Promoter Mutation Analysis of *LEPROTL1* Gene in Acute Leukemias and Solid Tumors

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Dear Editor,

The leptin receptor overlapping transcript-like 1 gene (*LEPROTL1*) encodes a membrane protein [1, 2]. To date, the only known function of this gene product is to suppress growth hormone activity in the liver [2]. The cancer-related functions of *LEPROTL1* remain unknown. According to the Human Protein Atlas database (www.proteinatlas.org), *LEPROTL1* is widely expressed in human tissues, but its expression in most cancer types is negative, suggesting that loss of *LEPROTL1* could be related to tumorigenesis. A recent study analyzed genome-wide somatic mutations in promoters in breast cancers and identified promoter mutations in several genes [3], including *LEPROTL1*, which resulted in its decreased expression, suggesting a loss of function [3]. Interestingly, the promoter mutations repeatedly occur at 2 hotspots (chr8:29952919 and chr8:29952921). Alterations in promoter regions such as somatic mutation and aberrant methylation are known to play a role in cancer pathogenesis [4]. Together, these data suggest that promoter mutation in the *LEPROTL1* gene might be prevalent and play a role in the development of breast cancer as well as other cancers.

For this study, we analyzed somatic promoter mutations of the *LEPROTL1* gene using genomic DNA from bone marrow aspirates of 1,011 hematologic tumors (acute myelogenous leukemia [AML], acute lymphoblastic leukemia [ALL], mul-

tiple myeloma, and myelodysplastic syndrome) (Table 1) by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay. We also analyzed the DNA from 127 non-Hodgkin lymphomas (NHL) and 1,545 epithelial and mesenchymal tumors of various origin (Table 1). For the solid tumors, malignant and normal cells were selectively procured by microdissection [5]. Approval for this study was obtained from the Institutional Review Board of the Catholic University of Korea's College of Medicine.

Because *LEPROTL1* promoter mutations have been focused in a narrow region (chr8:29952919 and chr8:29952921) [3], we amplified this region by PCR with the following primers: forward: 5-ACACCCGC-GGAGAAGAGAG-3, reverse: 5-GGAGACCCAAGACGCGCTAC-3, and subsequently analyzed the PCR products by SSCP. Genomic DNA from tumor cells and normal cells (remission bone marrow cells from leukemia cases) was used in this study. The PCR-SSCP method has previously been described [5]. After SSCP, direct DNA sequencing reactions were performed in the cancers with mobility shifts.

PCR and subsequent SSCP analysis detected somatic promoter mutations of *LEPROTL1* in 3 cancers (1 case of childhood ALL, 1 laryngeal carcinoma, and 1 angiosarcoma). The matched normal samples were also sequenced but did not show the mutations. All of these mutation sites de-

tected in our study (Table 1) overlap with the hotspots identified in the earlier study [3]. The ALL patient (a 6-year-old girl) with the *LEPROTL1* mutation was diagnosed as having B lymphoblastic leukemia with t(12;21)(p13;q22);TEL-AML1 (*ETV6-RUNX1*). Cytogenetic analysis of the bone marrow demonstrated the following karyotype in detail: 46,XX,del(11)(q14q21),t(11;18)(p15;q12),add(12)(p11.2)[3]/46,XX[17].ish t(12;21)(p13;q22)(*ETV6*+, *RUNX1*+;*RUNX1* + 1,*ETV6* + 1)[20]. After chemotherapy, she achieved complete remission and has been healthy for 2.5 years without relapse. While the specificity of SSCP used in this study was addressed by the recurrent sequencing of positives with the earlier data, its sensitivity was not. It is possible that the negative mutation status could be a result of the failure of SSCP to detect a specific variant. Under suitable conditions, SSCP is capable of detecting >90% of mutations occurring within any sequence, and the sensitivity of PCR-SSCP is generally believed to be high if the fragments are <200 bp [6]. Because we analyzed the samples with products <200 bp by SSCP, it is unlikely that we would miss the *LEPROTL1* mutations.

One goal of this study was to identify whether the promoter mutation found in

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Table 1. *LEPROTL1* promoter mutation in tumors from 2,683 patients

Type of tumor	Tumors, <i>n</i>	<i>LEPROTL1</i> promoter		Location
		wild-type	mutation (%)	
Adulthood AML	299	299	0 (0)	chr8:29952919G>C
Adulthood ALL	176	176	0 (0)	
Childhood AML	31	31	0 (0)	
Childhood ALL	361	360	1 (0.5)	
Multiple myeloma	76	76	0 (0)	
Myelodysplasia	68	68	0 (0)	
Non-Hodgkin lymphoma	127	127	0 (0)	
Non-small-cell lung cancer	170	170	0 (0)	
Gastric carcinoma	247	247	0 (0)	
Colorectal carcinoma	438	438	0 (0)	
Prostate carcinoma	270	270	0 (0)	
Ovarian epithelial tumors	25	25	0 (0)	
Ovarian stromal tumors	115	115	0 (0)	
Hepatocellular carcinomas	43	43	0 (0)	
Hepatoblastomas	13	13	0 (0)	chr8:29952921C>T
Esophageal squamous-cell carcinomas	32	32	0 (0)	
Laryngeal squamous-cell carcinomas	19	18	1 (5.3)	
Leiomyoma	68	68	0 (0)	chr8:29952921C>G
Gastrointestinal stromal tumors	21	21	0 (0)	
Sarcomas	56	55	1 (1.8)	
Meningioma	28	28	0 (0)	
Total	2,683	2,680	3 (0.11)	

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

breast cancer is common to other cancer types, including hematologic malignancies. The mutation prevalence in ALL (0.5%), laryngeal carcinoma (5.3%), and sarcoma (1.8%) is not significantly different ($p > 0.05$) from that in breast cancers (1.7%) [3]. Because *LEPROTL1* is ubiquitously expressed in tissues including breast tissue, and is lost in almost all types of cancers, we expected to find some mutations in the large cohort of hematologic and solid tumors. However, we detected the promoter mutation in only 3 cancers, which is a very low prevalence (3/2,683 [0.11%]). In

the hematologic tumors, only 1 ALL case exhibited the mutation.

These data indicate that *LEPROTL1* promoter mutations may be rare in both common hematologic and solid cancers. The discovery of the recurrent *LEPROTL1* promoter mutations in breast cancers offered an opportunity for developing therapeutic and diagnostic tools targeting the mutations. Our results, however, indicate that applications targeting the *LEPROTL1* mutations may not be clinically available in acute leukemia in the future due to the low incidence of the *LEPROTL1* mutation.

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Disclosure Statement

The authors report no conflicts of interest.

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