STAT3 exon 21 mutation is rare in common human cancers

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To the Editor,

To find cancer-related genome alterations in T-cell large granular lymphocytic leukemia, Koskela et al. [1] analyzed whole exomes of the cancer cells by next-generation exome sequencing. They found that signal transducer and activator of transcription 3 gene (*STAT3*) was frequently mutated in T-cell large granular lymphocytic leukemia (31 of 77 patients (40%) [1]. Of note, all of the *STAT3* mutations were found in exon 21, encoding the Src homology 2 (SH2) domain that mediates dimerization and activation of *STAT3* protein. The recurrent *STAT3* mutations consisted of p.Y640F, p.D661V, p.D661Y and p.N647I. The mutations increased transcriptional activities of *STAT3*, suggesting their roles in the tumorigenesis [1].

STAT3 is a transcription factor that is activated in response to a large number of cytokines and growth factors [2]. *STAT3* transcriptional targets include bcl-2, mcl-1 and cyclin D1, reflecting its oncogenic roles in promoting cell survival and cell cycle progression [1,3]. Also, activated *STAT3* maintains constitutive NF-κB activity in cancers [4]. STAT signaling is frequently activated in a wide spectrum of hematologic malignancies and solid tumors [4]. To see whether *STAT3* exon 21 mutation is responsible for activated STAT signaling in common human malignancies, we analyzed the mutation in a large number of human tumors from various origins by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay in this study.

For this, 2677 randomly collected tumor tissues from Korean patients (Table I) were used for this study. The hepatocellular carcinomas consisted of two grade I, 14 grade II and 20 grade III cancers by Edmondson's classification. All of the breast carcinomas were invasive ductal carcinomas [estrogen receptor positive (ER+): 66%, progesterone receptor positive (PR+): 58%, ER+/PR+: 51%]. The lung carcinomas consisted of 81 adenocarcinomas and 75 squamous cell carcinomas. The colorectal carcinomas originated from cecum (N = 10), ascending colon (N = 58), transverse colon (N = 18), descending colon (N = 20), sigmoid colon (N = 114) and rectum (N = 191). The GC consisted of 110 diffuse-type, 95 intestinal-type and 35 mixed-type gastric adenocarcinomas by Lauren's classification, and 220 advanced and 20 early gastric carcinomas according to the depth of invasion. The PCA consisted of 17 Gleason's score 6, 125 score 7, 100 score 8 and 33 score 9 cancers. The tumors did not include T-cell large granular lymphocytic leukemia. For the solid tumors, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides by microdissection as described previously [5]. Approval for this study was obtained from the Catholic University of Korea, College of Medicine's institutional review board. Genomic DNA

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Table I. *STAT3* exon 21 mutations analyzed in 2677 tumors.

Type of cancers	Number of tumors	*STAT3* exon 21		
		Wild type	Mutation	Mutation (%)
Adulthood AML	356	356	0	0
Adulthood ALL	201	201	0	0
Childhood AML	21	21	0	0
Childhood ALL	191	191	0	0
Multiple myeloma	75	75	0	0
Non-Hodgkin lymphoma	118	117	1	0.8
Non-small cell lung cancer	156	156	0	0
Gastric carcinoma	240	240	0	0
Colorectal carcinoma	411	410	1	0.2
Breast carcinoma	94	94	0	0
Prostate carcinoma	275	275	0	0
Ovarian epithelial tumors	105	105	0	0
Ovarian stromal tumors	99	99	0	0
Hepatocellular carcinomas	36	36	0	0
Hepatoblastomas	26	26	0	0
Esophageal squamous cell carcinomas	67	67	0	0
Laryngeal squamous cell carcinomas	44	44	0	0
Basal cell carcinomas of skin	36	36	0	0
Gastrointestinal stromal tumors	22	22	0	0
Sarcomas	104	104	0	0

ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia.

each from tumor cells and normal cells of the same patients were amplified by PCR with a primer pair (5′-CTGTGACAGGTAAGACCCAGAT-3′ and 5′-CGACAATACTTTCCGAATGC-3′; product size: 184 base pairs). Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. Other procedures of the PCR-SSCP were described in our previous studies [5]. After SSCP, direct DNA sequencing reactions were performed in the cancers with the mobility shifts in the SSCP.

PCR and subsequent SSCP analysis identified two tumors with aberrantly migrating bands. None of the corresponding normal samples from the same patients showed evidence of mutations by SSCP, indicating the mutations had risen somatically. Direct DNA sequencing analysis of the tumors with the aberrantly migrating bands led us to identify that these aberrant bands represented *STAT3* mutations. One of the *STAT3* mutations [c.1967G>A (p.G656D)] was detected in a peripheral T-cell lymphoma and the other [c.1927C>A (p.C643K)] was detected in a rectal adenocarcinoma. None of these were the recurrent mutations identified in T-cell large granular lymphocytic leukemia. We repeated the experiments twice in the two mutated cases, including tissue microdissection, PCR, SSCP and direct DNA sequencing analysis to ensure the specificity of the results, and found that the data were consistent.

One of the main concerns in cancer genetics is to identify whether any mutation found in a cancer is common in other cancer types. In this study, however, we could not indentify *STAT3* exon 21 mutations that had been recurrently detected in T-cell large granular lymphocytic leukemia. Also, the incidence of *STAT3* exon 21 mutations in our study was very low (2/2,677), suggesting that these mutations may be passenger mutations. Taken together, our results suggest that *STAT3* exon 21 mutation is rare in most human cancers and that the recurrent mutation may be specific to T-cell large granular lymphocytic leukemia. As activated STAT3 is considered to play a role in cancer development, the discovery of *STAT3* recurrent mutations in T-cell large granular lymphocytic leukemia offered an opportunity for developing therapeutic tools targeting human cancers. Our data, however, suggest that development and application of therapies targeting *STAT3* exon 21 mutants should be limited to T-cell large granular lymphocytic leukemias.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

[1] Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmäki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med 2012;366:1905–13.

[2] Germain D, Frank DA. Targeting the cytoplasmic and nuclear functions of signal transducers and activators of transcription 3 for cancer therapy. Clin Cancer Res 2007;13:5665–9.
[3] Puthier D, Bataille R, Amiot M. IL-6 up-regulates mcl-1 in human myeloma cells through JAK/STAT rather than ras/MAP kinase pathway. Eur J Immunol 1999;29:3945–50.
[4] Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, et al. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell 2009;15:283–93.
[5] Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 2005;24:1477–80.