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Research report

Microarray analysis of an synthetic α-synuclein induced cellular model reveals the expression profile of long non-coding RNA in Parkinson's disease

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ABSTRACT

Long non-coding RNAs (lncRNAs) are a new research focus that are reported to influence the pathogenetic process of neurodegenerative disorders. To uncover new disease-associated genes and their relevant mechanisms, we carried out a gene microarray analysis based on a Parkinson's disease (PD) *in vitro* model induced by α-synuclein oligomers. This cellular model induced by 25 μmol/L α-synuclein oligomers has been confirmed to show the stable, transmissible neurotoxicity of α-synuclein, a typical PD pathological marker. And several differentially expressed lncRNAs and mRNAs were identified in this model, such as G046036, G030771, AC009365.4, RPS14P3, CTB-11I22.1, and G007549. Subsequent ceRNA analysis determined the potential relationships between these lncRNAs and their associated mRNAs and microRNAs. The results of the present study widen our horizon of PD susceptibility genes and provide new pathways towards efficient diagnostic biomarkers and therapeutic targets for PD.

1. Introduction

Parkinson's disease is a chronic neurodegenerative disorder that is characterized by progressive movement disorder and dysmyotonia. Decades ago, Lewy bodies (LBs), comprising mostly misfolded α-synuclein filament tangles, were found in the substantia nigra of patients with PD, and are regarded as an important pathological maker of Parkinson's disease. As research into α-synuclein progressed, the presence of LBs was noted in certain other neurodegenerative diseases, such as multiple system atrophy (MSA), Lewy body disease, and Alzheimer's disease. As a consequence, some scientists considered such diseases associated with Lewy bodies as "α-synuclein induced diseases" (Fung and Kordower, 2015; Junn et al., 2003; Peelaerts et al., 2015).

In recent decades, to seek efficient therapies for PD, researchers have concentrated on PD's pathogenesis at both the microscopic biological level and the macroscopic anatomy level. Susceptibility genes (such as PAK1, PARKIN, PINK1, and DJ-1) (Garcia et al., 2014; Kim et al., 2016; Norris et al., 2015), oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction (Jeon et al., 2017; Wang et al., 2017; Xiang et al., 2017) and certain other related mechanisms have been proven to be involved in the molecular mechanism of PD. These revelations concerning PD's molecular mechanism were conducive to the discovery of new medicines, such as dopamine (DA) replacement drugs (Cruz-Monteagudo et al., 2016; Hammer et al., 2013; Kaut et al., 2017; Kim et al., 2017). Besides, the precise location and component analysis based on the gross anatomy contributed to therapeutic development and operative treatment of PD (Horn et al., 2017).

Recently, the discovery of long non-coding RNAs (lncRNAs) prompted a new wave of exploration. Disease-associated lncRNAs provided effective reference clinical diagnosis biomarkers, new hypotheses of diseases' pathogenesis, and potential therapeutic targets for the relevant disorders. Even though lncRNAs do not encode any proteins, they have crucial regulatory potential in transcription and post-transcription processing of proteins. Thus, they were marked as "transcriptional noise" (Liang et al., 2016; Liu et al., 2015; Wang et al., 2016). To date, research has shown that lncRNAs could compete with microRNAs for the mRNA binding sites and co-interfere with mRNAs' transcription and translation. Under the conditions of diverse diseases or pathological states,

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certain specific lncRNAs or microRNAs are expressed aberrantly, and the exceptional interaction among lncRNAs, microRNAs, and mRNAs contribute to changes in the mRNA's transcription level, followed by changes in the encoded protein's translation and conformation (Tay et al., 2011). In the present study, we hypothesized that the exploration of differentially expressed lncRNA factors in PD might identify novel biomarkers and therapeutic targets in Parkinson's disease.

Considerable research on chronic disease has been carried out using relevant cellular or animal models (Siracusa et al., 2017). Therefore, we constructed a stable *in vitro* model of PD induced by α-synuclein oligomers for our gene microarray analysis.

Accumulating evidence suggested the feasibility and mechanism of cellular or animal models of Parkinson's disease induced by α-synuclein oligomers, the oligomeric forms of misfolded α-synuclein. In PD patients' substantia nigra, affected dopaminergic neurons die gradually and release much pathological α-synuclein, which has been considered as a key pathogenic agent in the development and spread of PD (Cremades et al., 2017). This aggregating α-synuclein remains in the intercellular space and is passed into other healthy dopaminergic neurons through microtubule transfer, exosome delivery, or receptor-mediated endocytosis (Christensen et al., 2016; Dieriks et al., 2017; Emmanouilidou and Vekrellis, 2016). Notably, recent research revealed that ectogenic α-synuclein could be recognized and received by lymphocyte activating 3 (LAG3), a receptor protein within the cytomembrane, and after which it is translocated into cells (Mao et al., 2016). After the influx of α-synuclein, the cells were induced to secrete much pathogenic phosphorylated α-synuclein. Therefore, abnormally aggregating α-synuclein oligomers and polymers in the cells increased significantly (Hornedo-Ortega et al., 2016).

In this study, we stimulated SY-SH5Y cells with α-synuclein oligomers and detected the stability of this *in vitro* PD model. A subsequent gene microarray experiment was carried out based on this cellular model to explore lncRNA expression profiles and potential biomolecular processes in Parkinson's disease.

2. Results

2.1. Neurotoxicity accumulated in the cells treated by α-synuclein oligomers

When SY-SH5Y cells were stimulated by different doses of α-synuclein oligomers, cell viability was not affected at doses less than 25 μmol/L (Fig. 1A). As the dose of α-synuclein increased, cell viability was inhibited. However, this decreasing tendency was not as obvious as that in the acute toxicity injury model. Subsequently, the identification of phosphorylated α-synuclein in SY-SH5Y cells indicated that the typical Parkinson's disease pathological protein marker was upregulated obviously in the cells induced by 25 μmol/L α-synuclein oligomers (Fig. 1B–F). This phenomenon might be associated with cytotoxicity caused by α-synuclein's abnormal aggregation. Consequently, we hypothesized that it was ectogenic α-synuclein oligomers that lead to excessive secretion of endogenous α-synuclein, which could be misfolded when the balance between its degradation and aggregation was disturbed.

2.2. Ectogenic α-synuclein oligomers pass into cells and contribute to the pathological α-synuclein fiber entanglement and microstructure damage

To reveal the detailed molecular mechanism of the phenomenon mentioned in Section 3.1, we detected the expression of α-synuclein in the cells using quantitative fluorescence and observed that the ectogenic α-synuclein entered cells after 3 h of co-culture (Fig. 2A). The tiny, evenly-distributed fluorescence of the α-synuclein oligomers revealed that the ectogenic α-synuclein passed into the cells smoothly at the early phase of mutual contact between SY-SH5Y cells and the ectogenic α-synuclein. With increasing time, these tiny light-spots dimmed and some small fluorescent conglomerations appeared after the cells were removed from α-synuclein oligomer stimulation after 3 h of co-culture and the fluorescence was observed at 12 h, or the cells were maintained in co-culture until 12 h (Fig. 2B, D). In addition, the well-proportioned fluorescent spots completely faded and were replaced by masses of sporadic cloudy conglomerations when the cells were stimulated by α-synuclein oligomers until 24 h (Fig. 2F). The same effects were observed when the cells were removed from α-synuclein oligomer co-culture after 3 or 12 hand then observed at 24 h (Fig. 2C, E).

Further observation of these sporadic luminous masses by electron microscopy showed that contents of these masses were randomly arranged protein filaments (Fig. 3). We observed that the microstructure of SY-SH5Y cells was disrupted obviously after 24 h of co-culture, with tangled organelles and the occasional autophagosome in the cells (Fig. 3F, G). Taken together, the results showed that the entrance of ectogenic α-synuclein oligomer into cells occurred from the beginning of contact. Under stimulation by the α-synuclein oligomers, the cells were forced to secrete more endogenous α-synuclein. Once the content of α-synuclein increased and reached the limit of the α-synuclein scavenging capacity of the cells, α-synuclein was misfolded and aggregated into a mass of α-synuclein polymers. Thus, more and more α-synuclein was secreted and aggregated as the extraneous α-synuclein oligomers. Ultimately, a large cloud of non-degradable aggregations formed.

2.3. Expression of long non-coding RNAs in PD model induced by α-synuclein oligomer

We confirmed that our *in vitro* model of Parkinson's disease was stable, and then compared the expression profile of lncRNAs and mRNAs in the PD model with that in the control group (with three samples per group) using the Arraystar Human LncRNA Microarray V4.0, which is designed for the global profiling of about 40,173 human LncRNAs and 20,730 human protein-coding transcripts. At a fold change threshold of $\geqq$1.5, and a p-value < .05, we found 53 upregulated lncRNAs and 69 downregulated lncRNAs, as well as 70 differentially expressed mRNAs in the PD model group (Fig. 4).

Among them, 25 higher-fold-change lncRNAs and two particular lncRNAs, G069488 and RP11-142J21.2, drew our attention at first glance (Table 1). Database prediction indicated that G069488 was located close to the gene encoding NEDD9 (neural precursor cell expressed, developmentally down-regulated 9), which is involved in the neuron regeneration and repair process. RP11-142J21.2 was probably associated with the gene encoding SEMA6D (Semaphorin 6D), which plays an important role in the MAPK pathway by conjugated with the cellular membrane protein, PlexinB. Accordingly, we verified their variation trends using qRT-PCR (Fig. 5B). As expected, G069488 was upregulated and its expression of its downstream mRNA, *NEDD9*, was down regulated in the PD model group. For RP11-142J21.2, its down-regulation led to higher expression of its downstream coding gene, *SEMA6D*, in PD model group. The increase in *SEMA6D* might be related induction of the MAPK pathway. Six higher-fold-change lncRNA factors showed consistent trends between the microarray and qRT-PCR: G046036, G030771, AC009365.4, RPS14P3, CTB-11I22.1, G007549 (Fig. 5A). Given that the fluorescent quantitation of G007549 was far below that of the other selected genes, G007549 was excluded from further analysis.