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LETTER TO THE EDITOR

Somatic mutation of PIK3R1 gene is rare in common human cancers

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To the Editor

The PI3K signaling pathway regulates many normal cellular processes, and perturbation of the PI3K signaling results in deregulated kinase activity and malignant transformation [1]. Phosphatidylinositol 3-kinase (PI3K) is made up of a catalytic subunit, p110 encoded by phosphatidylinositol 3-kinase catalytic alpha (PIK3CA) and a regulatory subunit of either 85 kD (p85), 55 kD, or 50 kD. The p85 subunit consists of two closely related proteins, p85α (phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1)) and p85β (PIK3R2) [1]. One of the main functions of PIK3R1 is to inhibit PIK signaling [1].

Activating oncogenic mutation of *PIK3CA* is common in human cancers [2]. By contrast, somatic mutation of *PIK3R1* has rarely been reported in human cancers. Ovary, colon and breast cancers have been reported to harbor *PIK3R1* mutations in low incidences [3,4]. The Cancer Genome Atlas (TCGA) project aims to discover cancer causing genome alterations through the application of genome analysis technologies, including large-scale genome sequencing. The first cancer studied by the TCGA was glioblastoma multiforme (GBM), and the TCGA research network discovered that *PIK3R1* was somatically mutated in GBM [5]. The TCGA found nine *PIK3R1* mutations out of 91 GBM (9/91; 9.9%). The mutations were detected in exon 12 (N = 5), exon 10 (N = 2), exon 9 (N = 1), and exon 13 (N = 1). By a similar approach, Parsons et al. [7] found *PIK3R1* mutation in GBM (9/105; 8.6%), which were detected in exon 12 (N = 2), exon 10 (N = 3), exon 9 (N = 3), and the acceptor splice site of the exon 12 (N = 1). All of these *PIK3R1* mutations have been detected in coding exons for the N-terminal SH2 domain (exon 9) or the inter-SH2 domain (exons 10, 12 and 13), or in the intron (intron 12) adjacent to these exons. These domains have shown an inhibitory function of PIK signaling, suggesting that the *PIK3R1* mutations may act as oncogenes by disrupting the inhibitory function of PIK3R1 [1]. Activation of PI3K is crucial in the pathogenesis of many human cancers, and mutations of PI3K complex have been a popular candidate target for cancer therapies. Therefore, it is interesting to determine whether *PIK3R1* gene mutation occurs in other cancers besides GBM.

For this, 820 cancer tissues from Korean patients (carcinomas from breast (N = 78), colon (N = 60), lung (N = 127), stomach (N = 109), esophagus (N = 46), liver (N = 87), prostate (N = 75), ovary (N = 31) and skin (squamous cell carcinomas) (N = 9), and GBM (N = 19), multiple myelomas (N = 30) and acute leukemias (N = 169)) were used for this study (Table I). The *PIK3R1* somatic mutations have been detected in exon 9, 10, 12, 13 and intron 12 [3–7]. Thus, we analyzed these regions in this study by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) analysis. Genomic DNA each from tumor cells and normal cells from the same patients were amplified by PCR with four primer pairs covering

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Table I. *PIK3R1* mutations in 840 cancers.

Type of cancers	Number of cancers	*PIK3R1*		
		Wild type	Mutation	Mutation (%)
Primary GBM	19	19	0	0
Prostate carcinoma	75	75	0	0
B-ALL	49	49	0	0
T-ALL	20	20	0	0
Acute myelogenous leukemia	100	100	0	0
Multiple myeloma	30	30	0	0
Non-small cell lung cancer	127	127	0	0
Gastric carcinoma	109	109	0	0
Colon carcinoma	60	60	0	0
Breast carcinoma	78	78	0	0
Hepatocellular carcinoma	68	68	0	0
Hepatoblastoma	19	19	0	0
Esophageal squamous cell carcinoma	46	46	0	0
Ovarian carcinoma	31	31	0	0
Squamous cell carcinoma, skin	9	8	1	11.1
Total	840	839	1	0.12

these regions in human *PIK3R1* gene (exon 9, 5′-aaaacat atttccttattcc-3′ and 5′- actgagctagagattcattc-3′; exon 10, 5?-atccattgaattta ttttaatc-3′ and 5′-atagaaaactc acctggg-3′; exon 12, 5′-agaagacttgaagaagcag-3′ and 5′-catgtataggattccatttc-3′; exon 13, 5′-tgctacaattcagg atgag-3′ and 5′-aaaaatcttctgctatcacc-3′). Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. After SSCP, direct DNA sequencing reactions were performed in the cancers with the mobility shifts in the SSCP.

On the SSCP autoradiograms, all of the PCR products from the cancers were clearly seen. Of them, PCR-SSCP analysis of *PIK3R1* exon 12 revealed that a skin squamous cell carcinoma (1/9; 11.1%) displayed aberrantly migrating SSCP bands compared to wild-type bands from the normal tissues of the same patient. Direct DNA sequencing analysis revealed that the aberrant bands represented a frameshift mutation (c.1735dupC (p.Gln579Profsx23)). This mutation would lead to premature stops of amino acid synthesis of the affected proteins and hence resembles a typical loss-of -function mutation. This is a novel *PIK3R1* mutation, and a similar frameshift mutation (p.Arg574Lysfsx27) had been detected in a GBM [5]. There was no aberrantly migrating SSCP band compared to wild-type bands from the normal tissues in other 839 cancers in the four exons nor their splice sites. The patient with the skin cancer on the face was a 66-year-old male. The tumor was a well differentiated squamous cell carcinoma without metastasis (T1N0M0; TNM stage I).

One of the main concerns in cancer genetics is to identify whether any mutation found in a cancer is common in other cancer types. As a possible mechanism of PI3K signaling activation in common human cancers, we analyzed *PIK3R1* mutation in a wide range of human cancers. However, except one mutation in a skin cancer, we detected no *PIK3R1*-mutation in the exons nor introns where *PIK3R1* mutations had been detected in cancers [3–7]. The sensitivity of PCR-coupled SSCP screening is known to be lower than that of PCR-coupled direct sequencing which might in part explain the low mutation prevalence in this study. Under suitable conditions SSCP is capable of detecting over 90% of mutations occurring within any sequence, and the sensitivity of PCR-SSCP is generally believed to be high if the fragments are shorter than 200 bp. Since we used primers shorter than 200bp (138–186 bp), it can be thought that the missing of *PIK3R1* mutations, if any, would be very rare in this study. In our earlier work, we detected *PIK3CA* mutations in 26.9% of breast cancers by PCR-SSCP [8], which was similar to the incidence of the same mutation in breast cancers by direct sequencing method (20.6%) [9]. In our analysis, we included the cancer types (GBM, colon cancer, breast cancer and ovarian cancer) that had been reported to harbor *PIK3R1* mutation [3–7]. In earlier studies, ovarian cancers harbored *PIK3R1* mutations in donor and acceptor splice sites of intron 12, and exon 12 (3/80; 3.8%) [3], while breast cancers harbored the mutation in exon 10 (1/35; 2.9%) [4]. Colon cancers was reported to harbor the mutation in the exon 12 (1/60; 1.7%) in one study [3], but none in other study [4]. Although we could not detect any *PIK3R1* mutation in these cancers, there was no significant difference of the mutation frequency between our and the earlier data (Fisher's exact test, $p > 0.05$),

suggesting that *PIK3R1* mutation may be rare and passenger mutations in these cancers. In addition to the *PIK3R1* mutation data by the TCGA [5] (9/91; 9.9%) and Parsons et al. [7] (9/105; 8.6%), Mizoguchi et al. also analyzed *PIK3R1* mutation in 30 cases of GBM and found only one mutation in the donor splice site of the exon 12 (1/30; 3.3%). However, there was not any significant difference of the mutation frequency between our and the earlier data (Fisher's exact test, $p > 0.05$), either. The wide range of *PIK3R1* mutation incidences (0–9.9%) in GBM might be explained by several points. One of them may be an ethnic or geologic difference of the mutation (western (the earlier studies) vs. Asian (our study)). We analyzed a small number of GBM (19 GBM) in the present study. Analysis of the mutation in more GBM cases from non-western countries may clear this point.

In summary, our data indicate that the *PIK3R1* mutation is rare in human cancers, and suggest that the mutation may not play an important role in the pathogenesis of common human cancers. The discovery of the *PIK3R1* mutation in cancers offered an opportunity for developing therapeutic tools targeting this mutation in human cancers [5,6]. Our data, however, suggest that development and application of therapies targeting mutant *PIK3R1* should be limited to cancers only with the mutation that may be rare in common cancers.

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