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Effect of HDAC-6 on PD cell induced by lactacystin

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ABSTRACT

Objective: To explore the effects of histone deacetylase 6(HDAC-6) on the PD cell model induced by proteasome inhibitor lactacystin.

Methods: Human neuroblastoma SK-N-SH cells were cultured. The wild type pcDNA3.1-alpha-synuclein eukaryotic expression plasmid was transferred into the cells which then were divided into control group, group L, group T and group T+L. The cells of group L were added with 5 μmol/L lactacystin dissolved indimethylsulfoxide (DMSO) to induce PD cell model with abnormal protein aggregation, the cells of control group were treated with 5 μmol/L DMSO, the cells of group T were treated with 5 μmol/L selective HDAC-6 inhibitor tubacin dissolved in DMSO, and the cells of group T+L were treated with 5 μmol/L lactacystin and 10 μmol/L tubacin dissolved in DMSO. The expression levels of alpha-synuclein oligomers, HSP-27 and HSP-70 were detected by Western blot and the cell survival rate of all the groups was detected by MTT colorimetric assay, and compared 24 h after the cells were treated.

Results: The expression levels of alpha-synuclein oligomers, HSP-27 and HSP-70 of the cells of group L were significantly higher than the control group, and the cell survival rate was significantly lower ($P < 0.05$); the expression level of alpha-synuclein oligomers of the cells of group T+L was significantly higher than group L, but the expression level of HSP-27 and HSP-70 were significantly lower, and so as the cell survival rate ($P < 0.05$); the differences of the expression level of alpha-synuclein oligomers, HSP-27 and HSP-70 and the cell survival rate of the cells of group T and the control group were not statistically significant ($P > 0.05$).

Conclusions: The expression level of alpha-synuclein oligomers can be improved and the cell survival rate can be reduced by the PD cell model induced by lactacystin and treated with selective HDAC-6 inhibitor tubacin, which means that alpha-synuclein oligomers of the PD cell model induced by lactacystin can be inhibited and the cell survival rate can be improved by HDAC-6, and the mechanism may be related to the increased of HSP-27 and HSP-70.

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1. Introduction

As a kind of protease, histone deacetylase 6 (HDAC-6) plays a very important role in the gene expression regulation and structure modification of chromosome [1]. Studies have shown that the histone acetylation is one of the necessary conditions for the dissociation of DNA and histone octamer, and can make nucleosome structure relaxed to enable various transcription factors to specifically bind with DMA binding sites, resulting in the activation of transcription of genes in the nucleus [2]. In the nucleus, histone acetylation and histone deacetylation reach equilibrium state under the common control of histone acetyltransferase (HAT) and histone deacetylase (HDAC). On the one hand, HAT can transfer the acetyl of acetyl coenzyme A to the specific lysine residue at the amino terminus of histone. On the other hand, HDAC-6 can make histone deacetylated and closely combine with negatively charged DNA to make chromatin dense and curly, thereby inhibiting the transcription of genes [3].

Alpha-synuclein is a soluble protein expressing in the presynaptic area and around the nucleus of the central nervous system. Its unique NAC domain constitutes the main component of Lewy body, and it is closely related to the pathogenesis of Parkinson's disease and related dysfunctions [4]. When the gene point of alpha-synuclein mutates or amplifies, it can form a beta lamellar like structure and rapidly gather into oligomers. Then it can resist the degradation of ubiquitin proteasome, induce mitochondrial dysfunction and increase free dopamine in the cytoplasm, ultimately leading to the destruction of dopaminergic neurons [5]. Studies have shown that HDAC-6 can reduce the formation of alpha-synuclein oligomers and promotes their degradation through a variety of ways, so as to relieve the symptoms of Parkinson patients [6]. In this study, the expression level of alpha-synuclein oligomers, HSP-27 and HSP-70 were observed and compared after the function of HDAC-6 were inhibited in the PD cell model induced by lactacystin, in order to explore the effects and mechanism of HDAC-6 on the PD cell model induced by lactacystin.

2. Materials and methods

2.1. Materials

Human neuroblastoma SK-N-SH cells, DMEM high glucose culture medium, 10% bovine serum, 0.05% trypsin, alpha-synuclein recombinant plasmid DNA, proteasome inhibitor lactacystin, selective HDAC-6 inhibitor tubacin, dimethylsulfoxide (DMSO), Thiazolyl blue tetrazolium bromide (MTT) (Shanghai Research Domain Biological Technology Co., Ltd.), G418 culture medium (Suzhou Tianke Trading Co., Ltd.), 5% CO_2 incubator (center lab of Hong Kong University Shenzhen Hospital) and Lipofectamine 2000 (Suzhou Biotsith Bioscience Co., Ltd.).

2.2. Methods

2.2.1. Cell culture

The SK-N-SH cells were cultured in the DMEM high glucose culture medium containing 10% bovine serum and placed in 5% CO_2 incubator with a temperature of 37 °C. The liquid was changed 1–2 times per week. When being subcultured to fifth generation, 0.05% trypsin was used for digestion.

2.2.2. Transfection

The extracted alpha-synuclein recombinant plasmid DNA was transferred into SK-N-SH cells after subculture with Lipofectamine 2000. After cells were cloned into groups, they were cultured in the whole plate containing 750 μg/mL G418 culture medium.

2.2.3. Grouping

The transfected cells were randomly divided into control group, group L, group T and group T+L according to the random number table method. The cells of group L were added with 5 μmol/L lactacystin dissolved in DMSO to induce PD cell model with abnormal protein aggregation, the cells of control group were treated with 5 μmol/L DMSO, the cells of group T were treated with 5 μmol/L tubacin dissolved in DMSO, and the cells of group T+L were induced by 5 μmol/L lactacystin dissolved in DMSO and treated with 10 μmol/L tubacin.

2.2.4. Detection indexes

The expression level of alpha-synuclein oligomers, HSP-27 and HSP-70 detected by Western blot [7] and the cell survival rate of all the groups detected by MTT colorimetric assay [8] were compared 24 h after the cells were treated.

2.3. Statistical methods

The data in the study were inputted into SPSS17.0 for data analysis. Measure data were analyzed with homogeneity of variance test and one-way analysis of variance. The data between every two groups were compared with SNK method. A P value < 0.05 was considered statistically significant difference.

3. Results

3.1. Comparison of expression level of alpha-synuclein oligomers

The expression level of alpha-synuclein oligomers of the cells of group L was significantly higher than the control group of DMSO, and the expression level of alpha-synuclein oligomers of the cells of group T+L was significantly higher than group L after inhibiting HDAC-6 ($P < 0.05$); the difference of the expression level of alpha-synuclein oligomers of the cells of group T after treating and the control group of DMSO was not statistically significant ($P > 0.05$) (Figures 1 and 2).

3.2. Comparison of expression level of HSP-27 and HSP-70

The expression level of HSP-27 and HSP-70 of the cells of group L were significantly higher than the control group, but the expression level of HSP-27 and HSP-70 of the cells of group T+L were significantly lower than group L after inhibiting HDAC-6 ($P < 0.05$); the differences of the expression level of HSP-27 and HSP-70 of the cells of group T after treating and the control group of DMSO were not statistically significant ($P > 0.05$) (Figures 3 and 4).

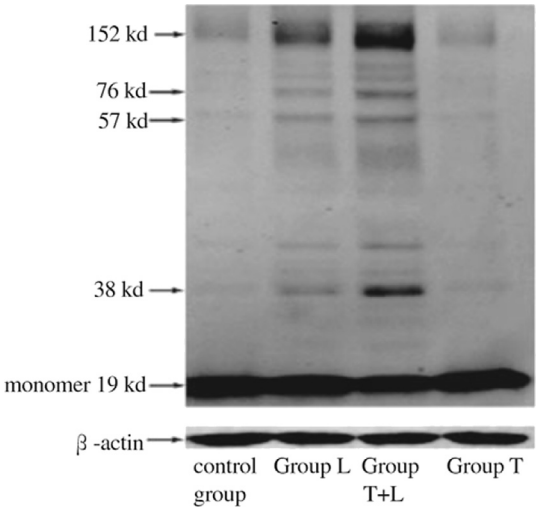

Figure 1. Expression map of alpha-synuclein oligomers.

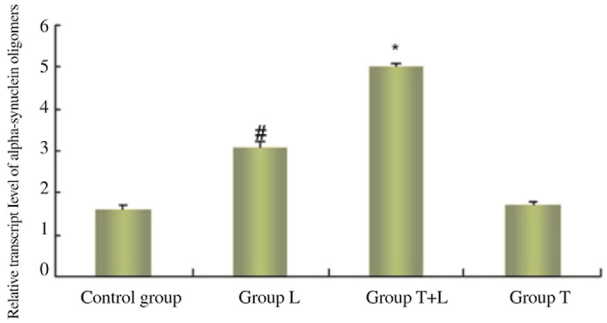

Figure 2. Relative expression of alpha-synuclein oligomers.

3.3. Comparison of cell survival rate

The cell survival rate of group L was significantly lower than the control group, and the cell survival rate of group T+L was significantly lower than group L after inhibiting HDAC-6 ($P < 0.05$); the difference of the cell survival rate of the cells of group T after treating and the control group was not statistically significant ($P > 0.05$) (Figure 5).

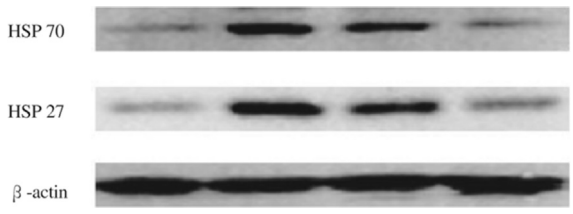

Figure 3. Expression map of HSP-27 and HSP-70 oligomers.

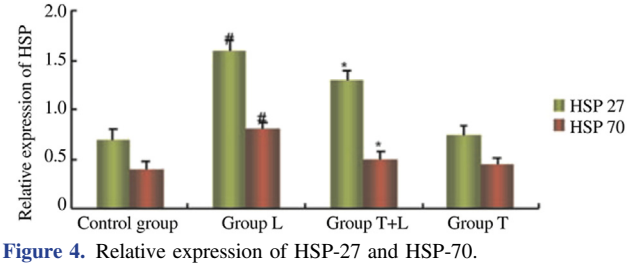

Figure 4. Relative expression of HSP-27 and HSP-70.

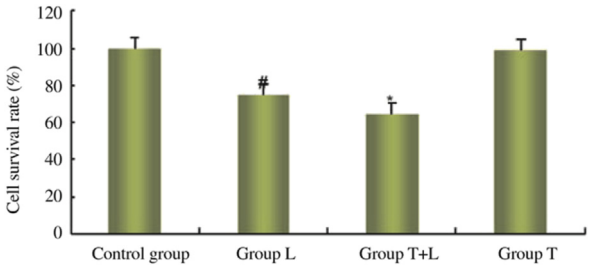

Figure 5. Comparison of cell survival rate in each group.

4. Discussion

Parkinson's disease, also known as idiopathic Parkinson's disease (PD) or Parkinson for short, referred to as paralysis agitans or shaking palsy, is a neurodegenerative disease common in middle aged and elderly people, and the most common extrapyramidal disease in middle aged and elderly people [9]. According to incomplete statistics, the morbidity rate of elderly people over 60 years old reaches 1000 per 100 thousand, and with the increase of age, the incidence of men is slightly more than women [10]. The main clinical manifestations of PD are static tremor, hypermyotonia, postural instability, movement delay and reduction and so on [11]. The cause of PD is still not very clear. It has been found that some central nervous system degeneration diseases can accompanied by the symptoms of PD, which mainly include degeneration in different parts of the central nervous system and other clinical features, such as striatonigral degeneration, SDS syndrome, progressive supranuclear paralysis, olivopontocerebellar atrophy and so on [12]. There are some diseases or factors that can produce clinical symptoms similar to PD, such as infection, poison (MPTP, carbon monoxide, manganese, *etc*.), vascular cause (multiple cerebral infarction), drugs (dopamine receptor blocking drugs, *etc*.), brain trauma and other causes, so they are all called Parkinson's syndrome or Parkinsonism clinically [13].

The most notable pathological changes of PD are degeneration and loss of neurons containing pigment, degeneration of DA neurons in the compact part of substantia nigra and so on [14]. Under microscope, melanin of the substantia negra cells disappears, or melanin granules are scattered in the tissues and macrophages, nerve cells decrease and neuroglia proliferates in different extent. It has been reported that the substantia nigra cells of normal people decrease with age. At the age of 80, they decrease from original 425 thousand to 200 thousand, and the substantia nigra cells of PD patients are less than 100 thousand. DA neurons can lose more than 50% when symptoms occur. Locus coeruleus, raphe nuclei, dorsal nucleus of vagus nerve, globuspallidus, putamen, caudate nucleus, subthalamic nucleus and so on also show mild changes. An important pathological feature of the disease is the appearance of eosinophilic inclusion Lewy body in the cytoplasm of residual neurons [15–18]. Lewy body is a kind of hyaloid mass composed of protein in the cytoplasm with a dense core in the centre and filamentous halos in the surrounding. Many Lewy bodies of different sizes sometimes can be seen in one of about 10% of the remaining cells. It is obvious in the substantia nigra and common in the globuspallidus, corpus striatum, locus coeruleus, *etc*. And it has been proved that alpha-synuclein and ubiquitin are the important components of

Lewy body [19]. Studies have found that there is a dynamic equilibrium in the normal, misfolding and oligomerization of alpha-synuclein, and when this equilibrium is broken, fibrils rapidly gather into large molecules, insoluble fine fibers; Alpha-synuclein may shows many kinds of forms under the influence of different factors, which include stretch form, ball form before dissolution, alpha-helix form (membrane binding), beta lamellar like form, dimer form, oligomer form, insoluble amorphous form and fiber form [20]; The point mutation of alpha-synuclein can lead to structural change, increase of intracellular content, accumulation of large amounts of protein molecules, truncated sequence, concentration of intracellular anions and salts (change of pH value), neurotoxic molecules (heavy metals, organic solvents, carbon monoxide, MPTP, pesticides and herbicides), post-translational modification (oxidation, phosphorylation and nitration) and so on, which can promote the alpha-synuclein to gather to form insoluble fibers [21]; The oligomer form of alpha-synuclein, which is considered the most cytotoxic currently, can not only resist the degradation of ubiquitin protease, but also induce mitochondrial dysfunction and increase the free dopamine in the cytoplasm, ultimately leading to the destruction of dopaminergic neurons [22]. And HDAC-6 can prevent the incidence of PD patients by reducing the formation of alpha-synuclein oligomers or promote their degradation [23]. Therefore, the effects and mechanism of HDAC-6 can be explored through the expression level of alpha-synuclein in the PD cell model induced by lactacystin.

In the study, the expression level of alpha-synuclein oligomers detected by protein immunosuppression method were observed and compared 24 h after the cells were treated with 4 different treatment methods. The change of expression level of alpha-synuclein oligomers was shown more intuitively and deeply. It was shown in the results that the expression level of alpha-synuclein oligomers of the cells induced by lactacystin in group L was significantly higher than that of the control group only treated with DMSO. And the expression level of alpha-synuclein oligomers of the cells treated with lactacystin and tubacin in group T+L after inhibiting HDAC-6 was significantly higher than group L. The difference of the expression level of alpha-synuclein oligomers of the cells only treated with tubacin in group T and the control group of DMSO was not statistically significant. The results indicate that the inhibition of HDAC-6 can promote the formation of alpha-synuclein oligomers in the PD cells, suggesting that HDAC-6 may have an effect of inhibiting the formation of alpha-synuclein oligomers on the PD cell model induced by lactacystin.

As an important member of low molecular weight heat shock protein family, heat shock protein 27 (HSP 27) is an important protein involved in the function of cell growth, apoptosis, drug resistance, occurrence and metastasis of tumor and so on, which may be related to its influence on other proteins [24]. When a cell is not stressed, the expression level of HSP-27 is low and it is mainly large polymer without activity generally. While a cell is stressed, the expression level of HSP-27 will increase significantly and HSP-27 is activated with phosphorylation to perform its specific function [25–27]. HSP-70 is also a very important member of heat shock protein family, including 20 kinds of proteins with molecular weight of 68, 72, 73, 75, 78 kDa and so on [28]. HSP-70 with molecular weight of 70 kDa (induction type) is the most one studied in heat shock protein, whose expression level is low in normal cells but significantly increases under stress. In particular, structure, function and expression regulation mechanism of HSP-70 family are studied a lot. Under normal condition, HSP-70 is in the cytoplasm. When stimulated by heat shock, HSP-70 increases rapidly in the nucleus and there are only a few in the cytoplasm. In the recovery phase of cells, the HSP-70 in the nucleus disappears and there is still a low expression level of HSP-70 in the cytoplasm [29]. Normally, HSP-70 shows a basic expression in the cell and the expression level is low. In the case of high temperature and all kinds of harmful stress or abnormal genes, the synthesis rate of HSP-70 significantly increases, which can reach the highest level within a few minutes generally, while the original protein synthesis is reduced, in order to improve the anti-stress ability of organism [30].

In the study, the expression level of HSP-27 and HSP-70 detected by protein immunosuppression method were observed and compared 24 h after the cells were treated with 4 different treatment methods. It was shown in the results that the expression level of HSP-27 and HSP-70 of the cells of group L were significantly higher than that of the control group. And the expression level of HSP-27 and HSP-70 of the cells of group T+L were lower than group L. The difference of the expression level of HSP-27 and HSP-70 of the cells of group T and the control group of DMSO was not statistically significant. The results indicate that after inhibiting HDAC-6, PD cell model can reduce the expression level of HSP-27 and HSP-70 to reduce the repair and removal of abnormal proteins, so that the formation of alpha-synuclein oligomers in the PD cells can be promoted, suggesting that HDAC-6 can inhibit the formation of alpha-synuclein oligomers in the PD cell model induced by lactacystin by increasing the expression level of HSP-27 and HSP-70.

At the same time, it can be found in this study that the cell survival rate can be sorted from high to low as the control group, group L and group T+L. There was no significant difference between the cell survival rate of group T and the control group after cell treatment. It can be indicated that the inhibition of HDAC-6 can serve to damage cell structure and function, which may be associated with the damage of mitochondria and phagocytic structure in the cell, suggesting that HDAC-6 may play a role on the protection of the PD cell model induced by lactacystin.

In summary, HDAC-6 may improve the expression level of HSP-27 and HSP-70 to inhibit the formation of alpha-synuclein oligomers in the PD cells, which plays a role in protecting cells. Bur it remains to be confirmed that whether the clinical symptoms of PD patients can be controlled by means of increasing the expression level of HSP-27 and HSP-70 to more inhibit the aggregation and folding of alpha-synuclein oligomers in the PD cells and whether it can be applied to the clinic.

Conflict of interest statement

We declare that we have no conflict of interest.

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