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Research Report

RNA interference targeting α-synuclein attenuates methamphetamine-induced neurotoxicity in SH-SY5Y cells

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ABSTRACT

The protein α-synuclein (α-syn) is abundant in neurons and has been claimed to play critical roles in the pathophysiology of Parkinson's disease. Overexpression of α-syn has been shown to be toxicity in methamphetamine (METH)-induced model in vivo and in vitro which has Parkinson's-like pathology. However, the exact mechanisms underlying toxicity of α-syn mediated METH-induced neuron remain unknown. In the present study, human dopaminergic-like neuroblastoma SH-SY5Y cells were used as METH-induced model in vitro. Cell viability was found to be dramatically increased after silencing α-syn expression followed by METH treatment compared with a-syn wild-type cells and the morphological damage to cells after METH treatment was abated through knockdown of α-syn expression in this model. The expression levels of tyrosine hydroxylase (TH), dopamine transporter (DAT) and vesicular monoamine transporter 2(VMAT-2) were significantly decreased and the activity/levels of reactive oxygen species (ROS), nitric oxide synthase (NOS) and nitrogen (NO) were notably increased after METH treatment. However, the changes of these expression levels were reversed in cells transfected with α-syn-shRNA. These results suggested that TH, DAT, VMAT-2, ROS and NOS maybe involved in α-syn mediated METH-induced neuronal toxicity.

Abbreviations: METH, methamphetamine; α-syn, α-synuclein; LBs, Lewy bodies; DA, dopamine; TH, tyrosine hydroxylase; DAT, dopamine transporter; VMAT-2, vesicular monoamine transporter 2; ROS, reactive oxygen species; NOS, nitric oxide synthase; NO, nitrogen monoxide

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1. Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder that is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of intracellular inclusions called Lewy bodies (LBs) (Schulz and Falkenburger, 2004; Thomas and Beal, 2007). The major protein component of LBs is α-synuclein (α-syn), a synaptic protein with the propensity to misfold and aggregate (Spillantini et al., 1997; Devine et al., 2011). Three missense mutations (A53T, A30P, E46K; Polymeropoulos et al., 1997; Kruger et al., 1998; Zarranz et al., 2004) in the α-syn gene which encodes α-syn have been found to cause an autosomal dominant form of PD. Moreover, duplications (Chartier-Harlin et al., 2004) or triplications (Singleton et al., 2003) of human wild-type α-syn are related to rare familial forms of early-onset PD with a severity proportional to the degree of α-syn overexpression (Eriksen et al., 2005). Although the exact function of α-syn is still unknown, increasing evidences have demonstrated that levels of a-syn protein are important in the pathogenesis of PD. Recently, it has been shown that patients with sporadic PD have increased levels of α-syn mRNA in the midbrain (Chiba-Falek et al., 2006). Furthermore, the age of onset and severity of PD are determined by the degree of a-syn overexpression (Farrer et al., 2004). It also has been found that increased a-syn levels can be toxic both in vitro (Moussa et al., 2004) and in vivo (Yamada et al., 2004). These observations have shown that abnormal structure or elevated levels of a-syn are sufficient to cause the degeneration of dopaminergic neurons. Therefore, therapeutic strategies targeting α-syn reduction may potentially affect the progression of dopaminergic cell death in PD.

METH is a well-known used potent psychostimulant drug of abuse and monoaminergic neurotoxin that can lead to toxicity effect on the dopaminergic nerve terminals and neurons. Increasing reports have shown that repeated high-dose METH administrations result to long-term dopaminergic system deficits, including reductions in dopamine (DA) content, tyrosine hydroxylase (TH) activity, DA transporter (DAT) levels and vesicular monoamine transporter-2 (VMAT-2) levels (Irina and Jean, 2009). Reactive oxygen species (ROS) generated by the elevation of DA auto-oxidization cause the degeneration and decrease of striatal DA neurons during METH-induced DA neuronal toxicity (Pubill et al., 2005). METH increases nitric oxide synthase activity, followed by raised NO production, a necessary step for METH toxicity. The above showed that pathologic change after METH exposure resembled experimental and human Parkinson's disease, so METH-induced cells or animal may be a putative experimental model for drug-induced Parkinsonism.

It has been reported that α-syn expression was increased after METH treatment in animals (Fornai et al., 2005) and cells (Ajjimaporn et al., 2007). α-Syn has been involved in a range of specific cellular activities, including effects on DA synthesis, storage and release and regulations of synaptic vesicle function (Cabin et al., 2002), TH (Perez et al., 2002) and monoamine transporters (Oaks and Sidhu, 2011). All of these indicated that increasing expression of α-syn after METH treatment resulted to neurotoxicity. However, there have no direct evidences to elucidate the effects of α-syn knockdown in the METH-induced death of SH-SY5Y cells. Therefore, the purpose of this study is to evaluate the mechanisms underlying reducing α-syn expression has potential neuroprotective effects in METH-mediated neurotoxicity.

2. Results

2.1. *Inhibitory effect on the expression of α-syn detected by quantitative real-time PCR and western blotting*

In order to get long-term suppression of gene expression, we used pLVTHM vector containing sequence targeting α-syn gene to reduce α-syn expression in SH-SY5Y cells. After transfection and selection, we performed quantitative real-time PCR and western blotting to examine the expression of α-syn in untransfected cells (normal control cells), cells transfected with empty vector (control vector), cells transfected with pLVTHM-α-syn-shRNA-A/B/C respectively. The result showed that, compared with the untransfected, the transfection of α-syn-shRNA-A/B/C vector led to a significant decrease of α-syn mRNA and protein expression in SH-SY5Y cells and the α-syn-shRNA-C inhibited α-syn expression the most effectively. In contrast, no difference was found in the expression of α-syn mRNA and protein between untransfected and transfected with empty vector (Fig. 1).Therefore, these results indicated that the down-regulation of α-syn by RNAi was effective and stable.

2.2. *Knockdown of α-syn abates METH-induced morphological changes*

We analyzed the effect of a-syn knockdown on cell morphologic changes by light microscope. Contrast to the control cells, the morphological changes of SH-SY5Y cells treated with 3.5 mM METH for 24 h were featured by cell shrinkage, dendrite disruption and floatation of cells. In cells transfected with α-syn-shRNA-C, these changes were abated (Fig. 2A).

2.3. *α-syn knockdown enhances cell survival against METH*

In this study, the cells were treated with a suitable concentration (3.5 mM) of METH for 24 h and cell viability was measured using the CCK-8 assay. The result revealed that METH strongly degraded the survival rate of SH-SY5Y cells. As shown in Fig. 2B, after treatment with METH for 24 h, the cell viability of control cells, empty vector transfected cells and α-syn-shRNA-C transfected cells decreased to $67.37 \pm 1.65\%$, $68.55 \pm 1.68\%$ and $91.29 \pm 1.73\%$, respectively. These results suggested that SH-SY5Y cells with down-regulated α-syn expression could enhance cell survival and resist the METH-induced neurotoxicity.

2.4. *Knockdown of α-syn raises DA level*

As shown in Fig. 3, in control cells, 113 ± 7.59 ng/L of DA was detected. In contrast, when cells were exposed to 3.5 mM