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Association study of integrins beta 1 and beta 2 gene polymorphism and papillary thyroid cancer

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Abstract

BACKGROUND: We investigated whether single nucleotide polymorphisms (SNPs) of integrin beta 1 *(ITGB1)* and integrin beta 2 *(ITGB2)* contribute to the development of papillary thyroid cancer (PTC).

METHODS: Two synonymous SNPs (rs2230396 and rs2298141) of *ITGB1* and 1 synonymous SNP (rs2352326), 1 5′ URT-region SNP (rs2070947), and 1 promoter SNP (rs2070946) of *ITGB2* SNPs were genotyped using direct sequencing in 94 patients with PTC and 213 healthy controls. Genetic data were analyzed using SNPStats (http://bioinfo.iconcologia.net/SNPstats), Helix Tree (Golden Helix Inc, Bozeman, MT), and SNPAnalyzer (ISTECH Corp, Goyang City, Republic of Korea).

RESULTS: The promoter SNP (rs2070946) of *ITGB2* was significantly associated with the development of PTC (dominant model, log-additive model). The *G* allele frequencies of the promoter SNP (rs2070946) of *ITBG2* in patients with PTC (19.9%) were increased by about 2-fold compared with controls (10.2%).

CONCLUSIONS: Our results suggest that a promoter SNP (rs2070946) of *ITGB2* might be associated with a risk of PTC.

Integrins are a diverse family of glycoproteins that form heterodimeric receptors for extracellular matrix (ECM) molecules.[1] The 18 alpha and 8 beta subunits combine to form at least 25 different integrins. Integrin beta 1 (ITGB1) adheres to vascular cell adhesion protein 1 on stromal cells and to fibronectin, and integrin beta 2 (ITGB2) binds to intercellular adhesion molecule 1 on hematopoietic or stromal cells.[2] In addition to regulating cell adhesion to the ECM, integrins relay molecular cues regarding the cellular environment; these cues influence cell shape, survival, proliferation, gene transcription, and migration and may play a major role in carcinogenesis, tumor behavior, and metastasis.[3,4] Integrin expression or signaling is often altered in squamous cell carcinomas (SCCs). In studies of chemically induced skin carcinogenesis, overexpression of integrins in the suprabasal layers alters susceptibility to tumor development.[5–7] A heterozygous mutation in the integrins can contribute to neoplasia, and the degree of differentiation in SCC of the tongue is inversely correlated with prognosis.[8]

A genetic predisposition for papillary thyroid cancer (PTC) has been suggested by case-control studies showing a 3- to 8-fold increase in risk in first-degree relatives, one of the highest such risks of all cancers.[9,10] Despite unequivocal evidence of heritability, large families displaying mendelian inheritance of PTC are rare, and no predisposing genetic

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factors have been convincingly described.[11,12] Genetic polymorphisms are responsible for interindividual variation and diversity. They have been recently considered as the main genetic elements involved in the development of common and complex diseases. Several single nucleotide polymorphisms (SNPs) have been evaluated for their roles in inflammatory diseases and cancer predisposition.[13–16] In this study, we investigated whether SNPs in ITGB1 and ITGB2 contribute to the development of PTC.

Methods

Subjects and controls

Patients with PTC were enrolled at the Kyung Hee University Medical Center, Seoul, Republic of Korea. All patients underwent total thyroidectomy with central neck dissection. Control subjects were selected from healthy individuals examined under a general health check-up program who had no clinical evidence of cancers, thyroid disease, or any other severe conditions. PTC diagnoses and the presence of regional lymph node metastases were confirmed by pathologic examination. The specimens that were diagnosed as variant forms of PTC, such as follicular variants, diffuse sclerosing variants, and tall cell variants, were excluded. This study was approved by the Institutional Review Board of the Medical Research Institute, Kyung Hee University Medical Center. Written informed consent was obtained directly from all subjects.

Single-nucleotide polymorphism selection and genotyping

We searched promoter and coding SNPs of *ITGB1* and *ITGB2* genes. The related information of the SNPs was obtained from the SNP database (www.ncbi.nlm.nih.gov/SNP, dbSNP Build 132) of the National Center of Biotechnology Information. Among SNPs of *ITGB1* and *ITGB2*, SNPs with unknown heterozygosity, minor allele frequency less than 10%, and unknown genotype in Asians were excluded. Two synonymous SNPs (rs2230396 and rs2298141) of *ITGB1* and 1 synonymous SNP (rs235326), 1 5' URT-region SNP (rs2070947), and 1 promoter SNP (rs2070946) of *ITGB2* SNPs were selected to analyze in this study. Blood samples for DNA extraction from each subject were collected in tubes with ethylenediaminetetraacetic acid and then stored in a −80°C refrigerator. Genomic DNA was extracted using a QIAamp DNA minikit (QIAGEN, Valencia, CA). SNP genotyping was conducted by direct sequencing. Polymerase chain reaction was performed using specific primers for the *ITGB1* and *ITGB2* SNPs that were selected for analysis (Table 1). Polymerase chain reaction products were sequenced using an ABI PRISM 3730XL analyzer (Applied Biosystems, Life Technologies, Carlsbad, CA), and sequence data were analyzed using SeqMan II software (DNASTAR Inc, Madison, WI).

Statistical analyses

Continuous variables are presented as mean ± standard deviation and were analyzed by independent *t* tests and chi-square tests. For all SNPs, the Hardy-Weinberg equilibrium (HWE) was assessed using SNPStats software (http://bioinfo.iconcologia.net/SNPstats) in both patients and controls and was adjusted for age and sex. We used Helix Tree (Golden Helix Inc, Bozeman, MT) and SNPAnalyzer (ISTECH Corp, Goyang City, Republic of Korea) to analyze genetic data. Multiple logistic regression models (codominant, dominant, recessive, and log-additive) were used to obtain odds ratios (ORs), 95% confidence intervals (CIs), and *P* values. All data analysis was performed using PASW Statistics, version 18.0 (IBM Corp, Armonk, NY). Statistical significance was set at P less than .05.

Results

The study sample was comprised of 27 male and 67 female patients. The mean age of the patients was 53.2 ± 12.0 years. The control sample included 213 healthy adults (55.4 ± 6.0 years), composed of 108 male and 105 female individuals. The genotypic distributions of 5 SNPs examined in this study were consistent with the HWE. The *P* values for the HWE of rs2230396, rs2298141, rs235326, rs2070947, and rs2070946 were .19, .10, .53, .22, and .14, respectively.

In analyses of genotype data from 94 patients with PTC and 213 controls, the promoter SNP (rs2070946) of *ITGB2* was significantly associated with the development of PTC (dominant model, *A/A* vs *A/G* + *G/G*; OR, 1.84; 95% CI, 1.04 to 3.25; $P = .038$; log-additive model, *A/A* vs *A/G*

Table 1 Primer sequences for the *ITGB1* and *ITGB2* SNPs analyzed in this study

SNP	Gene	Forward	Reverse	Product size (bp)
rs2230396	*ITGB1*	CTGTTTCTCTGGCCTCTGTG	CAATGTTTTCTACAGAAAATGC	337
rs2298141	*ITGB1*	AACACCAGCTAAGCTCAGGAAC	GGCACTTTATGTATACAGGCAA	362
rs235326	*ITGB2*	ATCTGAGCATCAGCTCTTCCTG	GCTCGGGGATGGTTCAACAG	380
rs2070946	*ITGB2*	GTTAACAGGACCTCATCCATCC	GCTTCCATCAGATTCACACTTG	339
rs2070947	*ITGB2*	GGACCTCATGTGAGAAATGACA	AGAGTGCTTCCCTCCAAAAATC	325

bp = base pair; ITGB1 = integrin beta 1; ITGB2 = integrin beta 2; SNP = single nucleotide polymorphism.