Clinical Surgery

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

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KEYWORDS:
Papillary thyroid cancer; Integrin; Polymorphism

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 SNStats (http://bioinfo.iconcologia.net/SNStats), 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 ITGB2 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.

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Integrins are a diverse family of glycoproteins that form heterodimeric receptors for extracellular matrix (ECM) molecules. 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. 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. 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. 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.

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. Despite unequivocal evidence of heritability, large families displaying mendelian inheritance of PTC are rare, and no predisposing genetic
factors have been convincingly described. 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. 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 (IS-TECH 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 + G/G; OR, 1.84).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2230396</td>
<td>ITGB1</td>
<td>CTGTTTCTGCGCTCCTGTG</td>
<td>CAATGTTCCTACAGAAAAATGC</td>
<td>337</td>
</tr>
<tr>
<td>rs2298141</td>
<td>ITGB1</td>
<td>AACACACCTAGTGCTGGAAAC</td>
<td>GGCACCTTATGTAACAGGGCA</td>
<td>362</td>
</tr>
<tr>
<td>rs235326</td>
<td>ITGB2</td>
<td>ATCTGAGCATCAGCTCTCGT</td>
<td>GCTCGGGATGTGTCCACAG</td>
<td>380</td>
</tr>
<tr>
<td>rs2070946</td>
<td>ITGB2</td>
<td>GTTACAGGACCTCATCCC</td>
<td>GCTTTCCATACAGTCACTTG</td>
<td>339</td>
</tr>
<tr>
<td>rs2070947</td>
<td>ITGB2</td>
<td>GACATCTATGTGAAATGACA</td>
<td>AGAGTGCTTCCTCCAAAAATC</td>
<td>325</td>
</tr>
</tbody>
</table>

bp = base pair; ITGB1 = integrin beta 1; ITGB2 = integrin beta 2; SNP = single nucleotide polymorphism.
vs G/G; OR, 1.99; 95% CI, 1.18 to 3.36; \( P = .009 \). The SNPs (rs2230396 and rs2298141) of ITGB1 and the SNPs (rs2352326 and rs2070947) of ITGB2 SNPs were not associated with PTC (Tables 2 and 3).

We also detected a significant difference between patients with PTC and controls with respect to gene allele frequencies of ITGB1 and ITGB2. The \( G \) allele frequencies of the promoter SNP (rs2070946) of ITGB2 in patients with PTC (19.9%) were increased by about 2-fold compared with controls (10.2%) (Table 2). However, we did not find significant differences in gene allele frequencies of the SNPs (rs2230396 and rs2298141) of ITGB1 and the SNPs (rs2352326 and rs2070947) of ITGB2 between patients with PTC and controls (Tables 2 and 3).

**Comments**

We investigated the relationships between the ITGB1 and ITGB2 SNPs and PTC. The finding of this study is that the promoter SNP (rs2070946) of ITGB2 is associated with the development of PTC.

Tumorigenesis is strongly affected by nonmalignant cells (ie, stromal cells) that compose the tumor microenvironment.\(^\text{17}\) A large number of genes abnormally expressed in human cancer encode secreted proteins and receptors, with paracrine and autocrine effects on other components of the tumor, such as stromal cells and ECM noncellular components.\(^\text{18,19}\) Dynamic and reciprocal interactions involving cell adhesion molecules (eg, integrins), ECM noncellular components, and soluble cytokines occur between tumor epithelial cells and tumor microenvironment stromal cells.\(^\text{20}\) The degree of these interactions may represent the basis of the triggering of intracellular signaling pathways that confer tissue-specific characteristics to the epithelium.\(^\text{20}\) ECM composition and organization undergo radical alterations in human cancers and could affect cell survival, proliferation, adhesion, migration, and other properties of both tumor and stromal cells. Many altered gene sets are involved in the composition and remodeling of the ECM, such as thrombospondin-1, transforming growth factor–\( \beta \)1, integrins, fibronectin, CD44, cathepsin B, and cathepsin S. These genes seem to be either targeted

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**Table 2** Frequencies of genotype and allele in SNPs of ITGB2 in patients with PTC and control subjects after adjustment for sex and age

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>PTC n (%)</th>
<th>Control n (%)</th>
<th>Model</th>
<th>OR (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ITGB2 promoter (rs2070946)</strong> -149</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>61 (65.6)</td>
<td>168 (79.6)</td>
<td>Codominant 1(^*)</td>
<td>1.58 (0.88–2.85)</td>
<td>.13</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>27 (29.0)</td>
<td>43 (20.4)</td>
<td>Codominant 2(^†)</td>
<td>NA</td>
<td>.33(^1)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>0 (0.0)</td>
<td>5 (5.4)</td>
<td>Dominant</td>
<td>1.84 (1.04–3.25)</td>
<td>.038</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td></td>
<td></td>
<td>Recessive</td>
<td>NA</td>
<td>.33(^1)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>149 (80.1)</td>
<td>379 (89.8)</td>
<td>Log-additive(^‡)</td>
<td>1.99 (1.18–3.36)</td>
<td>.0091</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>37 (19.9)</td>
<td>43 (10.2)</td>
<td></td>
<td>2.19 (1.36–3.53)</td>
<td>.001</td>
</tr>
<tr>
<td><strong>ITGB2 synonymous (rs235326)</strong> Val441Val</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>66 (70.2)</td>
<td>135 (63.4)</td>
<td>Codominant 1</td>
<td>0.66 (0.37–1.18)</td>
<td>.15</td>
</tr>
<tr>
<td></td>
<td>T/C</td>
<td>23 (24.5)</td>
<td>67 (31.5)</td>
<td>Codominant 2</td>
<td>1.09 (0.34–3.44)</td>
<td>.95</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>5 (5.3)</td>
<td>11 (5.2)</td>
<td>Dominant</td>
<td>0.71 (0.41–1.23)</td>
<td>.22</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td></td>
<td></td>
<td>Recessive</td>
<td>NA</td>
<td>.33(^1)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>155 (82.4)</td>
<td>337 (79.1)</td>
<td>Log-additive(^‡)</td>
<td>1.24 (0.40–3.87)</td>
<td>.71</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>33 (17.6)</td>
<td>89 (10.9)</td>
<td></td>
<td>0.82 (0.52–1.29)</td>
<td>.38</td>
</tr>
<tr>
<td><strong>ITGB2 5’ UTR (rs2070947)</strong></td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>63 (67.0)</td>
<td>112 (52.6)</td>
<td>Codominant 1</td>
<td>0.58 (0.33–1.03)</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>T/C</td>
<td>25 (26.6)</td>
<td>90 (42.2)</td>
<td>Codominant 2</td>
<td>1.15 (0.39–3.43)</td>
<td>.65</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>6 (6.4)</td>
<td>11 (5.2)</td>
<td>Dominant</td>
<td>0.64 (0.38–1.10)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td></td>
<td></td>
<td>Recessive</td>
<td>NA</td>
<td>.33(^1)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>151 (80.3)</td>
<td>314 (73.7)</td>
<td>Log-additive(^‡)</td>
<td>1.42 (0.48–4.15)</td>
<td>.53</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>37 (19.7)</td>
<td>112 (16.3)</td>
<td></td>
<td>0.78 (0.50–1.22)</td>
<td>.28</td>
</tr>
</tbody>
</table>

CI = confidence interval; ITGB2 = integrin beta 2; NA = not applicable; OR = odds ratio; PTC = papillary thyroid cancer; SNP = single nucleotide polymorphism.

\(^*\)Codominant 1, A/A vs A/G.

\(^†\)Codominant 2, A/A vs G/G.

\(^‡\)Log-additive, A/A vs A/G vs G/G.

\(^\text{Fischer exact test was used.}\)
or affected by the BRAF V600E mutation in PTC. They might act in concert and elicit important biologic crosstalk during tumor cell adhesion, migration, and invasion processes involving the tumor microenvironment and ultimately trigger thyroid cancer progression. Key integrins showed significantly higher mRNA levels in BRAF V600E-positive PTC compared with wild-type BRAF PTC or normal thyroid tissue and may mediate thyroid tumor cell migration and invasion.

Activation of integrins leads to recruitment of intracellular proteins, resulting in focal adhesion formation, which then provides a platform for cell adhesion and integrin signaling. Integrins are regulated by both environmental (outside-in) and intracellular (inside-out) signals. Outside-in activation occurs when components of the ECM bind and activate integrin, resulting in functional integrin heterodimers. Conversely, inside-out integrin activation can be initiated through a variety of mechanisms, including signaling downstream of RET receptor tyrosine kinase. Receptor tyrosine kinases can regulate integrin activity either directly through interaction or indirectly through transcriptional upregulation of integrins or regulation of other signaling pathways. Recently, inhibiting agents for RET or integrin have shown efficacy in preventing migration of pancreatic cancer cells, suggesting a cooperative regulation of cell migration and, ultimately, tumor invasion, progression, and metastasis. The ability of tumors to exploit multiple integrin heterodimers provides advantages in adapting to pressures from the changing microenvironment during tumor progression. In addition, ITGB1 is also fundamental in activating the FAK signaling axis to control the initial proliferation of micrometastatic mouse breast cancer cells disseminated in the lungs.

There have been several studies about the association with cancer and the polymorphisms of integrin. The integrin alpha 2 1648 AA genotype was significantly associated with breast cancer. Functional polymorphisms in integrin genes integrin alpha2 and integrin beta3 influence the development and progression of breast cancer, respectively. In the large prospective Copenhagen City Heart Study, the homozygous integrin beta3 176 CC genotype was associated with a higher risk for breast cancer. The integrin alpha2 807C>T polymorphism may be associated with reduced colorectal cancer risk. The contribution of integrin engagement to RET-mediated cell migration in neoplastic models of neural and thyroid cell migration has been documented. Heterozygous mutation was identified in the ITGB1 subunit of a poorly differentiated SCC. In this result, promoter SNP of the ITGB2 gene was associated with the development of PTC.

To determine whether alleles of rs2070946 of HLA-G relate to transcription factors, we used an online program (AliBaba 2.1; available at: http://www.gene-regulation.com/pub/programs.html#alibaba2). At the rs2070946 SNP site, it was shown that A-containing sequences can act with AP-1, C/EBPα1P, Oct-1, and Sp1 transcription factor, but AP-1, C/EBPα1P, and Oct-1 transcription factor disappear in G-containing sequences. Assuming that transcription factor binding varies with promoter SNPs, this promoter SNP may influence gene and protein expression of ITGB2.

Our study indicates that it will be worthwhile to develop mouse models of additional integrin polymorphisms. Knowledge about this new SNP of ITGB2 may help identify biomarkers or targets, or both, for innovative therapeutic strategies in PTC. Biomarkers for PTC could provide a tool to identify high-risk individuals. High-risk individuals...
may need to have more detailed examinations to detect occult PTC. Therapeutic strategies aimed at modulating the host microenvironment may offer a complementary perspective for the treatment of patients with PTC. Also, if our findings are replicated in different populations, ITGB2 might have a clinical application in helping to establish a definitive diagnosis of PTC preoperatively in patients with thyroid nodules.

Conclusions

In conclusion, in a case-controlled study of SNPs in the ITGB1 and ITGB2 genes in patients with PTC and in control subjects, we observed a significant association between promoter polymorphisms of the ITGB2 gene and the development of PTC.

References