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LETTER TO THE EDITOR

Mutational analysis of P-loop domains of proapoptotic Nod1 and ARTS genes in colon carcinomas

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Mounting evidence indicates that deregulation of apoptosis is involved in the mechanisms of cancer development. The proteins that directly control the apoptosis pathways often exist as families that can be recognized based on their amino acid sequence and structural similarity. P-loop or G1 motif (GXSGXGKST) is a phosphate-binding site that is conserved in the septin family and also found in many ATP/GTPases, including apoptosis regulators CED-4, Apaf-1, Nod1, Nod2 and apoptosis-related protein in the TGF-β signaling pathway (ARTS; also known as H5/SEPT4/PNUTL2) [1,2]. ARTS is released from mitochondria into the cytosol during apoptosis, promoting caspase activation by neutralizing the inhibition of inhibitor of apoptosis protein (IAP) on caspases [2]. ARTS is also important for apoptosis induced by a variety of proapoptotic factors, including TGF-β, etoposide, staurosporine and Fas [3]. The P-loop in ARTS is essential for the apoptosis function of ARTS [2,3]. Nod1 binds to caspase-9 and promotes caspase-9-induced apoptosis [4]. The P-loop domain of Nod1 is also essential for Nod1 to activate caspase-9 [4].

It is now believed that clonal expansion and tumor growth are the results of the deregulation of intrinsic proliferation (cell division) and cell death (apoptosis) [1]. Failure of apoptosis could allow the survival of transformed cells that are prone to undergo further genetic damage and play an important role in the pathogenesis of tumors. Apoptosis of cancer cells can be delayed or blocked by several ways, including somatic mutation and loss of expression of pro-apoptotic molecules, and expression of apoptosis-inhibitory molecules [1]. Somatic mutations of apoptosis-related genes, including the death receptors and caspases, have been common in human cancers, and many of the mutations were proven to inactivate cell death [4–6]. Similarly, it could be hypothesized that P-loop-containing proapoptotic proteins that 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 mutation status of ARTS and Nod1genes in human cancer is lacking.

To see whether ARTS and Nod1gene mutations are involved in the development of human cancers, we analyzed DNA sequences encoding the P-loop domains of ARTS and Nod1 proteins in the methacarn-fixed tissues of 90 colorectal carcinomas by a polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) assay. All of the patients of the cancers were Asians (Koreans). 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). 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, as described previously [4–6]. DNA extraction was performed by a modified single-step

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DNA extraction method [4–6]. Genomic DNAs from tumor cells and normal cells from the same patients were amplified with each primer pair covering the P-loop-encoding sequence of human ARTS gene and Nod1 gene. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. The procedures of PCR and SSCP analysis were performed as described previously [4–6].

However, the SSCP from the 90 colorectal carcinomas did not reveal any aberrantly migrating band compared to the wild-type bands from the normal tissues (Figure 1). 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 (data not shown).

We previously reported several pro-apoptosis gene mutations, including death receptors, caspases and Bcl-2 family in many types of human cancers [4–6], suggesting that genes encoding the proteins in the apoptosis machinery are popular targets for the somatic mutations in the cancer cells. Because both ARTS and Nod1 behave as components of the apoptosis machinery, we expected to detect some mutations of them in the cancer samples. However, the present data indicates that somatic mutation of both ARTS and Nod1 is uncommon in the colon cancers, and suggests that mutational events in both ARTS and Nod1 genes may not contribute to the development of colorectal carcinomas.

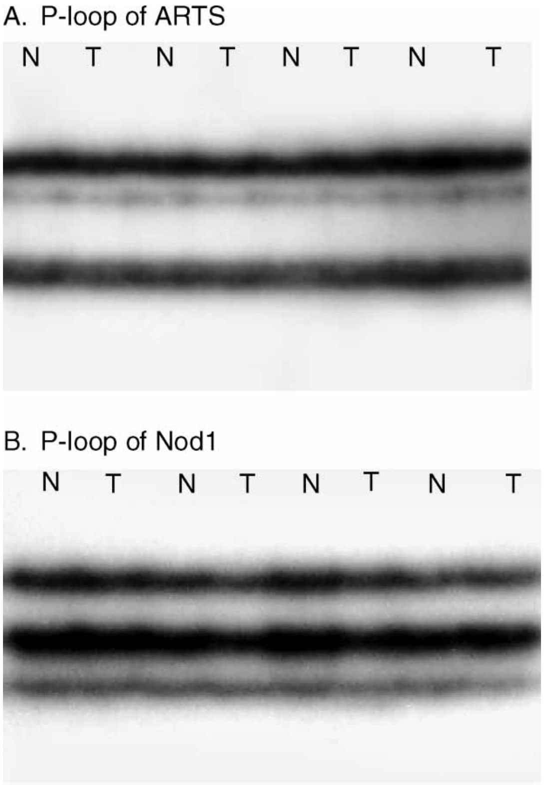

Figure 1. SSCP of P-loop domains of *ARTS* and *Nod1* in colon cancer tissues. Part of the exon 4 of *ARTS* gene and part of exon 6 of *Nod1* gene that encode the P-loop domains were amplified by PCR. Subsequent SSCPs of the PCR products (**A:** *ARTS* gene, **B:** *Nod1* gene) from colorecral carcinomas (Lane T) shows no aberrant band as compared to SSCP from their normal tissues (Lane N) of the same patients.

Somatic mutations of apoptosis-related genes frequently occurred in specific domains that are important in the apoptosis signaling. For example, most of the death receptor mutations have been found in the death domain, and caspase gene mutations frequently affected protease subunits [4–6]. Because the P-loop domain is conserved in some proapoptosis genes such as Apaf-1, Nod1, ARTS and CED-4, and essential for the apoptosis, we focused the mutational analysis of ATRS and Nod1 genes in the P-loop domain. It is possible that mutations besides the P-loop domain might exist and such mutations may promote the survival of the cancer cells by inactivating the proapoptotic function of the proteins. Also, there may be other ways of inactivation of ARTS and Nod1 in the cancer cells. For instance, the loss of ARTS expression by promoter methylation of ARTS gene was observed in 70% of acute lymphoblastic leukemias, and the lack of ARTS expression in the cancer cells inhibited apoptosis. Currently, however, the expression status of both ARTS and Nod1 in colorectal cancers remains unknown.

Acknowledgements

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