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Immunohistochemical expression of Insulin-like growth factor-1, Transforming growth factor-beta1, and Vascular endothelial growth factor in parathyroid adenoma and hyperplasia

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Abstract

Background: Insulin-like growth factor (IGF), transforming growth factor-beta1 (TGF-β1), and vascular endothelial growth factor (VEGF) are commonly studied growth factors, but little data are available on the immunohistochemical expression of these factors in parathyroid lesions. **Materials and Methods:** Tissue specimens from 36 patients with primary hyperparathyroidism (P-HPT) (26 adenomas and 10 primary hyperplasias) were examined. Normal parathyroid tissue adjacent to the adenoma or area of hyperplasia was used as control tissue. Preoperative laboratory testing [serum Ca and P, creatinine and parathormone levels (PTH)] which led to the diagnosis of P-HPT had been performed, the size and weight of the parathyroid glands measured, and postoperative serum PTH levels determined. Paraffin-embedded parathyroid tissue specimens were stained with antibodies to IGF-1, VEGF, and TGF-β1 using standard immunohistochemical procedures. **Results:** IGF-1 immunoreactivity was seen in 50% of hyperplasia and in 46% of adenoma samples, but in 87% of normal parathyroid tissue in the vicinity of the adenomas ($P = 0.005$). TGF-β1 immunoreactivity was observed in 90% of hyperplasia, in 92% of adenoma samples, and in 95% of normal tissues around adenomas. VEGF immunoreactivity was observed in 70% of hyperplastic and 65% of adenomatous tissues, as well as in 54% of normal tissues in the vicinity of the adenoma. No significant differences in the expression of IGF-1, TGF-β1, and VEGF were observed between primary adenomas compared to hyperplasia samples ($P > 0.05$). **Conclusions:** Parathyroid tissue is clearly a site for production of IGF-1, TGF-β1, and VEGF. IGF-1 receptor activity was higher in normal parathyroid tissue compared to hyperplastic and adenomatous tissue.

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Full Text

Introduction

Primary hyperparathyroidism (P-HPT) is characterized by the inappropriate or deregulated overproduction of parathyroid hormone (PTH), causing hypercalcemia. [1],[2] It is caused by sporadic adenomas (85%), hyperplasia (10%), multiple adenomas (4%), and carcinoma (1%). [2] Making the histopathologic distinction between adenoma and hyperplastic parathyroid tissue is an important, but not always easy, task. [3] While the morphologic appearance of hyperplasia is similar to that of an adenoma, their biologic behavior is assumed to be different. [4] On a molecular level, adenomas are monoclonal neoplasms whereas hyperplastic parathyroid tissues are proliferative polyclonal lesions. [5],[6],[7] The biomolecular mechanisms causing parathyroid adenomas and hyperplasia are not completely understood; recent studies on reasons for their development have focused on angiogenesis and growth factors. [8]

Insulin-like growth factor (IGF), transforming growth factor-beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF) are the most commonly studied growth factors in this field. [9] IGF has a mitogenic and anti-apoptotic effect on tissue. [10] Two types of IGF have been identified: IGF-1 and IGF-2. Parathyroid cells respond to the mitogenic actions of IGF-1. [11] TGF-β1 is a multifunctional cytokine, similar to IGF, and relates to a wide variety of physiological and pathological processes such as growth, differentiation, apoptosis and carcinogenesis. [12] Studies using cultured human parathyroid cells indicate that TGF-β1 affects the proliferation of parathyroid endocrine cells. [9] VEGF acts as a specific mitogen for vascular endothelial cells, fibroblasts, and epithelial cells, but its most important action is stimulating angiogenesis by increasing vascular permeability and endothelial cell proliferation, which is essential for tumor growth. [13] VEGF has a proangiogenic effect on parathyroid proliferative lesions. [14]

Information about the role of these growth factor receptors in parathyroid tissue is sparse. Making the pathological distinction between various parathyroid lesions is, in many cases, difficult. Our study was planned to compare parathyroid tissues (adenomas and hyperplasia) regarding IGF-1, TGF-β1, and VEGF growth factor receptors. The immunexpression status of these growth factor receptors may be useful in distinguishing between parathyroid hyperplasia and adenomas.

Materials and Methods

Local research ethical committee approval was granted for this study. The parathyroid tissues used in this study (26 patients with parathyroid adenoma and 10 patients with parathyroid hyperplasia, differentiated using Rosai's criteria [15] had been surgically removed at our tertiary care university hospital. Preoperative laboratory testing [serum Ca and P, creatinine and parathormone levels (PTH)] which led to the diagnosis of P-HPT had been performed, the size and weight of the parathyroid glands measured, and postoperative serum PTH levels determined.

Histopathological study

For immunohistochemical staining using monoclonal antibodies to IGF-1, TGF-β1, and VEGF, formalin-fixed and paraffin-embedded parathyroid tissue specimens were retrieved from the surgical pathology files of our department. Tissue sections (4 μm) were prepared from paraffin-embedded tissue. Normal parathyroid tissue adjacent to the adenoma or area of hyperplasia was used as control tissue. The slides were deparaffinized with no antigen retrieval step. Endogenous peroxidase activity was blocked by incubation with 0.3% H 2 O 2 for 10

min. The sections were blocked with 10% normal goat serum. Mouse monoclonal antibodies recognizing human TGF-β1 (1:150 dilution, clone TGFβ17, Novocastra, UK), VEGF (1:100 dilution, clone FFPE, Biogenex, USA), and IGF-1 (1:250 dilution, IGF 1R, Biocare, USA) were applied as primary antibodies at room temperature for 30 min. The slides were incubated with a secondary antibody (rabbit-anti-mouse IgG, Novocastra, UK) for 8 min at room temperature, then DAB chromogen (Novocastra, UK) was applied for 15 min. Then they were counter-stained with hematoxylin for 5 min (Novocastra, UK), then cleared and mounted. The areas of highest protein expression evident at low-power scanning were used for analysis. Each tumor field was graded as follows: 0 (no staining), +1 (weak staining), +2 (moderate staining), +3 (strong staining) for IGF-1, TGF-β1, and VEGF. Chief cells were considered immunopositive when a moderate or strong immunoreaction pattern was observed with these markers in the cell membranes and/or cytoplasm. Because oxyphilic cells may be slightly immunopositive under normal circumstances, they were deemed immunopositive only when they were intensely stained.

Statistical analysis

Using SPSS 12.0 for Windows®, differences in staining between normal and pathological tissues were analyzed using Mann-Whitney U and chi-squared tests. Correlation between different parameters was determined by Spearman's correlation test. Results with a P-value of less than 0.05 were considered statistically significant. Data are given as mean ± SEM.

RESULTS

Demographical, clinical and biohumoral data collected from hyperplastic tissue and adenomas are shown in [Table 1]. Mean patient age and tumor diameter were 57 ± 6 years and 1.4 ± 0.3 cm in hyperplastic parathyroids and 55 ± 3 years and 2.0 ± 0.3 cm in adenomatous glands, respectively. Adenoma was more common in women (17 women, 9 men) and hyperplasia in men (2 women, 8 men). As expected, serum calcium and PTH levels were higher than normal in both hyperplasia and adenoma, and levels were higher in patients with hyperplasia compared to those with adenoma. IGF-1 immunoreactivity was characterized by a membranous staining pattern and was seen in 50% of hyperplasia samples [Figure 1]a, in 46% of adenoma samples [Figure 1]b, and 87% of normal parathyroid tissue in the vicinity of the adenomas [Figure 2], P = 0.005]. TGF-β1 immunoreactivity was most commonly seen as membranous staining and occasionally as cytoplasmic staining. TGF-β1 immunoreactivity was observed in 90% of hyperplasia samples [Figure 3]a and in 92% of adenoma samples [Figure 3]b. In normal tissue around adenomas, TGF-β1 expression was seen in 95% of samples. VEGF immunostaining was seen as granular staining in the cytoplasm, stromal fibroblasts, parathyroid chief and oxyphilic cells, and rarely in endothelial cells. Overall, VEGF expression was seen in 58% of the parathyroid samples: έn 70% of hyperplastic [Figure 4]a and in 65% of adenomatous tissues [Figure 4]b, and in 54% of normal tissue in the vicinity of the adenoma. Staining results of the hyperplastic and adenomatous tissues are listed in [Table 2]. No significant differences in the expression of IGF-1, TGF-β1, and VEGF were observed between primary adenomas and hyperplasia samples (P > 0.05).{Table 1}{Table 2}{Figure 1}{Figure 2}{Figure 3}{Figure 4}

Discussion

In this study, we used immunohistochemical methods to demonstrate the presence of IGF-1, TGF-β1, and VEGF growth factor receptors in normal, adenomatous, and hyperplastic parathyroid tissue. Prior studies used a variety of methods to determine the expression of particular growth factors in the parathyroid gland including PCR, Western ligand blot testing, cell culture, in-situ hybridization, immunohistochemical methods, and blood levels. [5],[6],[7],[9],[11],[16],[17] Little data are available on the immunohistochemical expression of IGF-1, TGF- β1 and VEGF in parathyroid hyperplasia and adenomas. [8],[11],[14],[18]

IGF-1 has been identified in hyperplastic and adenomatous human parathyroid tissues. [11] Its effects are mediated primarily through the IGF-1 receptor (IGF-1R). [19] Tanaka et al. found that IGF-1R concentrations were much higher in parathyroid carcinomas than in adenomas or hyperplastic parathyroid tissue. [11] In the current study, IGF-1R activity in parathyroid adenomas was not significantly different from that in hyperplastic parathyroid tissues. Tanaka et al. also found no relation between IGF-1R activity and tumor weight, parathyroid hormone levels, or serum calcium levels. [11] While we found similar levels of IGF-1R in hyperplastic and adenomatous parathyroid tissues, the normal tissue surrounding adenomatous tissue had much greater IGF-1R activity. This suggests that IGF-1R are important for the function of normal parathyroid cells. The decrease in IGF-1R activity in adenomatous tissue may reflect a mutation in the receptor which is causing it to malfunction. Parathyroid adenomas are neoplasms that have been found to develop as a result of genetic abnormalities. [5],[6],[7]

Sowa et al. reported that TGF-β1 was expressed mainly in parathyroid endocrine cells rather than in its other cells, and stated that it was an important autocrine/paracrine negative regulator of parathyroid tumorigenesis and PTH secretion. [20] TGF-β1 is involved in pleiotropic activities that suppress and enhance tumor growth. In their parathyroid cell culture study, Cavallaro et al. found a significant decrease in numbers of cells if the sample had been stimulated by high-dose TGF-β1. In the same study, TGF-β1 was found to have a proliferative effect in low doses. [9] In our study, we found levels of immunohistochemical expression of TGF-β1 receptor to be similar in adenomatous and hyperplastic parathyroid tissues. Our finding supports TGF-β1's action as a proliferative factor in parathyroid tissues.

Lazaris et al. found VEGF in 66% of parathyroid tissue samples from their 38 patients with P-HPT primary hyperparathyroidism and from 30 patients with secondary HPT. In their study, VEGF expression was significantly higher in parathyroid adenomas than in controls, and higher in adenomas than in primary and secondary hyperplasias. [8] Our results were similar, in that VEGF expression was seen in 58% of parathyroid adenoma and hyperplasia samples from our patients with primary hyperparathyroidism. However, we found no significant difference in VEGF expression between hyperplasia and adenoma tissues. As in other studies, we found VEGF immunoreactivity to be greater in hyperplastic and adenomatous parathyroid tissue than in normal tissue. [12] Previous reports suggest that fibroblast growth factor-2, not VEGF, be considered a primary pro-angiogenic factor in parathyroid tissue. [14]

The concerted activity of several growth factors control parathyroid growth. [16] The three growth factors in this study probably play important roles in the development of hyperplasia and adenomas in parathyroid tissues, but we did not find a difference in the various tissues regarding the growth factor receptors, as measured immunochemically.

In current study, we clearly identified the parathyroid as a site for production for IGF-1, TGF-β1, and VEGF, their presence was not able to discriminate between adenomatous and hyperplastic tissue. This may be due to the small number of samples in our study, or may be related to the presence of other, heretofore undefined mechanisms in the genesis of parathyroid adenomas and hyperplasia. Further studies of IGF-1, TGF-β1, and VEGF should be planned to determine the possible mechanism of these receptors in the development of parathyroid adenomas and hyperplasia. Using molecular and immunochemical techniques together might allow correlations to be determined between histological appearance and gene expression.

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