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LETTER TO THE EDITOR

GNAS Mutation Affecting Codon 201 is Rare in Most Human Tumors

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To the Editors:

Guanine Nucleotide-binding protein, Alpha-Stimulating activity polypeptide 1 (GNAS) is a subunit of the stimulatory G protein that transmits signals to generate cAMP [1]. Mutations in *GNAS* gene result in several inherited diseases, including pseudohypoparathyroidism, pseudopseudohypoparathyroidism, progressive osseous heteroplasia, McCune-Albright syndrome, polyostotic fibrous dysplasia and several endocrine tumors [2]. Also, somatic mutations of *GNAS* have been reported in many tumors, including pituitary adenoma, colorectal carcinoma/adenoma, thyroid adenoma, juvenile ovarian granulosa cell tumor, low-grade appendix mucinous neoplasm, hepatocellular carcinomas, pancreatic mucinous neoplasm, fluke-associated cholangiocarcinoma and myelodysplasia [2–9]. Of note, most of the somatic *GNAS* mutations affected codon 201 [3–9]. Functionally, tumor-derived GNAS mutant constitutively activates cAMP signaling [3]. These data indicate that somatic mutations of *GNAS* are detected not only in endocrine tumors, but also in non-endocrine tumors [2–9].

To see whether the recurrent *GNAS* mutations occur in other tumor types as well, we analyzed the recurrent mutation site in 1,126 tumor tissues (Table 1) by polymerase chain reaction (PCR) - single strand conformation polymorphism (SSCP) assay. All of the patients of the cancers were Asians (Koreans). We included myelodysplasia in this study that had been analyzed already, because in Korean population incidence of myelodysplasia subtypes were reported to be different from those of western countries. For example, incidence of MDS without RS is far less common than that of MDS with RS in Korean population [10]. In solid tumors, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides using a microdissection method. We analyzed exon 8 that encompassed the most common *GNAS* mutation site (codon 201). The primer sequences for amplification were as follows (forward and reverse, respectively): 5′-CTGTTTCGGTTGGCTTTGGTG -3′ and 5′- AGGGACTGGGGTGAATGTCAAG -3′. SSCP analysis with this primer pair was proven to detect *GNAS* codon 201 mutation in our previous study [11]. Genomic DNA each from tumor cells and corresponding normal cells were amplified with the primer pairs by PCR. Radioisotope was incorporated into the PCR products for detection by autoradiogram.

However, SSCPs from the 1,126 cancers did not reveal any aberrantly migrating band compared to bands from normal tissues, indicating there was no evidence of mutation in the recurrent mutation sites in *GNAS* gene in the tumors. In a positive control (intraductal papillary mucinous neoplasm of the pancreas), the SSCP detected aberrantly migrating bands. We repeated the experiments twice, including tissue microdissection, PCR and SSCP analysis to ensure the results, and found that the data was consistent.

Prior to this study, we expected to find the recurrent mutations of *GNAS* in our samples analyzed, because *GNAS* recurrent mutations have been detected in many tumor types [3–9] and our

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Table 1 Tumor tissues analyzed in this study

Type of tumors	Number of tumors
Head/neck squamous carcinoma	13
Esophageal squamous carcinoma	59
Ovarian carcinoma	54
Prostate carcinoma	267
Non-malanomaous skin cancers	26
Hepatoblastoma	14
Sarcomas	64
Uterine leiomyoma	68
Meningioma	13
Gastrointestinal stromal tumor	19
Ovarian stromal tumor	43
Acute myelogenous leukemia	180
Acute lymphoblastic leukemia	180
Myelodysplasia	68
Non-Hodgkin lymphoma	58
Total	1,126

cancer samples included diverse cancer types (Table 1). However, we detected no *GNAS* mutations in those cancers (Table 1). Moreover, there was no *GNAS* mutation in myelodysplasia where the *GNAS* mutation had been detected [8]. Comparing the incidence of *GNAS* mutation in myelodysplasia between our data (0/68) and the earlier data (3/439), we found no significant difference between the two (Fisher's exact test, $p=0.649$), indicating that the recurrent *GNAS* mutation may be a rare event in myelodysplasia. In non-endocrine tumors, high incidences of the *GNAS* mutations have been reported in papillary mucinous neoplasm of digestive system [4, 7]. In other tumors, the incidences were negative or very low [5, 10]. Together, the data suggest that recurrent *GNAS* mutations are present in special types of tumors (endocrine tumors and papillary mucinous neoplasm), but rare in other tumors.

In summary, our study did not find any *GNAS* recurrent mutation in carcinomas (head/neck, esophagus, ovary, skin, liver and prostate), stromal tumors (sarcomas, leiomyomas, meningiomas, ovarian stromal tumors and gastrointestinal stromal tumors) and hematologic tumors (leukemias, myelodysplasia and lymphomas), indicating that *GNAS* codon 201 may be rarely mutated in most tumor types.

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