Parathyroid hormone related peptide and receptor expression in paired primary prostate cancer and bone metastases

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Parathyroid hormone-related peptide is a regulatory protein implicated in the pathogenesis of bone metastases, particularly in breast carcinoma. Parathyroid hormone-related peptide is widely expressed in primary prostate cancers but there are few reports of its expression in prostatic metastases. The aim of this study was to examine the expression of parathyroid hormone-related peptide and its receptor in matched primary and in bone metastatic tissue from patients with untreated adenocarcinoma of the prostate. Eight-millimetre trephine iliac crest bone biopsies containing metastatic prostate cancer were obtained from 14 patients from whom matched primary tumour tissue was also available. Histological grading was performed by an independent pathologist. The cellular location of mRNA for parathyroid hormone-related peptide and parathyroid hormone-related peptide receptor was identified using in situ hybridization with 35S-labelled probe. Expression of parathyroid hormone-related peptide and its receptor was described as uniform, heterogeneous or negative within the tumour cell population. Parathyroid hormone-related peptide expression was positive in 13 out of 14 primary tumours and in all 14 metastases. Receptor expression was evident in all 14 primaries and 12 out of 14 metastases. Co-expression of parathyroid hormone-related peptide and parathyroid hormone-related peptide receptor was common (13 primary tumours, 12 metastases). The co-expression of parathyroid hormone-related peptide and its receptor suggest that autocrine parathyroid hormone-related peptide mediated stimulation may be a mechanism of escape from normal growth regulatory pathways. The high frequency of parathyroid hormone-related peptide expression in metastases is consistent with a role in the pathogenesis of bone metastases.

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Despite increasing detection and treatment of early prostate cancer, this disease still accounts for 9000 deaths annually in England and Wales (Office for National Statistics, 1998). The development of bone metastases is undoubtedly a major cause of morbidity and mortality; patients presenting with osseous involvement have a mean survival of just 2.5 years (George, 1988).

The overwhelming majority of prostate cancer bone metastases are osteoblastic, characterized by increased rates of lamellar bone resorption and replacement with abnormal woven bone. Therefore, in common with osteolytic metastases, osteoclast activation is a key step in the pathogenesis of osteoblastic bone metastases (Clarke et al, 1991, 1993). Consequently osteo-active cytokines and growth factors produced by cancer cells may influence the tropism of certain tumours for metastasis to bone, and offer an advantage in the genesis of bony secondaries.

Parathyroid hormone related peptide (PTHrP) is a protein with N-terminal homology to PTH (Guise et al, 1997). It is able to bind to and activate the PTH receptor and is consequently a potent stimulant of osteoclasts and osteoblasts (Abou-Samra et al, 1992). Work in breast carcinoma has suggested that PTHrP may be an important factor in bone metastasis. Serum PTHrP levels are elevated in patients with bone metastases (Bundred et al, 1991) and that there is up regulation of PTHrP expression in the metastatic tissue (Powell et al, 1991). Additionally there are also found to be high levels of expression of the receptor for PTHrP in breast cancer bone metastases (Downey et al, 1996).

PTHrP is expressed in benign prostate, although its function is unclear (Cramer et al, 1996). In primary prostate cancer PTHrP staining intensity increases with tumour grade (Asadi et al, 1996), and in vitro cell line studies have suggested that it may be a significant autocrine growth factor (Iwashima et al, 1994a). Drawing on the work in breast cancer and the parallels between the two tumours, it has been suggested that PTHrP may be a potential factor in the pathogenesis of prostate cancer bone metastases (Guise, 1997).

It is hypothesized that expression of PTHrP and PTHrP receptor (PTHrPr) offer an advantage in the genesis of bone metastases in prostate cancer through paracrine and autocrine mechanisms. The aim of this study was to test this hypothesis by investigation of the co-expression of PTHrP and PTHrP receptor in matched pairs of untreated primary prostate cancer and their corresponding bone metastases.

MATERIALS AND METHODS

Having obtained informed consent, iliac crest bone biopsies were taken from men known or strongly suspected to have untreated
metastatic prostate cancer from whom primary prostatic tissue was also available. Local Ethics Committee approval was granted for the study. All specimens were fixed in formalin and the bone biopsies decalcified in EDTA. All tissue was embedded in paraffin wax. On analysis of the bone specimens 14 were found to contain metastatic prostate cancer. All primary and metastatic tumours were graded according to the Gleason system (Gleason and Mellinger, 1974) by an independent pathologist.

The expression of PTHrP and PTHrPr were determined by in situ hybridization on 7 μm tissue sections. The cDNA probes were kind gifts to the Department of Osteoarticular Pathology from Dr MT Gillespie (St Vincent’s Institute for Medical Research, Victoria, Australia) and Dr E Schipani (Massachusetts General Hospital, MA, USA) respectively.

**Probe labelling**

Radioactive labelling of the probe using [35S] was achieved using the Amersham Megaprime™ DNA labelling system. The probe and primer were mixed, then the double stranded DNA probe denatured by immersion in a boiling bath. The labelling reaction was initiated by mixing the denatured DNA, reaction buffer, unlabelled deoxynucleotide triphosphate, [35S]-deoxycytosine triphosphate, and Kenlow fragment of DNA polymerase. Following incubation at 37°C for 1 h, the reaction was stopped by the addition of 0.2 M EDTA pH 8.0. The labelled probe was then purified by centrifugation.

**Prehybridization**

Following dewaxing, tissue sections mounted on sialane coated slides were subjected to the following procedures. Tissue permeabilization was carried out by incubation with proteinase K for 1 h (5 μg ml⁻¹ for prostate and 10 μg ml⁻¹ for bone sections). Control sections were created by elimination of the hybridization signal by incubation for 2 h at 37°C with RNase A. Post-fixation was carried out in 0.4% paraformaldehyde at 4°C.

**Hybridization**

The hybridization buffer containing the [35S]-labelled probe was applied to all tissue sections and then incubated overnight at 37°C.

**Post-hybridization washes**

A series of progressively higher stringency washes were performed, using 0.5 x SSC/1 mM EDTA/10 mM Dithiotreitol, then 0.5 x SSC/1 mM EDTA, and then in 50% formalin: 50% 0.15 M NaCl/5 mM Tris pH 7.4/0.5 mM EDTA pH 8.0, (SSC – standard saline citrate solution: 7.5 mM NaCl/0.75 mM NaCitrate). Final washes were then performed in 0.5 x SSC, initially at 55°C then at room temperature. Sections were dehydrated in industrial methylated spirit and air dried.

**Detection**

Binding of the radiolabelled probe was detected by autoradiography. After exposure at 4°C for 2–3 weeks to a layer of Ilford K3 emulsion, slides were developed using Ilford D-19 developer, and fixed with Ilford Hypam. Sections were counter stained with Mayer’s haematoxylin, dehydrated and mounted with Xam.

**Analysis**

Precipitation of the silver granules produced by autoradiography was assessed using a Leitz Laborlux 12 microscope with the ×20 objective lens, with transmitted light and dark field condensers.

The distribution of signal across the tumour cell was described as uniform, heterogeneous or negative.

**RESULTS**

Only three of the primary tumours were moderately differentiated, the remainder being poorly differentiated (Gleason ≥8). All metastases were poorly differentiated. The distribution of tumour grades are shown in Table 1.

**Table 1 Distribution of Gleason score in the matched untreated primary prostate cancers and corresponding bone metastases**

| No. of tumours | Primary prostate cancers | Bone metastases |
|----------------|--------------------------|-----------------|
| 5              | 1                        | 2               |
| 7              | 2                        | 4               |
| 8              | 4                        | 2               |
| 9              | 5                        | 5               |
| 10             | 2                        | 8               |

**PTHrP and receptor in prostate cancer and bone metastases**

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DISCUSSION

Animal models of bone metastasis have demonstrated the importance of PTHrP in the metastatic cascade. The activation of osteoclasts by PTHrP in conjunction with interleukin 1 has been shown to be essential for bone metastasis development (Arguello et al, 1988). In mice inoculated with highly metastatic breast and lung cancer cell lines, treatment with an anti-PTHrP antibody, reduced both tumour burden and osteoclastic bone resorption (Guise et al, 1996; Iguchi et al, 1996).

PTHrP has been most extensively investigated in breast carcinoma, where 60% of primary tumours are found to have positive expression of PTHrP (Southby et al, 1990; Bunded et al, 1992). In metastases in soft tissues only 17% are positive for PTHrP in contrast to 92% of those in bone (Powell et al, 1991). These findings support the view that PTHrP has a role in the development and progression of bone metastases.

Although PTHrP is expressed in normal prostate (Cramer et al, 1996), benign prostatic epithelial cells do not show enhancement of growth in the presence of PTHrP in tissue culture (Peehl et al, 1997). PTHrP is secreted by all three commonly studied human malignant prostatic cell lines (PC3, DU-145, and LNCaP) and levels are highest in the bone metastasis derived PC3 line (Iwamura et al, 1994a). The same study demonstrated that culture with synthetic PTHrP lead to increased proliferation of PC3 and DU-145 cells (Iwamura et al, 1994a), suggesting that PTHrP may be a significant autocrine factor in prostate tumour growth, particularly in bone metastases.

PTHrP expression in primary prostatic carcinoma is common (Iwamura et al, 1993; Lee et al, 1997). Lymph node metastases exhibit similar levels of PTHrP expression to those of poorly differ-
entiated primary tumours (Asadi et al, 1996). In this study mRNA for PTHrP was detected in all 14 primary prostate tumours, although expression was heterogeneous in four cases. In the corre-
sponding bone metastases, all 14 were positively staining, all but
three being uniformly stained. Previous studies of 10 (Bryden
sponding bone metastases, all 14 were positively staining, all but
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correspon-

Figure 1 Section of prostatic bone metastasis having undergone in-situ hybridization with the probe for PTHrP receptor. Micrograph (A) standard light ground field, stained with H&E, and micrograph (B) is the same sec-
section but with dark ground field, and the silver grains show as the bright
spots. The distribution of the bright spots over the metastatic cells indicate uniform signal for PTHrP receptor.

This difference may be due to variation in PTHrP expression with
tumour grade as described for primary prostate carcinoma
(Iwamura et al, 1993). In our previous study 7 out of 10 specimens
were uniformly stained 

Table 2 Distribution of PTHrP and PTHrP receptor expression in 14 pairs of untreated primary prostate cancer and matched bone metastases

| Expression of PTHrP or PTHrP receptor | Primary tumour | Bone metastasis |
|--------------------------------------|----------------|----------------|
|                                      | Uniform | Heterogenous | Negative | Uniform | Heterogenous | Negative |
| PTHrP                                | 9       | 4            | 1         | 11      | 3            | 2         |
| PTHrP receptor                       | 12      | 2            | 1         | 7       | 5            | 2         |
Overall, this study has shown a high level of expression of PTHrP and its receptor in bone metastases of prostate cancer and their corresponding primary tumours. This supports the previous described work in prostate and other tumour systems, proposing that PTHrP is a likely mediator predisposing to the formation of bone metastases. The co-expression of PTHrP and its receptor would suggest that PTHrP is able to act as an autocrine and/or paracrine growth factor in prostate cancer.

Further work to define and investigate the expression of the PTHrP receptor in benign and malignant prostatic tissue would be important to determine whether this is a feature exclusive to malignant prostatic cells and whether there is any temporal relationship to the presence of bone metastases.

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REFERENCES

Abou-Samra A, Juppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Forts Jr JT (1992) Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol triphosphates and increases intracellular free calcium. Proc Natl Acad Sci USA 89: 2732 – 2736

Arguello F, Baggs RB, Frantz CN (1988) A murine model of experimental metastasis to bone and bone marrow. Cancer Res 48: 6876 – 6881

Asadi F, Farraj M, Sharifi R, Malakouti S, Antar S, Sukreja S (1996) Enhanced expression of parathyroid hormone-related protein in prostate cancer as compared with benign prostatic hyperplasia. Hum Pathol 27: 1319 – 1323

Blomme E, Sugimoto Y, McCauley LK, Lin YC, Capen CC, Rosol TJ (1998) Stromal and epithelial cells of the canine prostate express parathyroid hormone-related protein, but not the PTH/PTHrP receptor. Prostate 36: 110 – 120

Bryden AAG, Islam S, Freemont AJ, Shanks JH, Clarke NW, George NJR (2002) Parathyroid hormone-related peptide expression in prostate bone metastases. Prostate Cancer Prostatic Dis, in press

Bundred NJ, Ratcliffe WA, Walker RA, Coley S, Morrison JM, Ratcliffe JG (1991) Parathyroid hormone related protein and hypercalcaemia in breast cancer. Br Med J 303: 1506 – 1509

Bundred NJ, Walker RA, Ratcliffe WA, Warwick J, Morrison JM, Ratcliffe JG (1992) Parathyroid hormone related protein and skeletal morbidity in breast cancer. Eur J Cancer 28: 690 – 692

Carron J, Fraser WD, Gallagher JA (1997) PTHrP and the PTH/PTHrP receptor are co-expressed in human breast and colon tumours. Br J Cancer 76: 1095 – 1098

Clarke NW, McLure J, George NJR (1993) Morphometric evidence for bone resorption and replacement in prostate cancer. Br J Urol 68: 74 – 80

Clarke NW, McLure J, George NJR (1993) Osteoblastic function and osteomalacia in metastatic prostate cancer. Eur Urol 24: 286 – 290

Coleman R, Reubens RD (1987) The clinical course of bone metastases from breast cancer. Br J Cancer 55: 61 – 66

Cramer S, Peehl DM, Edgar MG, Wong ST, Defos LJ, Feldman D (1996) Parathyroid hormone-related protein (PTHrP) is an epidermal growth factor-regulated secretory product of human prostatic epithelial cells. Prostate 29: 20 – 29

Downey S, Hoyland JA, Bundred NJ, Freemont AJ (1996) Is overexpression of parathyroid hormone-related peptide receptor a predisposing factor in bone metastases from breast cancer? Br J Surg 83: 699

Downey S, Hoyland J, Freemont AJ, Knox F, Walls J, Bundred NJ (1997) Expression of the receptor for parathyroid hormone-related protein in normal and malignant breast tissue. J Pathol 183: 212 – 217

Dunne F, Lee S, Ratcliffe WA, Hutchesson AC, Bundred NJ, Heath DA (1993) Parathyroid hormone-related protein (PTHrP) gene expression in solid tumours associated with normocalcaemia and hypercalcaemia. J Pathol 171: 215 – 221

George NJR (1988) Natural history of localised prostate cancer managed by conservative therapy alone. Lancet i: 494 – 497

Gleason D, Mellinger GT (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111: 58 – 64

Guise T, Yin Jj, Taylor SD, Kumagai Y, Dallas M, Boyce BF, Yoneda T, Mundy GR (1996) Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J Clin Invest 98: 1544 – 1549

Guise T (1997) Parathyroid hormone-related protein and bone metastases. Cancer 80: 1572 – 1580

Iddon J, Bundred NJ, Hoyland J, Downey SE, Baird P, Salter D, McMahan R, Freemont AJ (2000) Expression of parathyroid hormone-related protein and its receptor in bone metastases from prostate cancer. J Pathol 191: 170 – 174

Iezzoni J, Bruns ME, Frierson HF, Scott MG, Penco RA, Deftos LJ, Bruns DE (1998) Coexpression of parathyroid hormone-related protein and its receptor in breast carcinoma: a potential autocrine effector system. Mod Pathol 11: 265 – 270

Iguchi H, Tanaka S, Ozawa Y, Kashiwakuma T, Kimura T, Hiraga T, Ozawa H, Kono A (1996) An experimental model of bone metastasis by human lung cancer cells: the role of parathyroid hormone-related protein in bone metastasis. Cancer Res 56: 4040 – 4043

Iwamura M, di Sant’Agnese, Wu G, Benning CM, Cockett AT, Deftos LJ, Abrahamsson P-A (1993) Immunohistochemical localization of parathyroid hormone-related protein in human prostate cancer. Cancer Res 53: 1724 – 1726

Iwamura M, Abrahamsson P-A, Foss KA, Wu G, Cockett AT, Deftos LJ (1994a) Parathyroid hormone related protein: a potential autocrine growth regulator in human prostate cancer cell lines. Urology 43: 675 – 679

Iwamura M, Wu G, Abrahamsson PA, di Sant’Agnese PA, Cockett AT, Deftos LJ (1994b) Parathyroid hormone-related protein is expressed by prostatic neuroendocrine cells. Urology 43: 667 – 674

Kao P, Klee GG, Taylor RL, Heath H (1990) Parathyroid hormone-related peptide in the plasma of patients with hypercalcaemia and malignant lesions. Mayo Clin Proc 65: 1399 – 1407

Lee C, Wojno KJ, Osterling JE, Singleton T, McCauley L, Lehr J, Montie JE, Pienta K (1997) Expression of parathyroid hormone-like protein in prostate cancer and prostate intraepithelial neoplasia. J Urol 157: 225

Minisola S, Perugia G, Scarada A, Scarneccia L, Tuzzolo D, Rossi W, Mazzuoli G (1987) Biochemical picture accompanying sclerotic bone metastases of prostatic origin. Br J Urol 60: 443 – 446

Office for National Statistics (1998) Estimates of newly diagnosed cancers, England and Wales. 1993 – 1997 In ONS Monitor Population and Health. The Stationery Office: London

Peehl D, Edgar MG, Cramer SD, Defos LJ (1997) Parathyroid hormone-related protein is not an autocrine growth factor for normal prostatic epithelial cells. Prostate 31: 47 – 52

Powell G, Southby J, Danks JA, Stillwell RG, Hayman JA, Henderson MA, Bennett RC, Martin TJ (1991) Localization of parathyroid hormone-related protein in breast cancer metastases: increased incidence in bone compared with other sites. Cancer Res 51: 3059 – 3061

Southby J, Kissin MW, Danks JA, Hayman JA, Moseley JM, Henderson MA, Bennett RC, Martin TJ (1990) Immunohistochemical localization of parathyroid hormone-related protein in human breast cancer. Cancer Res 50: 7710 – 7716