Luciferase assay technology and the application

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Abstract

We found Klokin 1, which binds to parkin and transports parkin to the mitochondria. Klokin 1 is a splice variant of Chondroitin Polymerizing Factor (ChPF). We found that ChPF increases the mRNA of the parkin. We considered whether ChPF promoted transcription of the parkin. We examined the transcription of parkin using the luciferase assay method.

Keywords: Luciferase Assay, Parkin, Klokin 1, Promoter

Introduction

We studied the association with mitochondria of parkin. Klokin 1 binds to parkin, and parkin is carried to the mitochondria. Klokin 1 is a variant of Chondroitin Polymerizing Factor (ChPF). ChPF was found to increase the mRNA of parkin. We planned to investigate whether ChPF promoted transcription of parkin. We analyzed transcription of parkin using the luciferase assay method.

Materials and Methods

We chose a promoter domain of the parkin [1] (Figure 1). We purchased pGL4.10[luc 2] Vector (Promega KK, Japan Tokyo), and a promoter domain was inserted (Figure 2).
We used the COS-1 cells in which manifestation of the parkin protein was high. Halo-tagged OGT, GalNAc T, B3 GALNT2 and B4 GALNT1 were supplied from Kazusa DNA Research Institute (Chiba, Japan). Vectors were transfected COS-1 cells using LipofectamineTM LTX Reagent (Life Technologies Corp.). ChPF⊿996, ChPF and Klokin 1 constructed in pcDNA4/HisMax (Invitrogen) were reported in our earlier paper [2,3]. pGV-C2 Vector (WAKO, Japan) was used as the Luciferase Vector Control. 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) [2]. Human genomic DNA isolated from PBMC was used as the template. The forward and reverse primers were used for amplification of the promoter region (Table1). PCR amplifications were performed in a TaKara PCR Thermal cycler (TaKaRa Inc., Japan) in standard mixtures of 25μL 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 visualised 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)

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and purified (Qiagen), according to the manufacturers' instructions. Vector arms were ligated to digested PCR fragments1.4.
A vector map is shown in Figure 2. 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 after 24h of transfection. Luciferase activities were measured using a PicaGene Luminescence Kit (WAKO Japan) according to the manufacturer's protocol [1-4].

Results and discussion

We were able to measure in pGV-C2 Control Vector. However, the objective sample could not measure measurements with nine samples (Figure 3). We were able to measure with the control sample but not the test samples. A different promoter domain [5] will be cloned in the future. The establishment of the luciferase assay method is essential for the identification of a new transcription factor. It is hoped that new knowledge will be obtained by pushing forward functional analysis of the parkin protein, which we aim at.

References

1. Andrew B.West, Demetrius Maraganore, Julla Crook, et al. Functional association of the parkin gene promoter with idiopathic Parkinson's disease. Hum Mol Genet. 2002;11(22) 2787-2792
2. Kuroda Y, Mitsui T, Kunishige M, et al. Parkin enhances mitochondrial biogenesis in proliferating cells. Hum Mol Genet. 2006;15: 883-895
3. Kuroda Y, Sako W, Goto S, et al. Parkin interacts with Klokin1 for mitochondrial import and maintenance of membrane potential. Hum Mol Genet. 2012; 21, 991-1003
4. Thi A Tran, Andrew D Nguyen, Jianjun Chang, et al. Lipopolysaccharide and Tumor Necrosis Factor Regulate Parkin Expression via Nuclear Factor-Kappa B. Hum Mol Genet. 2011;6(8) e23660
5. L Bouman, A Schlierf, AK Lutz, et al. Parkin is transcriptionally regulated by ATF4: evidence for an interconnection between mitochondrial stress. Cell Death and Differentiation. 2011;18: 769-782

Table 1. The forward and reverse primers used for amplification of promoter region of *parkin* gene

	5'→3'
Forward Primer(+Xho Ⅰ)	CTCGAGGGAAGAGGGCAGGACCTTGGCTA
Reverse Primer(+Bgl Ⅱ)	AGATCTCATGGTCACTGGGTAGGTGGC

Forward Primer

-300 GGAGCTGGAC CCTAGGGGCG GGGCGGGAAG AGGGCAGGAC CTTGGCTAGA GCTGCAACAA
-240 GCTTGGAAAG GTAAGCCTCC CGGTTGCTAA GCGACTGGTC AACACGGCGG GCGCATAGCC
-180 CCGCCCCCCG GTGACGTAAG ATTGCTGGGC CTGAAGCCGG AAAGGGCGGC GGTGGGGGGC
-120 TGGGGGCAGG AGGCGTGAGG AGAAACTACG CGTTAGAACT ACGACTCCCA GCAGGCCCTG
-60 GGATTTAACC CAGGAGAGCC CGCATTCCTA GGGCCGGGCG CGGGGCGGG GAGGCCTGAA
+1 **GGATTTAACC CAGGAGAGCC GCTGGTGGGA GGCGCGCTG GCGCCGCTGC GCGCATGGGC**
+60 CTGTTCCTGG CCCGCAGCCG CAACCTACCC AGTGACCATG ATAGGTACGT GGGTACCTGC

Reverse Primer

Figure 1. Promoter domain of *parkin* gene. The parkin exon 1 is shown as bold sequence, with the parkin start codon double-underlined.

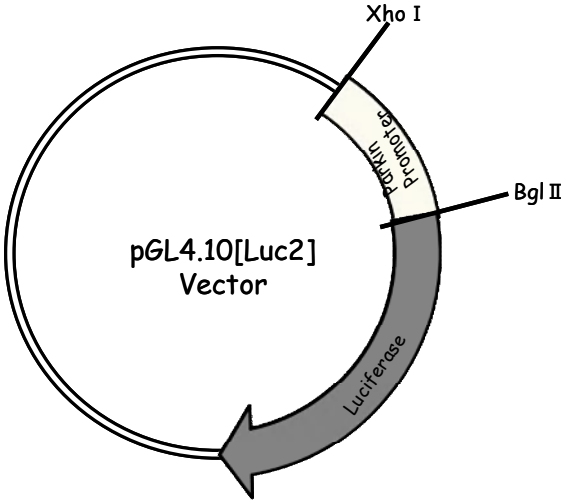

Figure 2. Vector map. A promoter domain was inserted.

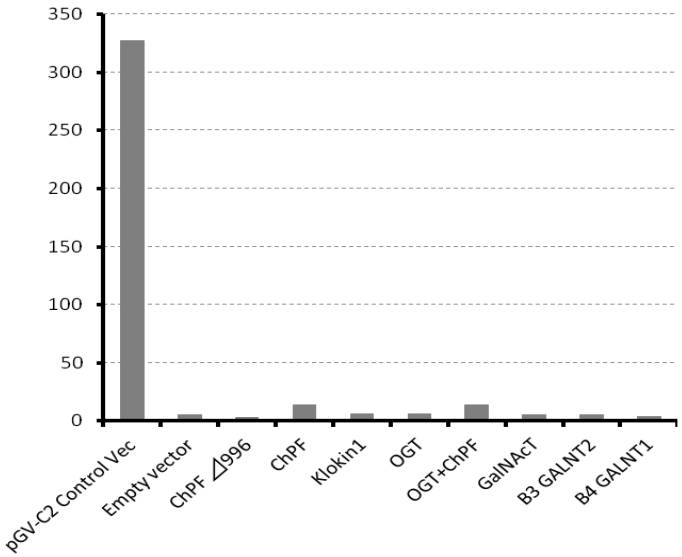

Figure 3. Results of Luciferase activities. We were able to measure with the control sample but the test samples could not be measured.