TGFβ, TGFβ Receptors, Ki-67, and p27Kip1 Expression in Papillary Thyroid Carcinomas

Ricardo V. Lloyd, MD, PhD, Jorge A. Ferreiro, MD, Long Jin, MD, and Tom J. Sebo, MD

Abstract

Although most papillary thyroid carcinomas behave as low-grade neoplasms and are generally associated with a good prognosis, some subgroups of these neoplasms represent more aggressive variants. In order to determine if differences in the behavior of these papillary carcinomas were related to expression of growth factors or cell-cycle proteins, we analyzed a series of papillary carcinomas including the conventional or usual type (n = 27), tall cell (n = 27), diffuse sclerosing (n = 5), and columnar cell (n = 2) variants for expression of transforming growth factor beta (TGFβ), TGFβ receptors (TGFβ-RI and II), the proliferation marker Ki-67, and for the cell-cycle inhibitory protein p27Kip1 (p27). All groups of thyroid tumors expressed TGFβ and TGFβ-RI and RII by immunohistochemical staining. There was a marked increase in the Ki-67 labeling index after staining with antibody MIB-1 in the columnar cell tumors compared to the other groups, but this difference was not significant because of the small number of tumors in this group. The cell-cycle inhibitory protein p27 was expressed in all groups and was not significantly different between groups. Normal thyroid cells had a higher labeling index for p27 compared to papillary carcinomas. These results indicate that TGFβ and TGFβ receptors I and II are commonly expressed in the usual and in variant forms of papillary thyroid carcinomas, and that there is decreased expression of p27 protein in all of these neoplasms compared to normal thyroid. The biological basis for the more aggressive behavior of these variants of papillary thyroid carcinoma remains uncertain.

Key Words: Thyroid; papillary carcinoma; p27; Ki-67; transforming growth factor beta.

Introduction

There are several variants of papillary thyroid carcinomas [1,2]. Some of these subtypes are associated with a more aggressive biological course [1–11]. The more aggressive variants include tall cell and columnar cell carcinomas and the diffuse sclerosing variants. The first two variants often develop in older patients and not infrequently lead to death from carcinomas [3–9]. The diffuse sclerosing variant occurs in younger patients, is frequently associated with metastatic disease, but is not a frequent cause of death in these patients [10,11]. The biological reasons for these differences in behavior of these variants is unknown.

Various markers have been used to analyze thyroid carcinomas including p53 and Ki-67 [12,13]. High-grade thyroid neoplasms such as anaplastic carcinomas are often positive for p53 suggesting mutations in this gene, and anaplastic carcinomas often have a high proliferative index detected by nuclear staining for Ki-67. Recent studies have detected TGFβ expression in thyroid tissues [14,15]. Other studies have shown that TGFβ has an
Table 1. Antibodies Used in the Analysis of Papillary Carcinomas

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Type</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIB-1</td>
<td>Monoclonal</td>
<td>AMAC (Westbrook, ME)</td>
<td>1/50</td>
</tr>
<tr>
<td>p27</td>
<td>Monoclonal</td>
<td>Transduction Labs (Lexington, KY)</td>
<td>1/1000</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Polyclonal</td>
<td>R and D Systems (Minneapolis, MN)</td>
<td>1/100</td>
</tr>
<tr>
<td>TGFβ-RI</td>
<td>Polyclonal</td>
<td>Santa Cruz Biotechnology (Santa Cruz, CA)</td>
<td>1/500</td>
</tr>
<tr>
<td>TGFβ-RII</td>
<td>Polyclonal</td>
<td>Santa Cruz Biotechnology</td>
<td>1/250</td>
</tr>
</tbody>
</table>

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inhibitory role on tumor growth and that loss of response to TGFβ by genetic changes in TGFβ receptor (TGFβ-R) may be associated with the development of more aggressive tumors [16–20]. Thus, an analysis of TGFβ and TGFβ-R in the usual and more aggressive variants of papillary carcinomas should allow us to explore the possibility of TGFβ-R loss in some papillary carcinomas.

TGFβ also has a direct role in regulating cell-cycle progression by affecting various cell-cycle proteins such as p27kip1 (p27) and p15ink4a (p15) [21–23]. Expression of p27 in thyroid neoplasms has not been previously examined. Because p27 is an inhibitory cell-cycle protein, the relationship between p27 and Ki-67 may provide insight into the biological action of thyroid neoplasms such as papillary carcinoma.

We analyzed a series of the usual type of papillary carcinomas and the more aggressive variants of these neoplasms to determine if differences in various immunohistochemical markers could explain differences in the behavior of these carcinomas.

**Materials and Methods**

**Cases**

Papillary carcinomas (1500) in the Mayo Clinic files were reviewed to identify various histological types. There were 33 tall cell carcinomas, three columnar cell carcinomas, and eight diffuse sclerosing variants. Six tall cells, one columnar cell, and three diffuse sclerosing variants were excluded because of the lack of immunoreactivity or insufficient tissues for immunohistochemical staining. Twenty-seven randomly selected cases of the usual type of papillary carcinomas were used as controls.

Histological analysis of all cases was performed. Tall cell carcinomas had 30% or more of the neoplasm composed of cells twice as tall as they were wide as previously described [3,4]. Other features such as squamous areas, lymphocytic and neutrophilic infiltrates, and mitoses were also examined.

**Immunohistochemical Analysis**

Immunohistochemical studies were performed with the antibodies and dilutions summarized in Table 1 using the Avidin Biotin Peroxidase method. Antigen retrieval by microwave treatment for 15 min in a 800-watt microwave oven was done for all antibodies before immunostaining.

Staining for MIB-1 and p27 was analyzed by counting 1000 cells/slide using at least 10 microscopic fields per slide. TGFβ and TGFβ-R were analyzed by positive or absent staining. The staining intensity was graded as: 0, absent staining; 1+, weak; 2+, moderate; and 3+, strong staining. p53 positive cases had more than 10% of cells positive in the nucleus for this protein.

Positive controls included a breast carcinoma for TGFβ and TGFβ receptors and tonsil tissue for Ki-67 and p27. Negative controls consisted of substituting normal rabbit or mouse serum for the primary antibodies.

Statistical analysis was done with the Wilcoxon rank-sum test.