Genetic Alterations in Papillary Thyroid Carcinoma and Hashimoto Thyroiditis

An Analysis of hOGG1 Loss of Heterozygosity

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Objectives: To determine the relationship between hOGG1 loss of heterozygosity (LOH), Hashimoto thyroiditis (HT), and papillary thyroid cancer (PTC). Hashimoto thyroiditis is an autoimmune-mediated chronic inflammatory disease previously shown to coexist with papillary PTC. To further define the relationship between HT and PTC, we report an analysis of hOGG1, a major repair gene for free radical–induced oxidative DNA damages, in thyroidectomy specimens.

Design: Tissue samples from 20 cases of PTC, 20 cases of HT, and 15 cases of benign goiter were included in this study. Samples of DNA collected from laser-capture microdissection of thyroidectomy specimens were analyzed for hOGG1 LOH by polymerase chain reaction (PCR) amplification using 5 fluorescent-labeled microsatellite markers followed by fragment analysis.

Setting: A university tertiary care center and regional veterans' hospital.

Results: Amplification by PCR was successful for all 5 markers in 18 cases of PTC, 15 cases of HT, and 12 cases of benign thyroid. Among these samples, hOGG1 LOH was found in 17 of 18 PTC specimens (94%), 11 of 15 HT specimens (73%), and 1 of 12 benign goiter specimens (8%).

Conclusions: hOGG1 LOH is strongly associated with PTC and HT but not with benign thyroid. We hypothesize that thyroid follicular epithelia accumulate aberrant genetic changes in long-standing HT, which may represent a precursor lesion of PTC.


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The mechanism of carcinoma formation in the presence of chronic inflammation is proposed to be due to large quantities of oxidative species (oxidative stress) produced by recruited inflammatory cells. These reactive oxidative species damage genetic material and, if the damage to critical tumor suppressor genes is not repaired, result in the development of malignant neoplasms. Loss of heterozygosity (LOH) of the gene (hOGG1) encoding human 8-oxoguanine DNA glycosylase (hOGG1), a key repair enzyme of DNA damaged by reactive oxygen species, has been strongly associated with head and neck squamous cell carcinoma. To our knowledge, no studies have evaluated the association between LOH of hOGG1 and PTC and/or HT.

To elucidate the possible relationship between the chronic inflammation known to occur in HT and PTC, we present herein an analysis of LOH of hOGG1 in HT, PTC, and goitrous thyroid specimens.
METHODS

Following approval by the institutional review board, we obtained thyroid tissue from partial or total thyroidectomy surgical specimens excised at the Central Arkansas Veterans Healthcare System and University of Arkansas for Medical Sciences. Paraffin-embedded thyroid tissue was analyzed from 55 patients (20 PTC cases, 20 HT cases, and 15 cases of benign thyroid). The clinical indication for partial or total thyroidectomy included PTC, HT, and benign goiter. Laser-capture microdissection (LCM) was used to obtain DNA from these thyroid specimens. Two DNA samples were obtained for each case: one from thyroid tissue (PTC, HT, or benign goiter) and the other from adjacent fibroadipose tissue. Analysis of LOH was performed by comparing the allelic profile (number and height of the allele) from the case tissue (thyroid) with that from the normal control tissue (fibroadipose tissue) (Figure). Polymerase chain reaction amplification was performed with 5 fluorescent-labeled microsatellite markers adjacent to the hOGG1 (OMIM 601982) locus (3S1297, 3S1289, 3S1300, 3S1261, and 3S1274). Fragment analysis was subsequently performed using an ABI PRISM 3100 Genetic Analyzer (Life Technologies, Carlsbad, California).

RESULTS

A total of 55 specimens were identified and sectioned (20 PTC, 20 HT, and 15 benign goiter). Of these tissue blocks, 45 of the collected DNA specimens were adequate for analysis (82%) (18 PTC [40%], 15 HT [33%], and 12 benign goiter specimens [27%]). The results of the hOGG1 LOH analysis in these specimens are listed in the Table. The hOGG1 LOH in at least 1 of the 5 microsatellites was noted in 17 of 18 PTC specimens (94%), 11 of 15 HT specimens (73%) and 1 of 12 benign goiter specimens (8%).

The DNA microsatellite analysis that we performed is illustrated in the Figure. In this example, the normal fibroadipose tissue (Figure, A and C) is informative, consisting of 2 separate alleles and with 2 analyzed microsatellite markers (D3S1297 and D3S1274). A papillary thyroid carcinoma specimen (Figure, B and D) retained heterozygosity (no hOGG1 LOH) at D3S1297 but lost 1 allele (hOGG1 LOH) at D3S1274.

Of note, 10 of the 18 PTC thyroid specimens also contained HT. Eight of these 10 HT specimens had LOH pat-
An association of HT and PTC was first proposed in 1955. Subsequently, multiple studies have confirmed this finding. Recently, Repplinger et al reviewed 1198 thyroid surgery cases and noted a 29% incidence of PTC in patients with HT, prompting the conclusion that the presence of HT is associated with an increased risk of developing PTC in women. No significant association was found for men in the study, but the authors attribute this to the small sample size of male thyroid specimens.

Similarly, Cipolla et al noted a 28% incidence of PTC in subjects with HT and concluded that HT may be a precursor of PTC. Kurukahvecioglu et al also found a significant association between HT and PTC in their analysis of 922 patients undergoing thyroid surgery. The results prompted their recommendation that total thyroidectomy be performed in young female patients with HT.

While the association between HT and PTC has been well documented, the biologic cause for the association has been less thoroughly investigated. A notable exception is a study by Unger et al evaluating the expression of p63 in PTC and HT. This study found that p63, a homolog of p53, was found in 78.8% of HT specimens (although rarely in Grave disease) and in 81.8% of PTC specimens. The authors note that p63 may be a potential pathobiologic link between HT and PTC.

The pathobiologic link of interest in our study, hOGG1, is a key repair enzyme of DNA damaged by reactive oxygen species. Previously, hOGG1 LOH has been strongly associated with head and neck squamous cell carcinoma. In addition, hOGG1 LOH has also been reported with other carcinomas including those of the prostate and lung. Despite these associations, to our knowledge, no previous studies have evaluated the presence of hOGG1 LOH in PTC and HT.

Our hypothesis that PTC may result from the long-standing chronic inflammation in HT is supported by the high incidence of hOGG1 LOH in both HT and PTC and the absence of hOGG1 LOH in benign goiter specimens. The presence of HT in 10 of the 18 PTC specimens is perhaps the most convincing evidence of a link between hOGG1 LOH, HT, and PTC. In 8 of the 10 cases, the LOH patterns between PTC and HT were identical. Despite these findings, however, a direct cause-effect relationship remains unclear and requires further investigation.

In conclusion, HT is a chronic inflammatory condition with a known histologic association with PTC. Our data reveal a high prevalence of hOGG1 LOH in both HT and PT but not in benign thyroid. While a direct cause-effect relationship between HT and PTC remains unclear, our data suggest the possibility that thyroid follicular epithelia accumulate aberrant genetic changes in long-standing HT. These genetically altered epithelia in HT may ultimately progress to PTC.

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Author Contributions: Dr Fan had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Royer and Zhang contributed equally to this article. Study concept and design: Kokoska. Acquisition of data: Zhang and Fan. Analysis and interpretation of data: Royer, Zhang, Fan, and Kokoska. Drafting of the manuscript: Royer. Critical revision of the manuscript for important intellectual content: Royer, Zhang, Fan, and Kokoska. Statistical analysis: Royer. Obtained funding: Fan. Administrative, technical, and material support: Zhang and Fan. Study supervision: Kokoska.

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Table. Detection of hOGG1 LOH

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>hOGG1 LOH</th>
<th>No hOGG1 LOH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTC</td>
<td>17 (94)</td>
<td>1 (6)</td>
<td>18</td>
</tr>
<tr>
<td>HT</td>
<td>11 (73)</td>
<td>4 (27)</td>
<td>15</td>
</tr>
<tr>
<td>Benign goiter</td>
<td>1 (8)</td>
<td>11 (92)</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>16</td>
<td>45</td>
</tr>
</tbody>
</table>

Abbreviations: HT, Hashimoto thyroiditis; LOH, loss of heterozygosity; PTC, papillary thyroid cancer.

a Unless otherwise noted, data are reported as number (percentage) of specimens.