Effect of oxidative stress on splicing of parkin gene

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Abstract

It is well known that reactive oxygen species play an important role in the development of sporadic Parkinson's disease. Parkin gene reportedly forms splicing variants in neuronal cells. In this paper, we investigate whether abnormal splicing of the parkin gene is caused by oxidative stress. Hydrogen peroxide solution was added to neuron cultures (SY-SY5Y) and then parkin mRNAs expressed by the cells were subjected to RT-PCR amplification. The PCR products were cloned and the nature of the splicing was examined using sequence analysis. After the addition of hydrogen peroxide solution, RT-PCR analysis showed the formation of parkin mRNAs that were shorter than the wild type. The findings suggest that abnormal splicing of the parkin gene is induced by oxidative stress.

Key Words: Reactive oxygen species, Parkinson's disease, Splicing

Introduction

Parkinson's disease is a representative neurodegenerative disease. Most of the time, the disease is isolated. The selective degeneration of nigral dopamine-producing cells has been observed. The involvement of mitochondrial functional disorder of the cells in Parkinson's disease is assumed. However, the cause and mechanism of the mitochondrial disorder are still unknown. On the other hand, in recent years, genes that cause hereditary Parkinson's disease were discovered in quick succession. Pathologic elucidation of isolated Parkinson's disease is attempted through the analysis of these genes. Parkin (PARK 2) was identified as the gene that is most frequently responsible for familial Parkinson's disease [1].

The parkin protein has ubiquitin ligase (E3) activity, and a reduction in E3 activity occurs owing to the mutation of the parkin gene. A mechanism to develop selective degeneration of nigral dopamine-producing cells because of their accumulation of harmful substrates was estimated [2, 3]. On the other hand, in recent years, the association of mitochondrial disorder with parkin gene defect has attracted attention. With regard to the condition of patients with various kinds of familial Parkinson's disease, the key point is whether symptoms are similar to the condition of a patient with isolated Parkinson's disease or one with mitochondrial disorder [4-6]. We found that parkin protein is closely associated with mitochondria in a study on hereditary Parkinson's disease, in particular PARK 2 [7-9]. Parkin was located in mitochondria in cells in growth phase, and it was reported that it promoted mitochondrial transcription / replication [10]. On the other hand, qualitative abnormality of the parkin protein may occur in patients with isolated Parkinson's disease without a deletion in the parkin gene. In other words, parkin protein is S-nitrosylated and may lose its function, resulting in the development of Parkinson's disease [11-13]. On the basis of these findings, we examined whether splicing disorder of the parkin gene occurred owing to oxidative stress.

Materials and Methods / Results

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Cell culture and reagents

PC -12, 10% of COS-1, SH-SY5Y, RD, and L6 cells were cultured at 37 ℃ (5% CO_2) in Dulbecco's modified Eagle's medium (DMEM) supplemented with (v/v) heat-incubated fetal bovine serum (FBS). Cells were partly treated with 200 uM H_2O_2 for 24 hr.

RT-PCR and Sequencing

Primers at exons 1 and 6 {forward primer 5′-atg ata gtg ttt gtc agg ttc aac tcc-3′ (nt102-125)}, exons 2 and 9 { forward primer 5′-ctg agg aat gac tgg act g-3′ (nt249-267) reverse primer 5′-aca ctc ctc tgc acc ata ctg-3′(nt1051-1069)}, exons 9 and 12 { forward primer 5′-cag tat ggt gca gtg gag tgt-3′(nt1051-1069), reverse primer 5′-cta cac gtc gaa cca gtg gtc ccc-3′(nt1476-1499)}(GenBank AB009973) were used. A 25-ul mixture for polymerase chain reaction (PCR) contained 50 ng of cDNA, 2.5 ul of 10xPCR buffer (Takara), 2 ul of 2.5 mM dNTP (Takara, Tokyo, Japan), 1.25 ul of each primer (10 pmol/ul), and 0.125 ul Ex Taq DNA polymerase (Takara). Thermal cycler (Takara) parameters were 33 cycles at 95℃for 30 s, 60℃for 2 min, 72℃for 3 min, with preheating at 95℃for 5 min and a final extension at 72℃for 7 min. Electrophoretic separation of RT-PCR fragments was performed on ethidium bromide-stained 1.5% agarose gels (Wako, Tokyo, Japan). The PCR fragments were purified from gel using Gel Extraction Kit (Qiagen, CA, USA). The fragments were cloned using TOPO TA Cloning Kit (Invitrogen, CA, USA) and DH5α （Toyobo, Tokyo, Japan）. Then, the fragments were subjected to sequence analysis using automated DNA sequencers (Applied Biosystems, CA, USA).

Cell Viability Assay

Cell viability was assessed using Quanti-BlueTM Cell Viability Assay Kits (BioAssay Systems, CA, USA) using 96-well plates. The fluorescence intensity (excitation wavelength=530-570 nm, emission wavelength = 590-620 nm) was measured in an Infinite 200 (Tecan, AG, Switzerland).

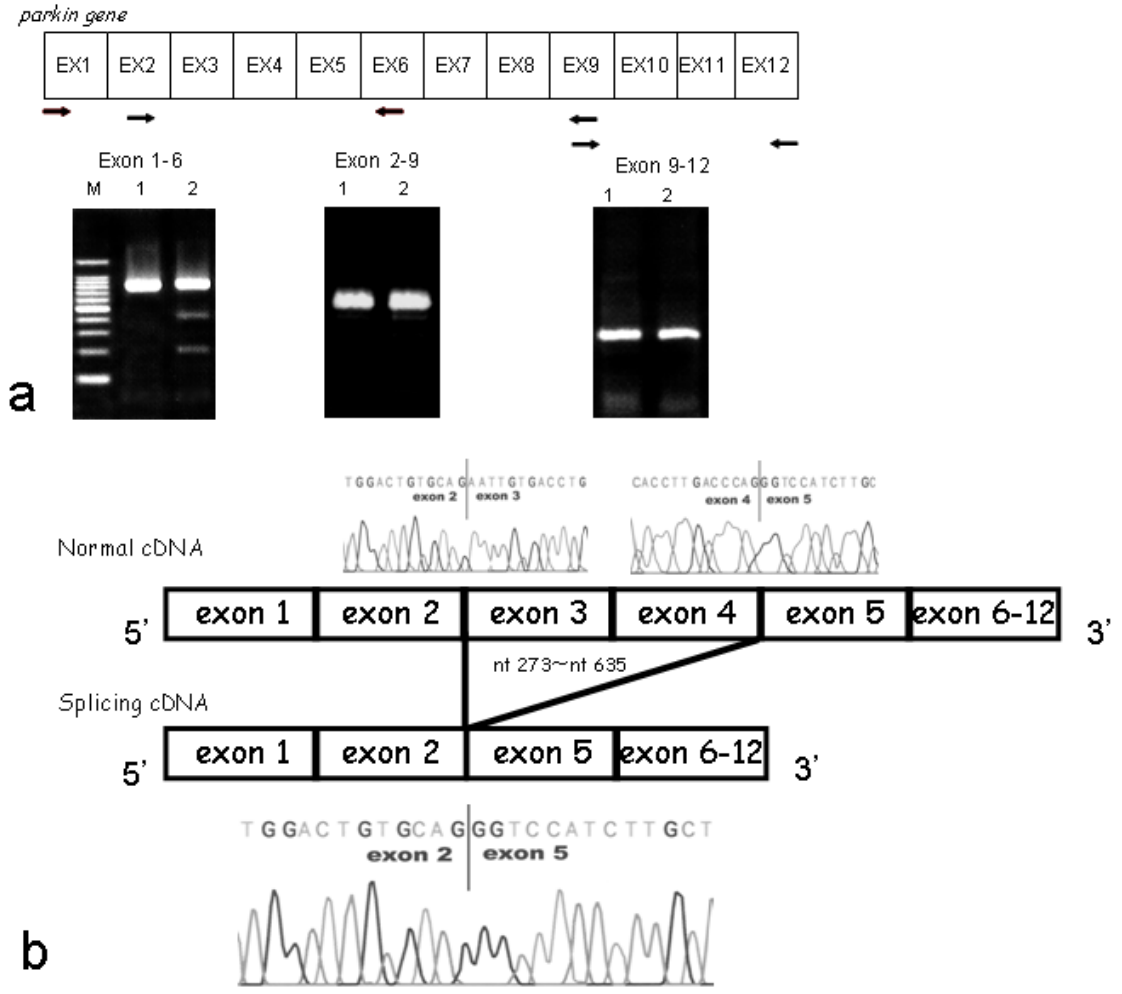

Figure 1. Effect of H_2O_2 on parkin mRNA. **a**, RT-PCR analysis of the parkin gene in cells with (lane 2) and without H_2O_2 treatment. Lane 1, no treatment; lane 2, H_2O_2 treatment. **b**, Sequence analysis of exons 1-6.

Results

Figure 1 shows the effect of H_2O_2 on parkin mRNA in cells without (lane 1) or with (lane 2) H_2O_2 treatment. RT-PCR analysis results of the parkin gene are shown in Figure 1a. When cells were treated with H_2O_2, RT-PCR analysis of exons 1-6 revealed two additional bands in lane 2 that were smaller than the main band. The main band of lane 2 showed a similar size to that of lane 1. The main fragment was confirmed to have a normal sequence corresponding to exons 1-6. Sequence analysis of the additional band with an intermediate size in lane 2 revealed a truncated fragment of exons 1-6 lacking exons 3-4 (Figure 1b).

The effect of H_2O_2 treatment was confirmed by cell viability assay. The result of the cell viability assay using PC-12 cells revealed that the count of viable cells with H_2O_2 treatment (200 uM, 24 h) was significantly decreased compared with that of non-treated cells (Figure 2). When various kinds of cells were examined, we obtained similar results (data not shown).

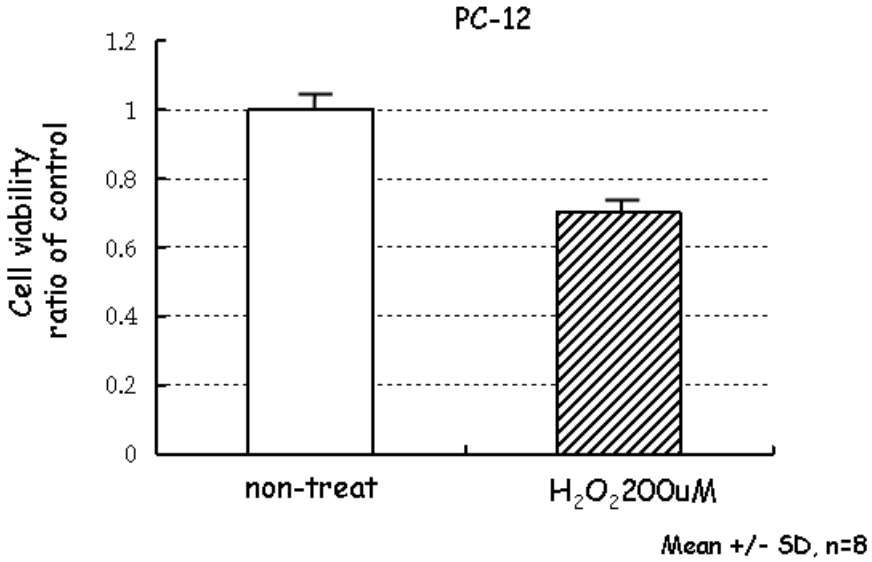

Figure 2. The effect of H_2O_2 treatment (200 uM, 24 h) on cell viability of PC-12 cells.

Discussion

Because parkin mRNA with a truncated form was formed in addition to the wild type when cultured cells were subjected to oxidative stress through hydrogen peroxide addition, it was thought that splicing disorder of the parkin gene had occurred. Parkin functions from a gene structure as ubiquitin ligase (E3), and it can definitely cause ER stress in a mutated form. However, it is still controversial whether parkin gene defect causes a reduction in the E3 activity of the parkin. Mitochondrial disorder is associated with parkin gene defect. Parkin non-specifically improves for a mitochondrial disorder. Parkin is associated with the transcription product of the gene responsible for other hereditary Parkinson's diseases in causing mitochondrial disorders. These findings suggest that parkin has an important role in mitochondrial function. When the parkin gene does not contain any abnormalities, it was reported that Parkinson's disease may develop by qualitatively abnormal S-nitrosylation of the parkin protein (11). Furthermore, the present results suggest that oxidative stress may induce splicing disorder of the parkin gene. Therefore, even in the absence of a defect in the parkin gene, the qualitative thing that could cause the above of the parkin protein was estimated.

References

1. Kitada T, Asakawa S, Hattori N, Masumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998; 392: 605–608.

2. Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R. An unfolded putative transmembrane polypeptide, which can lead to endoplasmin reticulum stress, is a substrate of Parkin. Cell 2001; 105: 891–902.

3. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N., Iwai K, Chiba T, Tanaka K et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000; 25, 302–305.

4. Abou-Sleiman PM, Muqit MM., Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. Nat Rev Neurosci 2006; 7: 207-19.

5. Wood-Kaczmar A, Gandhi S, Wood NW. Understanding the molecular causes of Parkinson's disease. Trends Mol Med 2006; 12: 521-8.

6. Klein C, Schlossmacher MG. The genetics of Parkinson disease: Implications for neurological care. Nat Clin Pract Neurol 2006; 2: 136-46.

7. Kunishige M, Mitsui T, Kuroda Y, Yoshida S, Kosaka M, Matsumoto T. Expanding phenotype and clinical heterogeneity in patients with identical mutation of the parkin gene. Eur Neurol 2004;51: 183-185.

8. Kuroda Y, Mitsui T. Parkin gene related neuronal multisystem disorder. J Neurol Neurosurg Psychiatry 2002;72: 419-420.

9. Kuroda Y, Mitsui T, Akaike M, Azuma H, Matsumoto T.. Homozygous deletion mutation of the parkin gene in patients with atypical parkinsonism. J Neurol Neurosurg Psychiatry 2001;71: 231-234.

10. Kuroda Y., Mitsui T., Kunishige M., Shono M., Akaike M., Azuma H., and Matsumoto T. Parkin enhances mitochondrial biogenesis in proliferating cells. Hum Mol Genet 2006; 15: 883-895.

11. Chung KK , Thomas B, Li X, Pletnikova O, Troncoso JC, Marsh L, Dawson VL, Dawson TM. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. Science. 2004; 304: 1328-31.

12. Yao D, Gu Z, Nakamura T, Shi ZQ, Ma Y, Gaston B, Palmer LA, Rockenstein EM, Zhang Z, Masliah E, Uehara T, Lipton SA. Nitrosative stress linked to sporadic Parkinson's disease: S-nitrosylation of parkin regulates its E3 ubiquitin ligase activity. Proc Natl Acad Sci USA 2004; 101: 10810-10814.