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LETTER TO THE EDITOR

Somatic mutation of *hCDC4* gene is rare in lung adenocarcinomas

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To the Editor

Perturbation of cell cycle regulation is important in the development and progression of human cancers. Many human cancers highly express cyclin E [1–3]. Cyclin E coupled with cyclin-dependent kinase 2 (Cdk2) involved in G1 progression of the cell cycle. Degradation of cyclin E depends on ubiquitin-mediated proteolysis by the SCF ubiquitin ligase complex [1,2]. *hCDC4* (Fbw7) is a component of the SCF complex [1,2]. The *hCDC4* gene was reported to be mutated in both cancer cell lines and primary human cancers. The *hCDC4* mutations were found in breast, ovary and leukemia cell lines, and functionally the mutations were associated with increased levels of cyclin E protein [1,2]. In primary tumor tissues, *hCDC4* mutations were detected in different tumors, including endometrial, colorectal and ovarian tumors (2.9–16% of the endometrial carcinomas, 13% of the colorectal tumors and 2% of the ovarian carcinomas) [4–8]. The *hCDC4* gene is considered a tumor suppressor gene, and the inactivation of *hCDC4* by the somatic mutations caused increased chromosomal instability of the affected cancer cells [4]. Although the mutational analysis of *hCDC4* gene has widely been performed in human tumors, the data on the lung adenocarcinoma is lacking. In this study, we investigated the occurrence of the *hCDC4* mutation in lung adenocarcinomas by a polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) assay.

Methacarn-fixed tissues of 50 lung adenocarcinomas (including 15 adenocarcinomas with bronchioloalveolar carcinoma features and 1 pure bronchioloalveolar carcinoma) were randomly selected for the study. All of the patients were Asians (Korean). We analyzed the primary tumors, but not the metastatic lesions of the tumors. The male to female ratio was 27:23. Ages of the patients ranged from 36–79 years with an average of 59.6 years. The patients consisted of 15 current smokers, 5 former smokers and 30 non-smokers. Approval was obtained from the Catholic University of Korea, College of Medicine's institutional review board for this study. Informed consent was provided according to the Declaration of Helsinki.

Malignant cells and normal cells from the same patients were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a micromanipulator by microdissection. To date, most of the *hCDC4* mutations in the tumor tissues have been reported within the exon 3–11 [4–8]. Thus, we analyzed the *hCDC4* mutation in these nine exons. Genomic DNAs from tumor cells and normal cells from the same patients were amplified by PCR with 15 primer pairs covering the exon 3–11 of human *hCDC4* gene. Radioisotope ($[^{32}P]$dCTP) was incorporated into the PCR products for detection by SSCP autoradiogram. After SSCP, DNAs showing mobility shifts were cut out from the dried gel, and the DNA sequencing of was carried out using the cyclic sequencing kit (Perkin-Elmer, Foster City, CA).

On the SSCP autoradiogram, all of the PCR products were clearly seen. However, there was no any aberrantly migrating band compared to the wild-type bands from the normal tissues, indicating no evidence of DNA sequence alterations in the PCR

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products. To confirm the SSCP results, we repeated the experiments twice, including tissue microdissection, PCR, SSCP and direct DNA sequencing analysis to ensure the specificity of the results, and found that the data were consistent. By comparison, we detected the known *hCDC4* R505L mutation in the SK-OV3 ovarian cancer cell line [2], demonstrating that the PCR-SSCP methods could detect a common *hCDC4* mutation.

The previous observations that *hCDC4* gene is mutated in colorectal, endometrial and ovarian cancers [4–8] led us to analyze whether *hCDC4* gene is mutated in lung adenocarcinomas. We found that the lung adenocarcinomas harbored no *hCDC4* mutation in the tumor samples. The incidence of *hCDC4* mutations in the endometrial carcinomas ranged from 3 to 16% [5–7], while the incidence in colorectal tumors was 13% [4]. In the ovarian carcinomas, the mutation rate was below 2% [8]. We also identified *hCDC4* mutations in approximately 3.5% of the gastric carcinomas (unpublished data). Together, these data indicates that the incidence of *hCDC4* mutation may vary depending on the types of the tumors.

The $hCDC4^{+/-}$ mice had high frequencies of epithelial tumor formations in the lung, raising the possibility that loss of *hCDC4* may be involved in the pathogenesis of lung cancers [9]. Our data, however, suggest that mutational events of *hCDC4* may not contribute to the development of human lung adenocarcinomas. Cyclin E overexpression was reported in one third of human lung adenocarcinomas [3]. The present data also suggest that the increased expression of cyclin E in lung adenocarcinomas may not arise from the mutational inactivation of *hCDC4*.

Acknowledgements

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