Examination of Cell Viability using the primary culture system of skin fibroblasts

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Abstract

We carried out a fibroblastic primary culture from a biopsy skin tissue of the person for a functional analysis of the parkin protein. Biopsy specimens were obtained from a patient with parkin gene mutation and a normal control. In this study, a Cell Viability assay was conducted using the first fibroblasts. The results of the present study demonstrated that a viable cell count decreased in the patients with PARK2 as compared with the control. It was suggested that the parkin had an antiapoptosis effect.

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

Introduction

Genetically modified mice are generally used for the molecular pathologic study of hereditary nervous diseases. However, in some inherited diseases, mice with a gene mutation similar to humans may not develop a disease. In this case, the molecular biological approach is impaired. The researcher may carry out a primary culture from histology obtained from patients with inherited disease. The parkin was discovered as the responsible gene (PARK 2) of familial Parkinson's disease to occur most frequently [1]. We carried out a fibroblastic primary culture
from a biopsy skin tissue of the person for a functional analysis of the parkin
protein.

Materials and Methods

Skin biopsy

The following biopsy procedure was used. In brief, a 4-mm diameter plug of skin was excised with a stainless steel punch and scissors from the anterior forearm 1-2 inches below the elbow crease, using xylocaine anesthesia without epinephrine. A full thickness one-quarter portion of the biopsy was divided with a sharp scalpel blade. The procedure involved mincing of the skin with fine scissors followed by sequential incubation with trypsin and DNase, and filtering through a sterile nylon mesh of 55 um pore size. Cells in the resulting filtrate were counted and 1,000-5,000 cells were plated into 100 x 20-mm Petri dishes. Attempts were also made to separate the

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epidermis from the dermis, (the latter being the precursor layer for cultured fibroblasts) to reduce the number of "background" epidermal cells, which would not contribute to colony formation under these conditions. Skin fragments were digested with collagenase and the epidermal layers removed with forceps under a dissecting microscope.
Biopsy specimens were obtained from Patient 1 with PARK2 (62 y, man) and a volunteer (32 y, woman). This study received the approval of the Tokushima National Hospital Ethical Review Board. The purpose of the study was explained to the study patients, and we obtained their consent.

Cell culture

Fibroblasts were cultured in Rat Fibroblast Growth Medium(cell application) with Rat Fibroblast Growth supplement (Cell application) and 1% penicillin-streptomycin (GIBCO BRL) at 37℃, 5%CO_2.

Nucleofection

4-5 x 10^5 cells were resuspended in 100ul of Human Dermal Fibroblast Nucleofector Kit Solution (Amaxa, Gaithersburg, MD) at room temperature. After 24hr, scramble RNA diluted in dH_2O, was transferred into the nucleofection cuvette. The cell suspension was added and nucleofection was performed as per the manufacturer's instructions (electrical setting U-023)[2]. Immediately after pulsing, the cells were transferred to pre-warmed culture medium and plated on gelatin-coated six-well plates. After 48hr, the cells were harvested and processed to assess cell viability.

Cell Viability Assay

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

Results

Fibroblast Culture

Fibroblasts multiplied from the primary culture in approximately one week, and groups of keratinocytes increased. On the other hand, the keratinocytes differed subsequently, and the increase stopped. Passage was performed two weeks later. After several passages, the cell groups became only fibroblasts.

Cell Viability Assay

Fibroblasts from Patient 1 and a control subject were assessed by cell viability. As shown in Figure 1, the viable cell count derived from the patient significantly decreased as compared with the healthy control ($p<0.05$).

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 occurs because a harmful substrate accumulates [3,4]. On the other hand, the participation of the mitochondrial disorder has attracted attention in the parkin gene defect. The parkin conducts mitochondrial quality control by taking a central role in mitochondrial autophagy (mitophagy) [5,6]. The parkin is located to cytoplasm, but is released to the mitochondria with oxidative stress [7]. In contrast, we reported that parkin promoted mitochondrial transcription/replication [8] and that parkin increased the mitochondrial membrane potential, and the apoptosis of cells was inhibited [9].
In recent years, genes responsible for hereditary Parkinson's disease have been discovered in succession. Pathologic elucidation of idiopathic Parkinson's disease has been attempted through analysis of these genes. For a molecular pathologic study of the parkin defect, parkin

knockout mice have been generated. However, it is known that this type of mouse does not develop parkinsonism as in humans [10]. We planned to establish a primary culture system from the skin of patients with parkin gene mutation to avoid this problem. In this study, a cell viability assay was conducted using the first fibroblasts. The results showed that the viable cell count decreased in the patients with PARK2 as compared with the control. It was suggested that the parkin had an antiapoptosis effect.

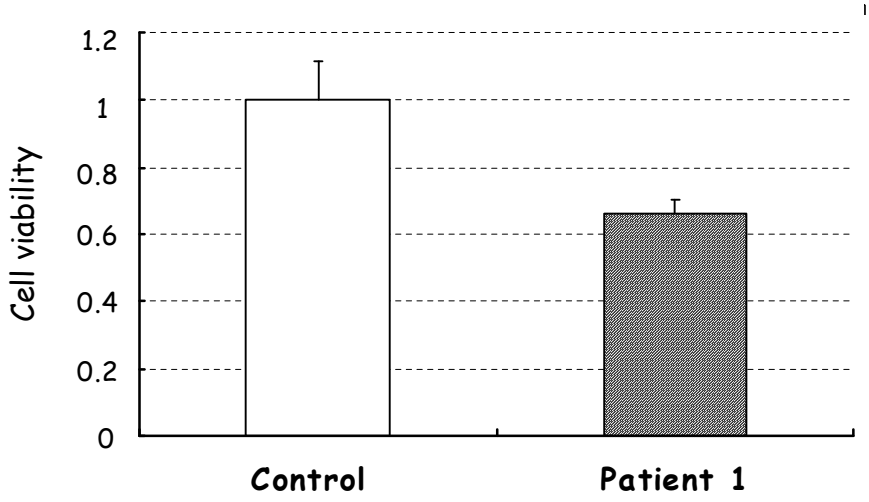

Figure 1. Cell Viability Assay of fibroblasts from Patient 1 and a control subject. The viable cell count derived from the patient significantly decreased as compared with the healthy control ($p < 0.05$).

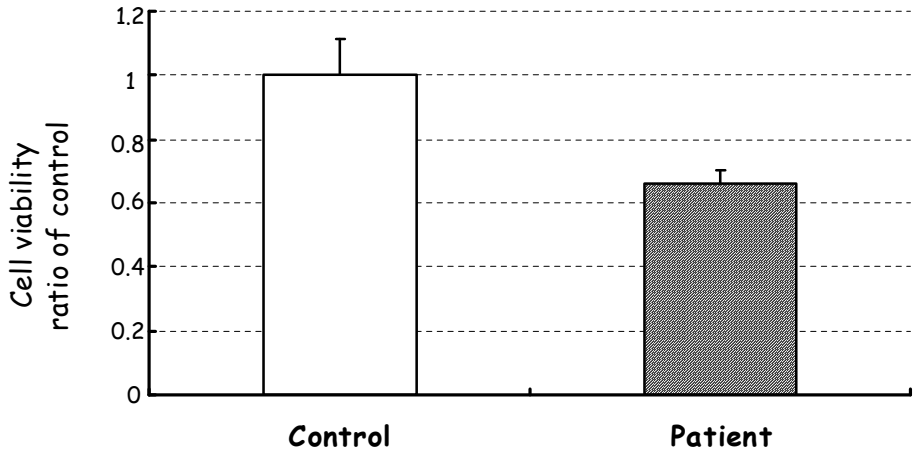

Figure 2. The viable cell count of cultured fibroblasts derived from a patient with PARK2 (patient1) and a normal control.

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