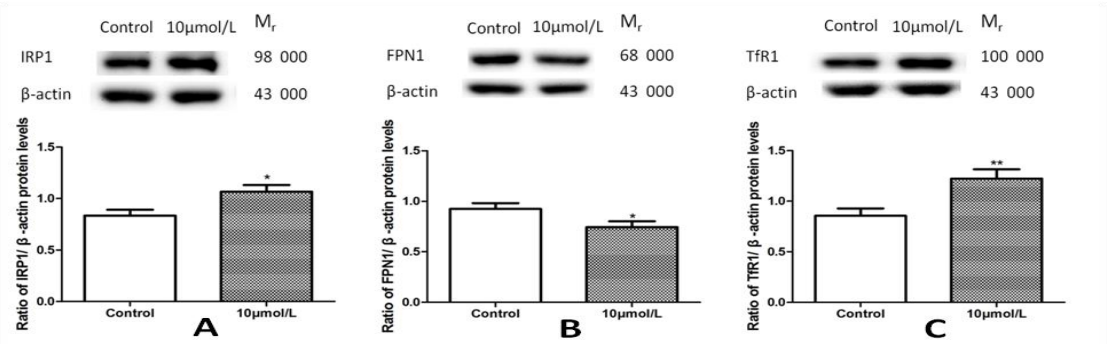

Fig 2 Western blot analysis of the effects of 10 μM 6-OHDA on primary OPCs for 24 hours.
(A) the expression of IRP1 protein was normalized with β-actin protein. The values are mean ± SEM, n=3. *p<0.05.
(B) the expression of FPN1 protein was normalized with β-actin protein, The values are mean ± SEM, n=5. *p<0.05.
(C) the expression of TfR1 protein was normalized with β-actin protein, The values are mean ± SEM, n=3. **p <0.01.

3 Discussion

Experiments were performed in vitro to obtain OPCs. Its purification and proliferation, to achieve the required concentration of the experiment. The purity of the OPCs was above 95% by immunofluorescence technique. With the deep understanding of glial cells, more and more experiments have shown that glial cells in the process of neuronal injury plays an important role.So now the study of PD is transferred from glaucoma dopaminergic neurons to glial cells.Iron is a key factor in PD, but it is also a necessary factor for maturation of oligodendrocytes. Therefore, it is important to study the changes in iron after 6-OHDA is acting on OPCs. The extraction of iron-related proteins IRP1, FPN1 and TfR1 was observed after the addition of 10μM 6-OHDA for 24 hours. Experiments shown that TfR1 protein expression increased significantly and the expression of FPN1 protein decreased when the expression of IRP1 increased significantly. IRPs can be combined with regulatory elements on FPN1 and TfR1 mRNAs. There are IRE structural elements can bind to IRPs in the 5 ' untranslated region of FPN1.When it binds to the 5 'UTR IRE of the mRNA, it is prevented from translating the protein expression of FPN1[12].At the same time, there are IRE structural elements in the 3 ' untranslated region of TfR1. When IRPs bind to the IRE of mRNA 3' UTR of TRf1, the stability of the mRNA molecule can be increased, and the protein expression of TfR1 raised[13]. This confirms the changes in iron-related proteins IRP1, FPN1 and TfR1 after 6-OHDA treatment of oligodendrocyte progenitor cells, which is consistent with the results obtained in the experiment. With the increase in the expression of TfR1, the iron transferred into the cells also increased. FPN1 is the only known iron-derived protein. With the decrease in the expression of FPN1, the removal of iron is reduced. In general, the intracellular iron content increases. This proves that OPCs in PD also produce iron aggregates similar to black dopaminergic neurons, which can lead to cell death when the iron content reaches the threshold of OPCs. This has a deeper understanding of PD, for the prevention and treatment of PD is of great significance.

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