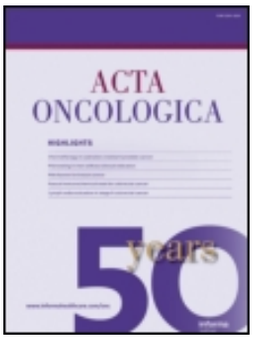

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LETTER TO THE EDITOR

Mutational analysis of proapoptotic death associated protein 3 (*DAP3*) P-loop domain in common human carcinomas

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To the Editor

Apoptosis is a biological process that plays a critical role in the development and homeostasis of multicellular organisms [1]. The extrinsic apoptosis pathway initiates at the cell surface through the ligation of the death receptors Fas or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors by their ligands [1]. While Fas directly binds with FADD, TRAIL receptors do not directly bond with FADD. Death-associated protein 3 (DAP3) serves as an adaptor protein, linking TRAIL receptor 1 and 2 to FADD [2]. Also, a recent study reported that DAP3 localizes in the mitochondria and is involved in the process of mitochondrial fragmentation during apoptosis [3]. DAP3 contains an ATP/GTP-binding motif (P-loop) that are also found in many apoptosis regulators, including CED-4, Apaf-1, ARTS, Nod1 and Nod2 [1,2]. The DAP3 P-loop associates with TRAIL receptors and is essential for the DAP3-mediated caspase-8 activation [2]. Also, the GTP-binding activity of DAP3 was essential for the fragmentation of mitochondria during apoptosis [3].

Cells from human cancers have a reduced capability to undergo apoptosis in response to some physiological stimuli [1]. Proapoptotic proteins are likely to be tumor suppressors and inactivation of apoptosis process by mutations of proapoptotic genes have been reported in many human cancers [1,5]. Similarly, it could be hypothesized that DAP3, which play an important role in the apoptosis, could be mutated and be responsible to the apoptosis resistance of the cancer cells. To date, however, the data on the mutational status of *DAP3* gene in human cancer is lacking. To explore the possibility, we analyzed 359 cases of human cancer tissues from various origins.

Methacarn-fixed tissues of 100 gastric carcinomas, 100 non-small cell lung cancers, 90 colorectal carcinomas and 69 hepatocellular carcinomas were randomly selected for this study. The gastric carcinoma samples consisted of 22 early and 78 advanced gastric carcinomas according to the depth of invasion. The non-small cell lung cancers consisted of 52 squamous cell carcinomas, 43 adenocarcinomas and five large cell carcinomas. The hepatocellular carcinomas consisted of Edmondson grade I (n = 8), grade II (n = 30) and grade III (n = 31) according to Edmondson and Steiner's criteria. The colorectal carcinomas originated from cecum (n = 2), ascending colon (n = 16), transverse colon (n = 5), descending colon (n = 2), sigmoid colon (n = 25) and rectum (n = 40). By microdissection, malignant cells and normal cells from the same patients were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 hypodermic needle affixed to a micromanipulator [5].

We analyzed the DNA sequences encoding the P-loop domain of DAP3 by a polymerase chain reaction (PCR) and a subsequent single strand conformation polymorphism (SSCP) analysis. Genomic DNA each from tumor cells and normal cells of the same patients were amplified by PCR with a primer pair covering the exon 5 of human *DAP3* gene. The primer sequences were as follows; forward: 5′-AAAACAAAACAAA

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GCATAACTACTG-3′ and reverse: 5′-GGATAA CTAAACCTAACAGACAGAAG-3′. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by SSCP autoradiogram.

On the SSCP autoradiograms, all of the PCR products were clearly seen. However, the SSCP from the 359 carcinomas did not reveal any aberrantly migrating band compared to the wild-type bands from the normal tissues. 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.

Because DAP3 acts as an important component of the apoptosis machinery both in intrinsic and extrinsic pathways, we expected to detect some *DAP3* mutations in the cancer samples. However, we detected no *DAP3* P-loop domain mutation in the samples, indicating that proapoptosis gene *DAP3* P-loop mutation is rare in the cancers, and suggesting that mutational events in DAP3 P-loop may not contribute to the development of common human cancers, including gastric, lung, colorectal and hepatocellular carcinomas. Along with this study, we also analyzed the P-loop mutations of *ARTS* and *Nod1* genes in cancer tissues from various origins, but found no mutation (unpublished data; Lee JW and Lee SH), suggesting that the P-loops in the proapoptotic proteins may not be mutational targets in human cancers.

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