

# The expression of K<sub>ATP</sub> channel subunits in alpha-synuclein-transfected MES23.5 cells

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**Background:** SUR1, one of the subunits of ATP-sensitive potassium (K<sub>ATP</sub>) channels, was found to be highly expressed in mRNA levels in the substantia nigra (SN) of Parkinson's disease (PD) brains. Though the mechanism of the selective dopamine (DA) neurons death is still unknown, some studies have demonstrated that selective activation of the K<sub>ATP</sub> channels in the SN might be associated with the degeneration of DA neurons. The objective of our study is to examine the expressions of K<sub>ATP</sub> channel subunits in dopaminergic cells with alpha-synuclein ( $\alpha$ -Syn) transfection.

**Methods:** In this study, we detected the K<sub>ATP</sub> channel subunits mRNA levels in MES23.5 cells by real-time quantitative PCR after the cells transfected with  $\alpha$ -Syn.

**Results:** Our results showed that the mRNA levels of SUR1 subunit were markedly increased by 35% in WT  $\alpha$ -Syn overexpression cells and by 31% in A53T  $\alpha$ -Syn overexpression cells, respectively. However, the mRNA levels of SUR2B and Kir6.2 subunit have no obviously differences from the controls.

**Conclusions:** We showed that the mRNA levels of SUR1 but not SUR2B or Kir6.2 were selectively upregulated in MES23.5 cells over-expressed with  $\alpha$ -Syn. The findings demonstrated that the SUR1 overexpressed might be involved in the process of PD.

**Keywords:** Alpha-synuclein ( $\alpha$ -Syn); ATP sensitive potassium channels (K<sub>ATP</sub>); Parkinson's disease (PD); SUR1

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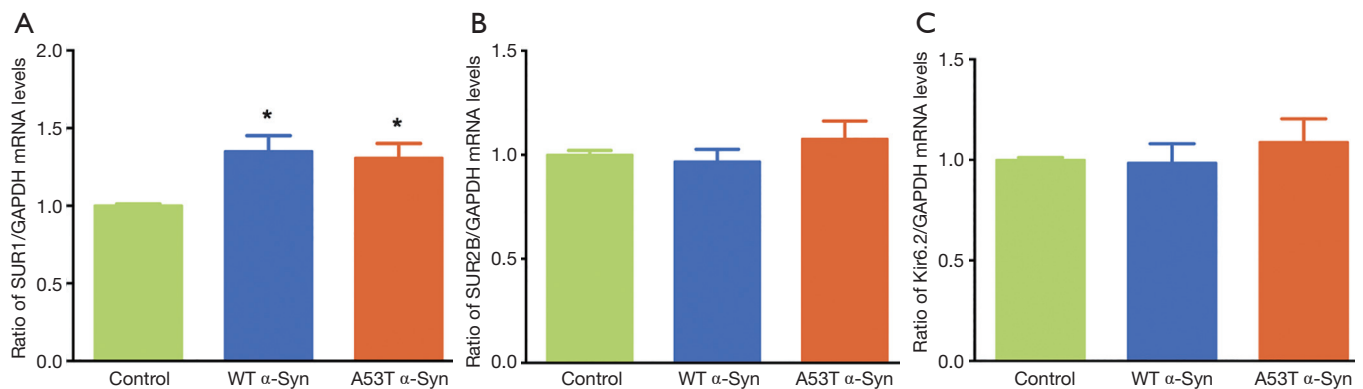
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## Introduction

Parkinson's disease (PD) is one of the leading neurodegenerative diseases in elderly, which is pathologically caused by the progressive loss of dopaminergic (DAergic) neurons in the substantia nigra (SN), and a formation of alpha-synuclein ( $\alpha$ -Syn) aggregates in Lewy bodies throughout the brain. However, the mechanism of the selective DAergic neurons death is still unknown (1-3).

The mRNA levels of SUR1 subunit of K<sub>ATP</sub> channels in the remaining DA neurons were increased in SN region of

PD brains, and some studies have also addressed that the mRNA expression of SUR1 in the nigral DAergic neurons was approximately two-fold to that in the ventral tegmental area (VTA) in a PD animal model (4,5), suggesting that specific activation of the K<sub>ATP</sub> channels in the SN might be related to the regression of DAergic neurons. K<sub>ATP</sub> channels were first demonstrated in the mammalian heart cells (6). In brain, K<sub>ATP</sub> channels are ubiquitously expressed, especially in the cortex, basal ganglia, hippocampus, hypothalamus, and nigral DAergic neurons (7,8). In nigral DAergic neurons, K<sub>ATP</sub> channels are more sensitive to some mitochondrial



**Figure 1** WT  $\alpha$ -Syn or A53T  $\alpha$ -Syn up-regulated the mRNA levels of SUR1 subunit. The results in the real-time quantitative PCR test showed that the mRNA expression of SUR1 (A) subunit was increased, and SUR2B (B) and Kir6.2 (C) were unchanged in MES23.5 cells-transfected with  $\alpha$ -Syn. All data were represented as mean  $\pm$  SEM of six independent experiments. \* $P < 0.05$ .

complex I inhibitors, like rotenone or 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (9).  $K_{ATP}$  channels consist of Kir6.x (Kir6.1 or Kir6.2) subunits and SUR (SUR1 or SUR2) subunits (10,11). Three subtypes of  $K_{ATP}$  channels, SUR1, SUR2B, and Kir6.2, have been identified in the midbrain DAergic neurons (12). Of these, SUR1/Kir6.2 subtype of  $K_{ATP}$  channels were extremely sensitive to mitochondrial complex I blockers, which is about 200 times as big as SUR2B/Kir6.2 subtype of  $K_{ATP}$  channels (13).

This study examines the expressions of  $K_{ATP}$  channel subunits in  $\alpha$ -Syn-transfected cells. The mRNA levels of SUR1 but not SUR2B or Kir6.2 were selectively upregulated in these cells.

## Methods

### Reagents

The MES23.5 cells were obtained from Stem Cell Bank (Chinese Academy of Sciences). The GV230 vectors that carrying cDNA encoding WT or A53T  $\alpha$ -Syn were purchased from GeneChem Co., Ltd. (Shanghai, China). Fetal Bovine Serum (FBS), Trizol Reagent and Lipofectamine 2000 were purchased from Invitrogen (CA, USA). Dulbecco's modified Eagle's medium (DMEM) and other chemical drugs were obtained from Sigma (St. Louis, MO, USA).

### Cell culture

The MES23.5 cell, a DAergic cell line, is obtained from the murine neuroblastoma glioma N18TG2 cells hybridized

with the rat mesencephalic neurons. It owns a number of properties which are resemble to the DAergic neurons from the SN. MES23.5 cells were maintained in DMEM which contained FBS (10%), Sato's (2%), penicillin (100 U/mL) and streptomycin (100 U/mL) in a humidified atmosphere containing 5%  $CO_2$  at 37 °C, and seeded into the 6 wells plastic plates ( $1 \times 10^5$  cells per well).

### Cell transfection

To observe the expressions of distinct  $K_{ATP}$  channel subunits, the cells were separated to three groups: GV230 vector group, WT  $\alpha$ -Syn group and A53T  $\alpha$ -Syn group. In the control group, GV230 vectors were transfected into MES23.5 cells alone. In the over-expression group, cells were transfected with the WT or A53T  $\alpha$ -Syn in a serum-free medium. This relatively high efficiency of infection of MES23.5 cells were harvested for the further studies. All vectors were transfected using Lipofectamine 2000. For optimal transfection efficiency, five volume of DNA relative to lipofectamine 2000 was used.

### Total RNA extraction and real-time quantitative PCR

Total RNA was harvested using Trizol Reagent based on the manufacturer's protocols. A total of 2  $\mu$ g RNA was reversely transcribed into cDNA using a First Strand cDNA Synthetic Kit. The mRNA levels of  $K_{ATP}$  channel subunits were detected using quantitative PCR with SYBR Green reagents. The primer sequences used as follows: SUR1 (forward: 5'-CCC TAG CTG TGG TGT GCT ACT

TCA-3'; reverse: 5'-GGG GCT GCG TTG TGT CATC-3'); and SUR2B (forward: 5'-TGG AGC TGA CAG ACA CGA ACA AC-3'; reverse: 5'-GAA CAA TGC ACG CTC CCA GA-3'); and Kir6.2 (forward: 5'-ATG GCC CTG ACA GGC AAG AG-3'; reverse: 5'-CCA AGT TGG CCA GAC AGA CAG A-3'). GAPDH purchased from Takara was served as a normalization control. The amplification was carried out according to the following steps: the mixture was preheated at 95 °C for 30 s, followed by 5 s, 95 °C and 34 s, 60 °C for total 40 cycles. Relative mRNA levels were calculated by the  $2^{-\Delta\Delta C_t}$  method.

### Statistical analysis

All data were presented as mean  $\pm$  SEM. Statistical analysis was performed using Graphpad 5.0. One-way analysis of variance (ANOVA) with Tukey's multiple comparison tests were carried out for multiple comparisons. Values of  $P < 0.05$  were deemed to be significant.

## Results

### Increased SUR1 mRNA levels were observed after $\alpha$ -Syn transfection

We detected the expressions of distinct subunits of  $K_{ATP}$  channels in transfected MES23.5 cells with WT or A53T  $\alpha$ -Syn for 24 hrs. The results illustrated that the mRNA levels of SUR1 were increased by 35% and by 31%, respectively, when were compared with that in the control (Figure 1A). SUR2B and Kir6.2 were slightly increased in A53T  $\alpha$ -Syn group, but there were no significances observed (Figure 1B,C).

## Discussion

In our study, the mRNA levels of SUR1 were found increased in either WT  $\alpha$ -syn or A53T  $\alpha$ -Syn transfected MES23.5 cells, while there were no effects on Kir6.2 and SUR2B transcriptions.

Though the precise mechanisms underlying the PD pathology are still unknown, some evidence has shown that selective activation of SUR1/Kir6.2 subtype of  $K_{ATP}$  channels in SN may lead to the degeneration of DA neurons. The transcript levels of SUR1 subunit of  $K_{ATP}$  channels in the remaining DA neurons were increased in SN of PD brains (4). The increased SUR1 subunit expression could promote the transport, expression and activation of

$K_{ATP}$  channel in membrane (14,15). Previously, our studies had reported that the activation of  $K_{ATP}$  channels enhanced iron uptake mediated by divalent metal transporter 1 (DMT1) in SK-N-SH cells, implicating an important role of  $K_{ATP}$  channels on the cell membrane hyperpolarization. Furthermore, a decrease in ratio of ATP/ADP and an increase in the production of ROS induce additional  $K_{ATP}$  channels activation in a feed-forward cycle (16). In both PD patients and animal models, neuroscientists found the SUR1 subunit of  $K_{ATP}$  channels was highly expressed in the remaining nigral DAergic neurons, and the SUR1 subunit was involved in  $K_{ATP}$  channels trafficking to the cell membrane (4,5). An increase in the number of functional  $K_{ATP}$  channels which triggered burst firing might compensate the loss of nigrostriatal dopamine-induced by the progressive degenerations of DAergic neurons (17). Nevertheless, the constant burst firing of DAergic neurons triggered by  $K_{ATP}$  channels could lead to excitotoxicity, elevate calcium loading, reduce calcium buffering capacities, cause the ROS production, and a variety of processes which result in detrimental effect to DAergic neurons in a long term (18-20).

In pancreatic  $\beta$ -cells, SUR1 expressions are regulated by many factors, such as Hsp90, insulin, PKA, FOXA1 and FOXA2 (21-23). FOXA1 and FOXA2 are so-called "pioneer proteins", which could facilitate access of other transcription factors by binding to condensed chromatin in promoters and enhancer regions tightly (24). FOXA1 and FOXA2 are members of the winged-helix/fork head transcription factors which play a major role in the development of midbrain DA neurons during the early and late embryonic period (25-27). Moreover, FOXA1 and FOXA2 are also necessary for the maintenance of appropriate firing patterns of SN pars compacta neurons. It has been showed that the burst firing of nigral DAergic neurons is reduced in response to the deletion of FOXA1 and FOXA2 (28). FOXA1 and FOXA2 may regulate the expression of SUR1 subunit of  $K_{ATP}$  channels in DA neurons.

In conclusion, we showed that the mRNA levels of SUR1 but not SUR2B or Kir6.2 were selectively upregulated in MES23.5 cells over-expressed  $\alpha$ -Syn. Therefore, the present study could provide a new evidence for the influence of  $K_{ATP}$  channels in the loss of DAergic neurons in PD.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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