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LETTER TO THE EDITOR

Leukemia Relapse-Associated Mutation of *NT5C2* Gene is Rare in de Novo Acute Leukemias and Solid Tumors

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To the editor:

The 5′-nucleotidase cytosolic II (NT5C2), also known as cN-II, encodes a hydrolase that plays an important role in cellular purine metabolism. It inactivates 6-thioinositol monophosphate (MP) and 6-thioguanosine MP, which mediate the cytotoxic effects of 6-MP and 6-thioguanine (6-TG) that are used in the treatment of acute lymphoblastic leukemias (ALL) [1]. Recent studies discovered that somatic mutations of *NT5C2* were common in relapsed T-ALL and less frequently in B-ALL [2, 3]. The *NT5C2* mutations were recurrent in specific amino acids (most frequently in p.R367Q and p.R238W) and appeared gain-of-function mutations that enhanced the enzymatic activity, resulting in inactivation of 6-MP and 6-TG [2, 3]. They also found that *NT5C2* mutations had existed before the relapses as a rare clone [2], indicating that *NT5C2* mutations exist at diagnosis in ALL and emerge after 6-MP is treated. In the COSMIC database, some solid cancers (gastric, colon and endometrial cancers) harbored the relapse-specific *NT5C2* mutation p.R367Q, which emerged without any history of chemotherapy, suggesting either that the *NT5C2* mutation had been raised by other factors besides chemotherapy or that it might play a role in development rather than relapse of the cancers. However, the status of *NT5C2* mutations remains unknown in primary tumors without exposure to 6-MP. A similar situation was also identified in the case of relapse-specific *EGFR* mutation p.T790M, which was also identified in lung cancers that had never been exposed to gefitinib therapy [4].

Thus, it is interesting to study whether the *NT5C2* mutations are present in primary hematologic neoplasia as well as in primary solid tumors. For this, we analyzed the *NT5C2* somatic mutations using genomic DNA from in fresh bone marrow aspirates of 705 hematologic tumors (acute myelogenous leukemias (AML), ALL, multiple myelomas and myelodysplastic syndromes) (Table 1) by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay. Also, we analyzed the gene in paraffin-embedded tissues of 150 non-Hodgkin lymphomas (NHL) and 1639 solid tumors (Table 1). Approval was obtained from the Catholic University of Korea, College of Medicine's institutional review board for this study. Genomic DNA each from tumor cells and normal cells (remission bone marrow cells in the cases of leukemias) were used in this study. Because the relapse-specific mutations of *NT5C2* have been detected in exons 11 and 15 [2, 3], we analyzed these two exons in this study by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP). Radioisotope was incorporated into the PCR products for detection by autoradiogram. Other procedures of the PCR-SSCP were described in our previous studies [4, 5]. After SSCP, direct DNA sequencing reactions were performed in the cancers with mobility shifts.

PCR and subsequent SSCP analysis detected aberrant migrating SSCP bands in two tumors (one AML and one colon cancer), but not in the other hematologic nor solid tumors (2/2496, 0.08 %). Direct DNA sequencing analyses for the two cases with aberrant bands led us to identify that the aberrant bands represented *NT5C2* somatic mutations. The mutations were missense mutations that substituted two different amino acids (p.Glu240Gln in the AML and p.Arg363Gln in

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Table 1 *NT5C2* mutations analyzed in tumors from 2444 patients

Type of cancers	Number of tumors	*NT5C2* mutations		
		Wild type	Mutation	Mutation (%)
Adulthood AML	245	244	1	0.4
Adulthood ALL	130	130	0	0
Childhood AML	17	17	0	0
Childhood ALL	170	170	0	0
Multiple myeloma	75	75	0	0
Myelodysplasia	68	68	0	0
Non-Hodgkin lymphoma	150	150	0	0
Non-small cell lung cancer	235	235	0	0
Gastric carcinoma	210	210	0	0
Colorectal carcinoma	402	401	1	0.2
Breast carcinoma	93	93	0	0
Prostate carcinoma	275	275	0	0
Ovarian epithelial tumors	15	15	0	0
Ovarian granulosa cell tumors	69	69	0	0
Hepatoblastomas	29	29	0	0
Esophageal squamous cell carcinomas	72	72	0	0
Laryngeal squamous cell carcinomas	44	44	0	0
Leiomyoma	68	68	0	0
Gastrointestinal stromal tumors	20	20	0	0
Malignant peripheral nerve sheath tumor	22	22	0	0
Malignant fibrohistiocytic tumors	15	15	0	0
Other sarcomas	42	42	0	0
Meningioma	30	30	0	0
Total	2496	2494	2	0.08

the colon cancer), which were not identical with the relapse-specific *NT5C2* mutations.

Unexpected presence of relapse tumor-specific *NT5C2* mutations in primary tumors in the COSMIC database led us to further analyze the mutations in diverse types of hematologic and non-hematologic neoplasia in this study. However, we detected only two mutations (one in adult AML and the other in colon cancer) in 2444 tumors in this study. The mutations detected were not even overlapped with the relapse-specific *NT5C2* mutations. Our results indicate that relapse-specific *NT5C2* mutations are not clonal in primary human tumors but that they, when present, may be subclonal. Also the data suggest that *NT5C2* mutations may not play an important role in the development of human tumors. Practically, an attempt to find subclonal *NT5C2* mutations in pretreated hematologic neoplasia using a sensitive method instead of the conventional sequencing may be needed to predict a therapy-related relapse.

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