Immunohistochemical Localization of Parathyroid Hormone-related Protein in Human Prostate Cancer

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Abstract

Parathyroid hormone-related protein (PTHrP) is produced by a variety of malignant tumors and has been implicated as a major cause of humoral hypercalcemia of malignancy. Expression of PTHrP in prostate cancer tissue was studied immunohistochemically using 33 radical prostatectomy specimens from patients with clinically localized carcinoma of the prostate. None of these patients demonstrated hypercalcemia prior to the surgery. Acetone-methyl benzate-xylene-processed, paraffin-embedded tissues were stained with a validated mouse monoclonal antibody to an amino acid fragment, PTHrP(109-141), using the streptavidin-peroxidase enzyme conjugate method. All cases (33 of 33; 100%) studied demonstrated some degree of immunoreactivity throughout the cytoplasm of the tumor cells, but immunostaining was absent from inflammatory and stromal cells. The intensity of the staining appeared to directly correlate with increasing tumor grade. The widespread immunohistochemical localization of PTHrP in carcinoma of the prostate suggests that PTHrP may play some local role in the growth of transformed cells in the prostate. Furthermore, overexpression of PTHrP may be a possible marker to evaluate the malignant potential of carcinoma of the prostate.

Introduction

Hypercalcemia associated with malignant tumors is one of the most common paraneoplastic syndromes (1, 2). Since many of the features of this phenomenon mimic those of primary hyperparathyroidism, including increased bone resorption, decreased calcium excretion, and increased nephrogenous cyclic AMP and phosphate excretion (1), it has been suggested that certain humoral factors secreted by tumors might be involved (1-3). Recently, PTHrP was isolated and characterized from hypercalcemic patients with various malignant tumors and regarded as the principal agent responsible for the humoral hypercalcemia of malignancy (4-6). The gene for PTHrP encodes three forms of the protein, PTHrP(1-141), PTHrP(1-139), and PTHrP(1-173), in each of which 8 of the first 13 amino acids are common to PTH, but thereafter diverge completely from that of PTH or any of the other known peptides (5, 7). Recent studies have indicated that PTHrP is not only expressed by tumors but is also produced by normal adult tissues and cell lines including parathyroid, adrenal, brain, lactating mammary gland, skin, and placenta (8-10). Additionally, it has been shown that PTHrP is highly expressed in both human and rat fetuses throughout gestation (11, 12). These findings suggest that this protein is likely to have its own functions which may be involved in cellular growth and development (11). Furthermore, there is accumulated evidence that PTHrP appears to be involved in an autocrine growth regulation of malignant tumors, which has been demonstrated in human renal cell carcinoma (13). Similarly, we reported recently that PTHrP is a potential autocrine growth factor in prostate cancer cells in culture. Although an elevated level of PTHrP in prostate cancer tissue has been isolated from one patient who showed severe hypercalcemia (14), the distribution and localization of this peptide in CAP are virtually unknown. We therefore applied immunohistochemistry to evaluate the expression of PTHrP in prostate tissue from 33 patients with clinically localized CAP.

Materials and Methods

Patients and Tissue Specimens. The study group comprised 33 consecutive patients (mean age 65 years; range 52-74 years) with clinically localized CAP undergoing radical prostatectomy at Strong Memorial Hospital. The diagnosis of CAP was established by transurethral needle biopsy prior to radical prostatectomy or by transurethral resection of the prostate. Clinical stage was determined by digital rectal examination, chest X-ray, and skeletal scintigraphy. Preoperative blood analysis revealed normocalcemia in all patients studied. None of the patients received any hormonal treatments prior to radical prostatectomy.

Prostatic tissue (obtained at operation) was immediately sent to the Department of Surgical Pathology and cut into thin slices. Tissue specimens were fixed using the AMEX method (15) for optimal preservation of the antigenic epitopes and embedded in paraffin.

Immunohistochemistry. PTHrP was identified by the streptavidin-peroxidase enzyme conjugate method (16) using a murine monoclonal antibody, 9H7 (17-19), and similarly shows no cross-reactivity with PTH (20). This antibody was raised to the carboxy-terminal amino acid fragment (109-141) of PTHrP with no homology to PTH and similarly shows no cross-reaction with PTH (17). The following procedures were performed at room temperature unless otherwise mentioned. AMEX-fixed, paraffin-embedded tissue sections (5 µm thickness) were deparaffinized and incubated with normal horse serum (1:20; Vector, Burlingame, CA) diluted in 0.5% BSA (Sigma, St. Louis, MO) in PBS (0.5% BSA/PBS) for 20 min to reduce background staining. Excess normal horse serum was drained, and anti-PTHrP(109-141) antibody 9H7 was applied (5 µg/ml in 0.5% BSA/PBS) and incubated overnight in humidity chambers. After 5 min of washing with PBS, sections were incubated with biotinylated horse anti-mouse IgG antibody (1:200; Vector) in 0.5% BSA/PBS for 30 min. Followed by 5 min of rinsing with PBS, enzyme complex streptavidin-horseradish peroxidase (1:250; Zymed, San Francisco, CA) diluted in PBS was applied and incubated for 30 min. Slides were then rinsed with PBS, and positive staining was developed by incubating with chromogen, 3-aminoo-9-ethylcarbazole, for 10 min. After rinsing with warm running tap water for 5 min, slides were counterstained with Mayer’s hematoxylin, washed, and mounted in dibutylphthalate xylene.

In order to determine whether the positive staining observed was specific for PTHrP, liquid-phase absorption control was used. The monoclonal antibody to PTHrP, 9H7, was suspended in 0.5% BSA/PBS at a concentration of 10 µg/ml and incubated with a equal volume of the corresponding antigen, PTHrP(109-146). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 To whom requests for reprints should be addressed, at the Department of Urology, Box 656, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642-8656.

3 The abbreviations used are: PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; CAP, carcinoma of the prostate; AMEX, acetone, methyl benzate, and xylene; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

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141) (7 μg/ml), or 0.5% BSA/PBS (control) overnight at 4°C. These were then applied to AMEX-processed prostate in the same manner as described above.

**Evaluation of Staining.** Hematoxylin and eosin slides of each case were reviewed. Histopathological diagnosis of the prostatic tissue specimens and the immunohistochemically stained sections was reviewed by two independent observers. All cases were graded (from 1 to 5) according to the Gleason grading system (20). The degree of staining was designated by semiquantitative analysis as 0 (negative), 1+ (focal areas with sparse staining or occasional individual positive cells), 2+ (at least one focus with extensive staining or numerous areas with weak to moderate staining), or 3+ (extensive staining of more than 50% of the neoplastic cells).

**Results**

Since the carboxy-terminal 33-amino acid fragment, PTHrP(109–141), is not homologous to any known protein (5, 6) but is common to all three forms of PTHrP, we applied a region-specific antibody, 9H7, for immunohistochemical study. Tumors were classified into three groups according to their Gleason score: 2–4 (n = 3); 5–7 (n = 15); and 8–10 (n = 15) (Table 1). All specimens (33 of 33, 100%) demonstrated various degrees of immunoreactivity to 9H7. Although a heterogeneous distribution of staining was observed in all cases, the degree of immunoreactivity appeared to directly correlate with the tumor grade (Table 1). The tumors with a Gleason score of less than 4 showed mainly weak staining (2 of 3, 66.7%), whereas more than 85% (13 of 15) of tumors with a score of 8–10 showed moderate (9 of 15, 60%) to strong (4 of 15, 26.7%) staining. Fig. 1 shows examples of positive staining in low- (Fig. 1A), moderate- (Fig. 1B), and high-grade tumors (C). Immunoreactivity was seen throughout the cytoplasm of tumor cells but was absent from inflammatory and stromal cells. The positive immunostaining was completely abolished when the antibody (5 μg/ml) was preincubated overnight at 4°C with PTHrP(109–141) at a concentration of 3.5 μg/ml (data not shown).

**Discussion**

This is the first report that shows an immunohistochemical localization of PTHrP in prostate cancer tissue. By using AMEX-fixed, paraffin-embedded tissue sections, we found that all tumors (33 of 33, 100%) showed various degrees of positive staining. Furthermore, the intensity and the distribution of staining appeared to directly correlate to the histopathological grade of the tumors, although the number of cases is too small for a statistical analysis.

Immunohistochemical studies of PTHrP in prostate tissue have been made by two independent groups of researchers. In the two studies, Danks et al. (21) used rabbit polyclonal antibodies to PTHrP-(1-16), whereas Kitazawa et al. (22) raised a murine monoclonal (IgM) antibody to PTHrP(1–34); the antibodies showed no positive immunostaining in neoplastic (21) or normal (22) prostate tissues, respectively. One likely explanation regarding this apparent discrepancy between previous results and our data may be attributed to the difference of tissue fixation methods. We used the AMEX method to preserve antigenicity, whereas previous studies used formalin fixation. In fact, we observed in our laboratory that the monoclonal antibody 9H7 used in the present study did not show any specific immunoreactivity in formalin-fixed sections. Another possible explanation is that we studied the carboxy-terminal peptide of PTHrP, whereas previous studies have been of the amino terminus of the molecule. Hypercalcemia occurs in less than 2% of patients with CAP (23). In this report, however, we demonstrated that 100% of tissues from clinically localized CAP express PTHrP immunoreactivity. This raises the possibility that PTHrP produced by prostate cancer cells may rarely be secreted into the circulation. A more likely explanation,

![Fig. 1. Immunohistochemical localization of PTHrP(109–141) in prostate cancer tissues. AMEX-fixed, paraffin-embedded tissue sections were immunostained with a monoclonal antibody 9H7 at a concentration of 5 μg/ml and counterstained with Mayer's hematoxylin as described in "Materials and Methods." PTHrP immunoreactivity was observed throughout the cytoplasm of well-differentiated (Gleason grade 3) (A), moderately differentiated (grade 6) (B), and poorly differentiated (grade 8) (C) prostate cancer tissues. The degree of immunoreactivity was scored as 1+, 2+, and 3+ for A, B, and C, respectively.](image-url)

Table 1

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<th>1+</th>
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<tr>
<td>n</td>
<td>Degree of immunoreactivity (%)</td>
<td></td>
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<tr>
<td>2–4</td>
<td>3</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
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<td>5–7</td>
<td>15</td>
<td>4 (26.7)</td>
<td>9 (60.0)</td>
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<tr>
<td>8–10</td>
<td>15</td>
<td>2 (13.3)</td>
<td>9 (60.0)</td>
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<tr>
<td>Total</td>
<td>33</td>
<td>8 (24.2)</td>
<td>19 (57.6)</td>
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M. Iwamura, P-A. Abrahamsson, and L. J. Deftos, unpublished data.
however, is that the hypercalcemic effect of PTHrP may be attenuated by other factors. It has been demonstrated that various PTHrP-producing tumors concordantly produce calcitonin (18). Calcitonin is frequently detected in neuroendocrine cells of the prostate gland (24). Therefore, it is speculated that calcitonin may counter-regulate the action of PTHrP. On the other hand, it appears that PTHrP, in addition to its systemic action mimicking PTH, may have some local role in prostate cancer cells. There is accumulated evidence that PTHrP may be involved in an autocrine regulation of the growth of malignant tumors (13). We recently reported that PTHrP(1–34) stimulates the DNA synthesis of prostate cancer cell lines in culture. These findings strongly suggest that PTHrP may be a potential autocrine growth factor in prostate cancer cells.

We did not evaluate any "normal" prostate tissues in the present study. Considering the fact that PTHrP is expressed by a number of nonmalignant cells and tissues (7–9), it seems unlikely that the expression of PTHrP is a specific phenomenon for only transformed cells of the prostate. Further studies are warranted to demonstrate the physiological role of PTHrP in human prostate under normal and pathological conditions.

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References

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