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RESEARCH

# Somatic Mutation of *PARK2* Tumor Suppressor Gene is not Common in Common Solid Cancers

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**Abstract** Recent studies identified that *PARK2* gene was a candidate tumor suppressor gene in colorectal cancers and glioblastomas. The aim of this study was identify whether *PARK2* somatic mutation is present in other solid tumor as well. In this study, we analyzed the entire coding sequences of human *PARK2* gene in gastric, colorectal, breast, lung and prostate carcinoma by single-strand conformation polymorphism (SSCP) and subsequent direct DNA sequencing. We found two missense mutations (p.Ser9Thr and p.Gly450Val) in colon carcinomas (4.3 %), which were not overlapped with the known *PARK2* mutations. Our data suggest that somatic mutational events in *PARK2* gene may be rare in colorectal, gastric, prostate, breast and lung carcinomas and may not play an important role in the development of these cancers.

**Keywords** PARK2 · Tumor suppressor · Mutation · Cancers

## Introduction

PARK2 (also known as parkin) is a RING domain-containing E3 ubiquitin ligase involved in proteosome-mediated protein degradation [1, 2]. Germline mutations of *PARK2* gene are known to cause a familial form of Parkinson's disease known as autosomal recessive juvenile Parkinson disease [3, 4]. The *PARK2* gene region is known as a fragile area that is unstable and prone to breakage and rearrangement [5]. Changes involving this region have been reported in several forms of human cancer. Loss of heterozygosity (LOH) of *PARK2* alleles and loss of PARK2 expression have been observed in breast, ovary, liver, lung and colon cancers [5–9]. As a result of these alterations, parkin activity is reduced or absent in the cells [8]. *PARK2*-deficient mice also showed increased development of colorectal and liver cancers [7, 8]. Because parkin is considered a tumor suppressor, a shortage of this protein could allow the cells to grow in an uncontrolled manner, leading to tumor formation [5]. In addition, somatic mutations of *PARK2* gene were reported in glioblastomas (9.3 %), lung cancers (6.5 %) and colon cancers (1.2 %) [9]. The somatic mutations also abrogated the growth-suppressive functions of PARK2 [9]. Together, these data suggest that PARK2 is a candidate tumor suppressor gene. To data, however, the data on *PARK2* somatic mutation in other common carcinomas are lacking. In the present study, we analyzed somatic mutations of *PARK2* gene in common human carcinomas.

## Materials and Methods

### Tissue Samples and Microdissection

Methacarn-fixed tissues of 47 gastric, 47 colorectal, 47 breast, 47 lung (non-small cell lung cancers) and 47 prostate carcinomas were randomly selected for the study. All of the patients were Korean. Approval was obtained from the Catholic University of Korea, College of Medicine's institutional review board for this study. The gastric carcinomas consisted of 20 diffuse-type, 16 intestinal-type, and 11 mixed-type gastric adenocarcinomas by Lauren classification, and ten early and 37 advanced gastric carcinomas according to the depth of invasion. The colorectal carcinomas originated from cecum ($N$=1), ascending colon ($N$=9), transverse colon ($N$=2), descending colon ($N$=2), sigmoid colon ($N$=13) and rectum ($N$=20). The lung carcinomas consisted of 25 adenocarcinomas and 22 squamous cell carcinomas. The breast carcinomas consisted of 7 ductal

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carcinomas in situ and 40 invasive ductal carcinomas. The prostate carcinomas consisted of 17 Gleason score 7, 19 Gleason score 8 and 11 Gleason score 9 cancers. TNM stages of the prostate carcinomas were 33 stage II and 14 stage III. Prostate-specific antigen (PSA) of the patients ranged 0.75–132.0 ng/mL with an average of 22.8 ng/mL. Tumor cells and normal cells from the same patients were selectively procured from hematoxylin and eosin-stained slides (5um thick) using a 30 G1/2 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a micromanipulator by the microdissection, as described previously [10–12]. Roughly, we used approximately 500 cells for each polymerase chain reaction (PCR). We used microdissected tissues that had purity of cancer cells over 90 %. Thus, false negativities by normal cell contamination might be low in our study. DNA extraction was performed by a modified single-step DNA extraction method by proteinase K treatment, as described previously [10–12].

### Single Strand Conformation Polymorphism (SSCP) Analysis

Genomic DNA each from tumor cells and corresponding normal cells was amplified with 12 primer pairs covering the entire coding region (exon 1–12) of the *PARK2* gene. Radioisotope was incorporated into the PCR reactions for detection by autoradiogram. The primers were the same in the SSCP as in the DNA sequencing reaction. The PCR products were subsequently displayed in SSCP gels. After SSCP, direct DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP. Other procedures of PCR and SSCP analysis were performed as described previously [10–13].

## Results and Discussion

SSCP analysis detected aberrantly migrating bands of *PARK2* gene in two colon adenocarcinoma compared to wild-type bands from its normal DNA (4.3 % of colorectal cancers). There was no aberrantly migrating band on the SSCP in the other cancers. Direct DNA sequencing of the tumors revealed that both of the *PARK2* mutations were missense mutations. One mutation (c.26C>A) detected in a rectal adenocarcinoma from a TNM stage III male patient substitutes an amino acid in the UBL domain (p.Ser9Thr) and the other (c.1349G>T) detected in a sigmoid colonic adenocarcinoma from a TNM stage II male patient substitutes an amino acid in the C-terminus (p.Gly450Val) (Fig. 1). These mutations were not associated with any available clinicopathologic features of the cancer patients ($\chi^2$ test, $p>0.05$). Neither of these mutations has been reported in human cancers

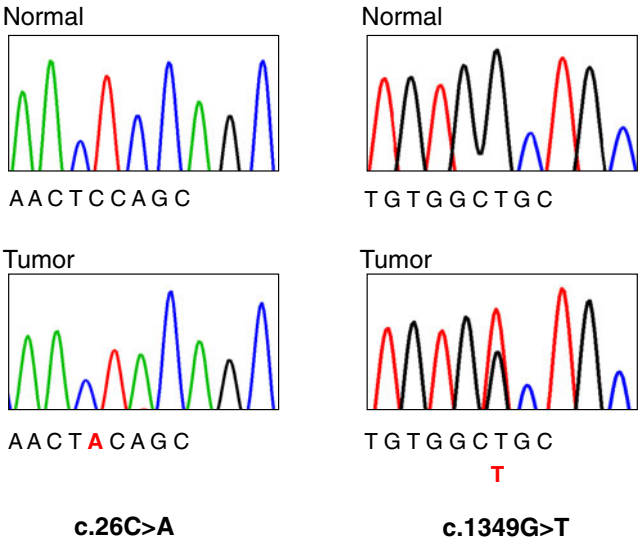


**Fig. 1** DNA sequencing of *PARK2*. Direct DNA sequencing analysis of *PARK2* gene show missense mutations in a rectal adenocarcinoma (c.26C>A) and a sigmoid colonic adenocarcinoma (c.1349G>T)

nor juvenile Parkinson disease [3–9]. To confirm the SSCP results, we repeated the experiments twice, including tissue microdissection, PCR and SSCP to ensure specificity of the results, and found that the data were consistent.

As a mechanism for *PARK2* inactivation in cancers, we analyzed its somatic mutation in colorectal, gastric, prostate, breast and lung carcinomas. The present study, however, detected somatic mutations only in colorectal cancers with a low incidence (4.3 %). Moreover, these are not overlapped with the known mutations. We detected no *PARK2* mutation in 47 lung cancers, while the previous study reported that *PARK2* mutation was detected in 4 of 61 lung cancers [9]. Statistically, however, there is no significant difference of the mutation frequencies between them (Fisher's exact test, $p=0.097$), suggesting that somatic mutation in *PARK2* may be rare in lung cancers as well.

Under suitable conditions SSCP is capable of detecting over 90 % of mutations occurring within any sequence, and the sensitivity of PCR-SSCP is generally believed to be high if the amplified fragments are 200 bps or less in size [14]. It is possible that some mutational changes may not be detected by SSCP in this study. However, since we have analyzed the samples by SSCP twice times and used primers around 200 bp (152–204 bps), it can be thought that the missing of *PARK2* mutations, if any, would be very rare in this study.

In summary, our and the previous data suggest that somatic mutational events in the *PARK2* may be rare in colorectal, gastric, prostate, breast and lung carcinomas and may not play an important role in the development of these common solid cancers.

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