Expression of ras p21 in benign and malignant thyroid lesions

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Received June 21, 1993; Accepted August 18, 1993

Abstract. It is now widely accepted that human neoplasms arise as a result of a sequence of mutations affecting the structure of genes involved in growth control. Identifying the nature of such genetic mutations in thyroid neoplasms and their prevalence in the various tumor phenotypes is critical to the understanding of their pathogenesis. Mutational activation of ras oncogenes has been associated with human thyroid neoplasia. We examined the expression of ras oncogene in benign hyperplastic or inflammatory lesions of the goiter, as well as in benign and malignant thyroid tumors. Although no significant differences of ras oncogene expression was found between benign and malignant lesions, the overexpression in proliferative areas generally suggests the possible involvement of ras oncogene in the trophic hormone control of the thyroid.

Introduction

Proto-oncogenes are thought to have regulatory roles in normal cell proliferation and differentiation (1,2). They may contribute to the development and/or maintenance of the malignant phenotype, when there is an alteration of their function. They are overexpressed in a wide variety of human cancers (3). In some cases tumor aggressivity and state of differentiation have been correlated to the expression of certain oncogenes (3).

The study of oncogene expression in the thyroid gland could be of particular interest, due to the existence of a wide range of both hyperplastic and neoplastic growth abnormalities, which are very common and are often subjected to surgical removal. ras oncogenes in particular have been found to be activated by qualitative (4) or quantitative (5-7) changes.

In this study we report data on the expression of ras-oncogene in benign hyperplastic or inflammatory lesions of the goiter in benign and malignant thyroid tumors. Our data show no significant differences in the expression of ras-oncogene between benign and malignant lesions. The overexpression in proliferative areas generally suggests the possible involvement of ras-oncogene in the trophic hormone control of the thyroid.

Materials and methods

The study was based on 66 cases of both benign and malignant conditions of thyroid. The tissue samples were collected from surgically removed thyroid lobes of patients subjected to either partial or total thyroidectomy. The cases included 16 nodular goiters (10 were of 'parenchymatous' type whereas the other 6 were of architecturally macrofollicular 'colloid' type), 2 cases of Grave's disease, 4 cases of Hashimoto's thyroiditis, 5 follicular adenomas and 39 cases of thyroid cancer and the following types of carcinomas: 23 papillary, 7 follicular, 8 medullary and 1 anaplastic. In 26 cases of thyroid the non-tumorous parenchyma appeared normal whereas in the 13 remaining tumors changes of lymphocytic thyroiditis of varying degree were demonstrable in the non-tumorous tissue.

The histological diagnosis was based on sections stained with H and E. The expression of H-ras-oncogene was studied on formalin-fixed and paraffin-embedded tissue sections by detection of ras p21 protein with the use of a 3-step immunoperoxidase technique as previously described (5). Paraffin sections were mounted on slides, incubated at 60°C for 24 hours and deparaffinized in xylene. The expression of the H-ras oncogene was examined in the sections of all cases with benign and malignant lesions as well as in the 'normal' parenchyma observed in the vicinity of thyroid tumors. The staining pattern was graded as negative or equivocal (±), moderate (+) or intense (++).

Results

The expression of H-ras oncogene was demonstrable by diffuse cytoplasmic immunoreactivity for ras p21 in the epithelial follicular cells or in the carcinomatous cells focally or in all regions of the examined tissue (Fig. 1). The non-tumorous parenchyma adjacent to follicular adenomas and to follicular and papillary carcinomas having normofollicular structure without any infiltration by inflammatory cells showed little or no reactivity for ras p21 (Fig. 2). The staining in the non-tumorous thyroid tissue by the presence of medullary carcinoma was more intense than by the presence of follicle cell-carcinomas. All cases of nodular
Figure 1. Papillary carcinoma of the thyroid. Intense (++) immunostaining for ras p21 in the cytoplasm of the carcinoma cells (X136).

Figure 2. Papillary carcinoma of the thyroid. Intense (++) immunostaining for ras p21 in the tumor cells and negative immunostaining in macrofollicular areas of the adjacent parenchyma (X136).

Figure 3. Nodular goiter of the thyroid. Intense (++) immunostaining for ras p21 in microfollicular areas and negative staining in macrofollicular areas (X136).

Figure 4. Hashimoto's thyroiditis. Positive (+) immunostaining for ras p21 of the metaplastic oxyphilic follicular cells (X136).

Figure 5. Follicular carcinoma of the thyroid, oxyphilic variant. Focally intense (++) immunostaining for ras p21 (X340).

goiter with focally or diffuse microfollicular ('adenomatous') pattern showed moderate to intense immunoreactivity (Fig. 3). Large epithelial cells in colloid-filled follicles were generally negative in ras expression as was demonstrable in the cases of 'colloid' goiters. Both cases of Crave's disease expressed moderate ras p21 as well as all cases of Hashimoto's thyroiditis, where the expression was even more intense particularly in cells with oxyphilic metaplasia (Fig. 4). Most of the neoplastic thyroid lesions expressed H-ras oncogene. The benign lesions (adenomas) showed slight or moderate immunoreactivity. Their malignant counterparts (follicular carcinomas) showed immunoreactivity to a slightly lesser extent (Fig. 5). The majority of papillary carcinomas showed strong staining for ras p21 and in only 2 cases no reaction was demonstrable. No significant differences were found in the staining pattern of the papillary carcinoma related to any histological characteristics, grade of differentiation, or stage of the disease. A summary of the immunohistological analysis of ras p21 expression in normal, benign and malignant thyroid lesions is shown in Table I. Finally, the ras p21 expression was weak in
tissues with prominent hyperplasia, such as Grave’s disease, may influence from the tumor tissue cannot be excluded. However, the presence of a tumor, though the possibility of normal tissue that can be obtained by the pathologist, indicates that the tissue examined lesions.

<table>
<thead>
<tr>
<th>Histological type of thyroid specimen</th>
<th>Total no. of cases</th>
<th>Staining intensity no. of cases (%)</th>
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<tr>
<td>Normal adjacent to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>papillary carcinoma</td>
<td>14</td>
<td>13(93) 1(7) -</td>
</tr>
<tr>
<td>follicular carcinoma</td>
<td>10</td>
<td>10(100) -</td>
</tr>
<tr>
<td>medullary carcinoma</td>
<td>6</td>
<td>3(50) 2(35) 1(17)</td>
</tr>
<tr>
<td>Benign</td>
<td></td>
<td></td>
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<tr>
<td>Nodular goiter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>’parenchymatous’</td>
<td>10</td>
<td>0 7(70) 3(30)</td>
</tr>
<tr>
<td>’colloid’</td>
<td>6</td>
<td>6(100) -</td>
</tr>
<tr>
<td>Grave’s disease</td>
<td>2</td>
<td>2(100) -</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>4</td>
<td>- 1(25) 3(75)</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>5</td>
<td>2(40) 3(60) -</td>
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<tr>
<td>Malignant carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>23</td>
<td>5(22) 12(52) 6(26)</td>
</tr>
<tr>
<td>Follicular.</td>
<td>7</td>
<td>4(57) 3(43) -</td>
</tr>
<tr>
<td>Medullary</td>
<td>8</td>
<td>5(62) 1(13) 2(25)</td>
</tr>
<tr>
<td>Anaplastic</td>
<td>1</td>
<td>1(100) -</td>
</tr>
</tbody>
</table>

Table I. Immunohistochemical analysis of ras p21 expression in normal, benign and malignant thyroid lesions.

Discussion

Thyroid glands subjected to surgical excision exhibit hyperplastic, inflammatory or neoplastic changes. Thus, the most ‘normal’ tissue that can be obtained by the pathologist is non-tumorous thyroid parenchyma from glands resected for the presence of a tumor, though the possibility of influence from the tumor tissue cannot be excluded.

The normal follicular thyroid parenchyma adjacent to follicular cell tumors (papillary and follicular carcinomas) which we examined showed only weak expression of ras- oncogenes or no expression at all, which is in agreement with reports of others (6,7) whereas the ‘normal’ thyroid tissue adjacent to medullary carcinoma showed moderate immunoreactivity for ras p21. This enhanced expression could be the result of epithelial alterations due to unknown peptides secreted by the neuroendocrine tumor cells.

Apparent enhanced expression of the ras- oncogene was demonstrable in most benign proliferative processes which produce goiters e.g. nodular hyperplasia, Grave’s disease and Hashimoto’s thyroiditis. Cell proliferation seemed to be the main histologic feature related to increased expression of ras-genes since the immunoreactivity was more intense in tissues with prominent hyperplasia, such as Grave’s disease and ‘adenomatous’ areas in nodular hyperplasia. The intense staining in Hashimoto’s thyroiditis could be explained by the fact that in many cases of Hashimoto’s thyroiditis cell proliferation can be prominent. Therefore, the intensive immunoreactivity for ras p21 of the oxyphilic cells suggests that oxyphilic metaplasia does not represent a degenerative change in Hashimoto’s thyroiditis. Our observations are in accordance with previous studies in rat thyroid carcinoma lines which have shown that TSH stimulates proto-oncogene expression (8).

The regulation of ras proto-oncogenes in normal thyroid tissue at different stages of differentiation is still unknown. Although ras oncogene mutations have been associated with thyroid neoplasia (9-13) it remains difficult to assess whether overexpression of ras-oncogenes could cause, or be a consequence of a carcinoma. We observed a significant overexpression of ras-oncogene in well-differentiated follicular-cell carcinoma, and even more intense overexpression in papillary carcinoma. We did not find, however, the elevated apical surface expression of the ras product reported by others (7). It is of interest that, the ras p21 expression in follicular adenomas was slightly more increased when compared to the malignant counterpart (follicular carcinoma). A similar finding has been reported for colon adenomas, where the expression for ras-oncogene was elevated as compared to that found in colon carcinoma (14,15). The similarity in the ras-oncogene expression of cellular hyperplastic nodules, follicular adenomas and follicular carcinomas suggest that ras p21 expression is unable to predict the potential biological behaviour of the examined lesions.

Another interesting finding was the observation that in medullary carcinoma ras p21 was expressed weakly as compared to the follicular cell carcinoma. Medullary carcinoma is a neuroendocrine tumor located in the thyroid gland and is characterized clinically and biochemically by its neurosecretory properties. It has been shown that, when a mutated ras-gene is transfected into a neuroendocrine small-cell lung carcinoma can change its phenotype to that suggesting a non-small cell lung carcinoma without neuroendocrine properties (16). This observation suggests that, ras-oncogene is not related to the neuro-endocrine differentiation.

Long term prognosis of well-differentiated follicular-cell thyroid carcinoma is favourable, modulated by several parameters such as age, histologic characteristics and sex (17). On the contrary, undifferentiated carcinoma or carcinoma with an anaplastic component are very aggressive tumors with rapid progression and fatal outcome.

A relationship has been sought between the overexpression of oncogenes and the prognosis of patients with thyroid cancers by using diverse prognostic indices (18). In our study we have not found any significant differences in the expression of ras oncogene in well-differentiated carcinoma when compared to prognostic parameters, such as size and extension of primary tumor, histological features or presence of metastases.

References


