The role of the study assistant in Tokushima National Hospital

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Introduction

We conduct clinical studies on neuromuscular diseases and fundamental research in Tokushima National Hospital. In the clinical research department, which I belong to, study of the molecular mechanisms of neurodegenerative diseases including Parkinson's disease is conducted as fundamental research. In recent years we conducted a functional analysis of Parkin. We proved that Parkin was located in the mitochondria of the cells in the cell growth phase and protected from apoptosis. We identified Klokin 1 protein as a related protein of Parkin. The study duties that I am engaged in as an assistant are reported.

There are three duties that I am engaged in, as follows.

1) Genetic screening

I separate DNA from the peripheral blood of patients with suspected hereditary Parkinson's disease. I conduct genetic PCR analysis and sequence analysis of the Parkin gene and 1 Klokin gene.

2) Real-time PCR

I assay the Parkin mRNA to examine the transcription control of the Parkin gene.

3) Luciferase assay method

I carry out luciferase assays to measure the activity of the transcription control factor of Parkin.

Materials and Methods

Materials and reagent

Halo-tagged OGT, GalNAc T, B3 GALNT2 and B4 GALNT1 were supplied by Kazusa DNA Research Institute (Chiba, Japan). Vectors were transfected COS-1 cells using LipofectamineTM LTX Reagent (Life Technologies Corp.). ChPF$^{\triangle 996}$, ChPF and Klokin 1 constructed in pcDNA4/HisMax(Invitrogen) were reported our previous paper [1,2].

Cell culture

COS-1 (a cell line derived from kidney cells) was cultured at 37°C (5% CO_2) in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-incubated fetal bovine serum (FBS) [1].

Construction of luciferase vector

Human genomic DNA isolated from PBMC was used as the template. The forward and reverse primers were used for amplification of the promoter region. PCR amplifications were performed in a TaKara PCR Thermal cycler (TaKaRa Inc., Japan) in standard mixtures of 25 μL containing 2× GC buffer I, 10 pmol of each primer, 2.5 nM dNTP, 2.5 U of LA 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. PCR product and the pGL 4.10[luc 2] vector (Promega KK, Japan Tokyo) were digested with Xho I and Bgl Ⅱ (Takara) and purified (Qiagen), according to the manufacturers' instructions. Vector arms

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were ligated to digested PCR fragments [3,4].

Real-time PCR

Parkin mRNA was quantified by real-time PCR in COS1 cells transfected with several plasmids.

Luciferase Assay

COS-1 cells were plated in 6-well plates at $1x10^5$ cells per well. The next day, the cells were transfected with 4ug pGL4.10-Parkin vector plasmid and pGL4.74 vector. Cells were harvested 24h after transfection. Luciferase activities were measured using PicaGene Luminescence Kit (WAKO Japan) according to the manufacturer's protocol [3,4].

Results

A genetic analysis

A genetic analysis database in our hospital was created. To date, the genetic analysis of 329 patients has been conducted.

Real-time PCR

The results of Parkin mRNA assays are shown in figure 1 . The expression 1,000 times greater than the control (lane C) was found in the lane of the positive control (lane 1).

Luciferase Assay

The results of a luciferase assay performed to exam the transcription control of the Parkin are shown in figure 2.The positive control (lane 1) showed activity four times greater than the control lane (C).

Discussion

My aim in my everyday duties is to achieve simple economy. I make apparatus and repair broken apparatus, if possible. I have made various account books to plan how to improve the efficiency of my duties. I described the safekeeping place of the sample in an account book. To date the genetic analysis of 329 patients has been conducted. I have participated positively in a technical class and utilized the tech support of the reagent supplier frequently. I have learned about real-time PCR and luciferase assay technology through these efforts. I hope that our work at the hospital will ultimately lead to pathologic elucidation of Parkinson's disease and the development of new therapies.

References

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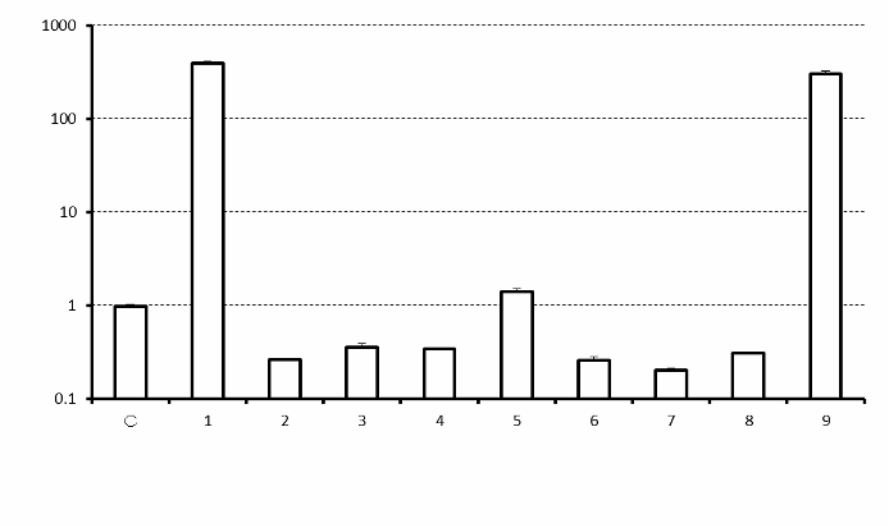

Figure 1. The results of real-time PCR for Parkin mRNA. The expression 1,000 times greater than the control (lane C) was found in the lane of the positive control (lane 1).

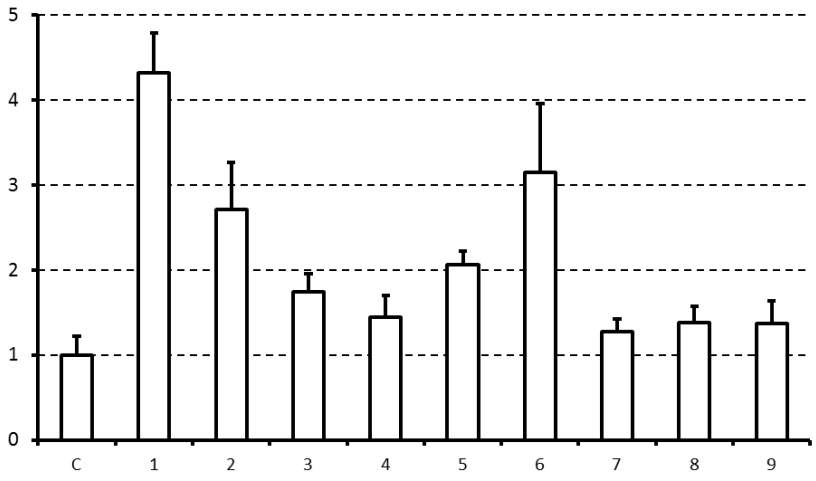

Figure 2. Results of luciferase assay for examination of the transcription control of Parkin. The positive control (lane 1) showed activity four times greater than the control lane (C).