Effect on mitochondrial gene of Parkin

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Abstract

The parkin plays an important part in the maintenance of the mitochondrial function. We studied whether the parkin increases mitochondrial DNA in cells and whether it is involved in the transcription of the mitochondrial gene. Parkin-GFP and BOBO-3 were co-localized in the cytoplasm in proliferating SH-SY5Y cells, indicating an increased amount of mitochondrial DNA. A northern blot study demonstrated that mitochondria-encoded mRNAs were markedly decreased by the siRNA. The present results suggest that parkin enhances the transcription and replication of mitochondrial DNA, leading to the activation of mitochondrial biogenesis.

Keywords: Parkinson's disease mitochondria transcription, replication respiratory chain complex, mitochondria, biogenesis, siRNA

Introduction

We found that the parkin protein had a close relation with mitochondria while a study about PARK 2 was conducted [1-4]. As for the parkin, the localization in cells changes by proliferative state of cells. The parkin is located to mitochondria with the cells of the growth phase, and is isolated from mitochondria for the non-growth phase. The parkin promotes mitochondrial transcription / replication. We reported that the parkin increased mitochondrial membrane potential, and the apoptosis of cells was inhibited [3]. In this study, we studied whether the parkin increases mitochondrial DNA in cells. Furthermore, using siRNA, we considered whether the parkin is involved in the transcription of the mitochondrial gene.

Materials and Methods

Cell culture and reagents

SH-SY5Y and RD were cultured at 37°C (5% CO_2) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-incubated fetal bovine serum (FBS).
To stain mitochondrial DNA, the cells were fixed with 4% paraformaldehyde (PFA) for 30 min, then treated with 0.5% Triton X-100 for 5 min and then with RNase A (50 μg/ml) for 15 min, and fixed with methanol for 3 min. The cells were then incubated with 150 nM BOBO-3 (Molecular Probes), followed by fixation with 4% PFA.

Mutagenesis, cell transfection and RNAi

Full-length parkin cDNA was constructed as previously described (9). Full-length or deletion-mutant parkin cDNA was subcloned into pEGFP-C2 (Clontech, CA). Cells were transfected using EffecteneTM Transfection Reagent (Qiagen, CA, USA) or

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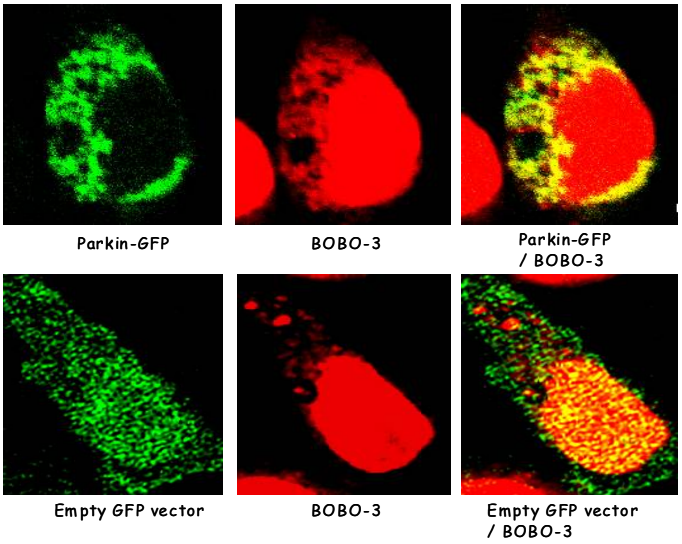

Figure 1. The localization of Parkin-GFP and BOBO-3 in proliferating SH-SY5Y cells. Parkin-GFP and BOBO-3 were co-localized in the cytoplasm.

LipofectamineTM 2000 (Invitrogen Corp.). A morphological study analysis was performed 48 h after transfection. Northern blot analysis was performed 24 h after transfection. Synthesized 21-mer dsRNA with a 2-base overhang corresponding to the parkin coding region 847-865 (Acc. No. AB009973) was purchased from Dharmacon Research (Lafayette, CO, USA). SH-SY5Y cells were cultured in 6-well plates, and the targeting siRNA duplex and scrambled siRNA duplex (Dharmacon Research) were transfected with LipofectamineTM 2000. The effects of siRNA were evaluated 12 to 30 h after transfection of siRNA duplex, 0.2 μg/well (15 nM) and 2.0 μg/well (150 nM).

Northern blot analyses

We prepared four mitochondrial cDNA probes for ND 3-4 (complex, nt 10125-11332), cytochrome b (complex 3, nt 14744-15678), COX 1-2 (complex 4, nt 7059-8190) and COX 3-ATPase 6 /8 (complex 4-5, nt 8290-9550) and three nuclear cDNA probes for COX 5a (Rizzuto et al., 1988)(complex 4), β-actin, and 18s rRNA. Ten μg of RNA were applied per lane in Northern blotting, respectively.

Results

Parkin overexpression enhances mitochondrial transcription and replication

Since we successfully stained both mitochondrial and nuclear DNAs with a DNA-specific probe, BOBO-3, in situ replication of mitochondrial DNA was morphologically analyzed using this probe. As shown in Figure 1, parkin-GFP and BOBO-3 were co-localized in the cytoplasm in proliferating SH-SY5Y cells, indicating an increased number of mitochondrial DNA. In cells transfected with an empty GFP vector, there was little co-localization of the parkin and mitochondrial DNA in the cytoplasm.

Influence of the silencing of an endogenous parkin gene on mitochondrial biogenesis

Figure 1a shows the result that the expression of the parkin was inhibited by siRNA (15nM and 150nM) in SH-5Y5Y and RD cells. An immunoblotting study demonstrated that parkin expression was more inhibited concentration-dependently as compared with scramble RNA induction. On the other hand, the expression of β-actin did not change in any of the lanes. As shown in Figure 2b, the Northern blot study demonstrated that mitochondria-encoded mRNAs were markedly decreased by the

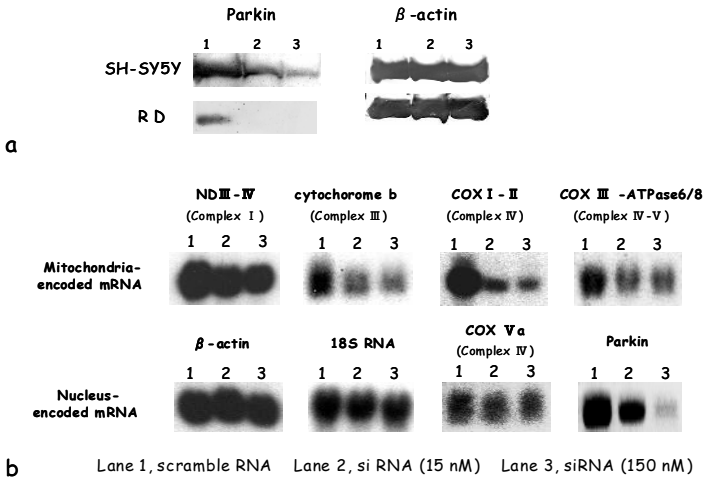

Figure 2. a. Immunoblotting study of parkin and ß-actin in SH-5Y5Y and RD cells. The expression of parkin was inhibited by siRNA (15nM and 150 nM). b. Northern blotting study of mitochondria-encoded mRNAs and nucleus-codes mRNAs. Mitochondria-encoded mRNAs were markedly decreased by the siRNA.

siRNA. On the other hand, the change was not found in gene (COXVa) encoded by β-actin, 18S rRNA, a nucleus.

Discussion

The parkin protein has ubiquitin ligase (E3) activity. When a parkin gene mutates, a fall in the E3 activity occurs. As a result, it is thought that the selective degeneration of nigral
dopamine-producing cells develops because a harmful substrate accumulates
[5,6]. On the other hand, the involvement of mitochondrial disorder has attracted attention in regard to parkin gene defects. The parkin conducts mitochondrial quality control by taking a central role in mitochondrial autophagy (mitophagy) [7,8]. The parkin is located to cytoplasm, but is released to mitochondria due to oxidative stress [9]. In contrast, we reported that parkin promoted mitochondrial transcription/replication [3] and that the parkin increased mitochondrial membrane potential, and the apoptosis of cells was inhibited [4]. On the other hand, there is a report that the parkin is localized in mitochondria, and that it promotes mitochondrial transcription/replication by binding to a mitochondrial gene [10]. There is a report that the parkin increases the mitochondrial membrane potential, and inhibits apoptosis [11]. The present results suggest that the parkin enhances transcription and replication of mitochondrial DNA, leading to the activation of mitochondrial biogenesis, which is a prerequisite for cell proliferation. The parkin plays an important role, at least in the maintenance of the mitochondrial function.

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