Parkin Gene analysis in Tokushima National Hospital

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Abstract

In the present study, we report the situation regarding Parkin genetic analysis in Tokushima National Hospital. PCR analysis has been completed for 284 patients. The mutation of the Parkin gene was found in ten cases in total: six patients with Exon3.4 deletion, one patient with Exon3 deletion, one patient with Exon10 deletion, and one patient with Exon4 deletion. A skin biopsy was performed in four of these cases. Elucidation of the cause and pathogenesis of Parkinson's disease may lead to the development of new treatments, and analysis of the genes PARK2 and Klokin 1 will take place in the future.

Keywords: Parkin, Klokin 1, mitochondria, biogenesis, PCR, biopsy

Introduction

Parkinson's disease (PD) is a prevalent age-associated progressive neurodegenerative movement disorder primarily characterized by the death of nigrostriatal dopaminergic neurons and the presence of intracytoplasmic proteinaceous inclusions, termed Lewy bodies. Although PD is a sporadic disorder of unclear etiology, recent studies have demonstrated the importance of genetic contributions to PD that may provide insights into the mechanistic details of the disease pathogenesis. Mutation of the gene encoding Parkin (PARK2) plays a major etiopathogenic role in autosomal recessive juvenile parkinsonism [1]. PARK2 contains RING finger motifs and functions as a ubiquitin–protein ligase for protein degradation [2,3].

In the present study, we report on situation regarding Parkin genetic analysis in Tokushima National Hospital.

Materials and Methods

Subjects and Sample Collection

Patients with Parkinson's disease that reputation examined our hospital as for the subject. Details of age at onset, the presence or absence of family history, presence or absence of consanguineous marriage, and information on neurologic symptoms were gathered. Healthy volunteers were used as control subjects.

Ethical Proceeding

This study was carried out after approval from the Tokushima National Hospital

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Ethical Review Board.

DNA Isolation

Peripheral blood mononuclear cell (PBMC) was isolated from fresh whole blood with heparin. Total DNA in PBMCs was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany) following the manufacturer's instructions.

PCR

All PCR amplifications were performed in a TaKara PCR Thermal cycler (TaKaRa Inc., Japan) in standard mixtures of 25 μL containing 1× PCR buffer, 10 pmol of each primer, 2.5 nM dNTP, and 2.5 U of Taq DNA polymerase, The PCR program included one incubation at 95 °C for 5 min and 40 amplification cycles (95 °C for 60 s,53 °C for 60 s and 72 °C for 60 s), followed by one final extension incubation of 7 min at 72 °C. The PCR products were separated on a 1.7% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. Primer sets are shown in Table 1.

Biopsy

Skin biopsies were obtained from three patients (mean age±SD: 62.25±3.75) and from a control subject.

Table 1. Primer sets of PCR analysis of Parkin gene

Primer sequences and sizes of expected PCR products				
Exon	primer	Forward(5'-3')	Reverse(5'-3')	product size(bp)
1	EX1	GCGCGGCTGGCGCCGCTGCGCGCA	GCGGCGCAGAGAGGCTGTAC	112
2	EX2	ATGTTGCTATCACCATTTAAGGG	AGATTGGCAGCGCAGGCGGCATG	308
3	EX3	ACATGTCACTTTTGCTTCCCT	AGGCCATGCTCCATGCAGACTGC	427
4	EX4inner	AGGTAGATCAATCTACAACAGCT	CAGGGTCAAGGTGAGCGTTGCCTGC	121
4	EX4outer	ACAAGCTTTTAAAGAGTTTCTTGT	AGGCAATGTGTTAGTACACA	261
5	EX5	ACATGTCTTAAGGAGTACATTT	TCTCTAATTTCCTGGCAAACAGTG	227
6	EX6	AGAGATTGTTTACTGTGGAAACA	GAGTGATGCTATTTTTAGATCCT	268
7	J-17inner	GAGCCCCGTCCTGGTTTCC	CCACACAAGGCAGGGAGTAGCCAA	137
7	J-17outer	TGCCTTTCCACACTGACAGGTACT	TCTGTTCTTCCATTAGCATTAGAGA	239
8	EX8	TGATAGTCATAACTGTGTGTAAG	ATCGTCTCATTAGCGTCTATCTT	206
9	EX9	GGGTGAAATTTGCAGTCAGT	AATATAATCCCAGCCCATGTGCA	278
10	EX10	ATTGCCAAATGCAACCTMTGTC	TTGGAGGAATGAGTAGGGCATT	165
11	EX11	ACAGGGAACATAAACTCTGACC	CAACACACCAGGCACCTTCAGA	303
12	EX12	GTTTGGGAATGCGTGTTTT	AGAATTAGAAAATGAAGGTAGACA	255

Table 2. Patients with Parkin gene mutation

Age	Sex	PARK2 Mutation	sample	
			PBMC	fibrobrast blood drawing date
61	M	deletion Exons3-4	○	●（2010/8/20）
63	F	deletion Exons3-4	○	×
64	M	deletion Exons3-4	○	●（2010/11/24）
70	M	deletion Exons3-4	○	×
63	M	deletion Exon10	○	×
71	M	deletion Exons3-4	○	×
68	F	deletion Exons3-4	○	×
65	M	deletion Exon3	○	×
61	F	deletion Exon4	○	×
55	F	deletion Exons3-4	○	●（2011/2/2）
67	F	deletion Exon4	○	●（2011/6/16）

Results

PCR analysis was completed for 284 patients. The mutation of the Parkin gene was found in ten cases in total (Table 2): six patients with Exon3.4 deletion, one patient with Exon3 deletion, one patient with Exon10 deletion, and one patient with Exon4 deletion. A skin biopsy was performed in four of these cases.

Discussion

Recently, important insights have been obtained into the mechanism by which Parkin regulates mitochondrial homeostasis. Parkin translocates from the cytoplasm to accumulate on depolarized mitochondria and promotes their degradation by autophagy [4]. Several studies have suggested that PTEN-induced putative kinase 1 (PINK1) is required for Parkin-mediated mitochondrial autophagy, wherein it recruits Parkin to dysfunctional mitochondria and promotes their degradation [5-7]. However, we and others have reported that Parkin can associate directly with mitochondria under basal conditions [8-10]. Recent studies have detected Parkin in the mitochondria of untreated cultured cells, although it is mainly present in the cytoplasm [5,10-12]. We previously reported that Parkin is localized in the mitochondrial matrix during proliferation and is rapidly released to the cytosol in differentiated or quiescent states. We also found that Parkin enhances mitochondrial transcription and replication in vitro and in vivo [9], which was confirmed by a recent study [10].

We reported that Parkin was present in both the cytoplasm and mitochondria in basal conditions and that its intracellular localization changes with growth phase. Parkin was mainly located in the cytoplasm

from the lag growth phase to the early log phase, but a portion of Parkin appeared to be located in the mitochondria from the late log growth phase to the plateau phase. Unlike previous reports, its mitochondrial localization was not associated with reduced membrane potential during the log growth phase. We found a novel protein—Klokin 1—that transports Parkin to the mitochondria. Klokin 1, a splice variant of human chondroitin polymerizing factor (ChPF), may attenuate cellular apoptosis with or without Parkin. The analysis of the PARK2 gene may lead to elucidation of the condition of patients with Parkinson's disease and development of a new treatment. Research in this regard should be pushed forward in future.

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