A positive correlation was found between advanced clinical stages and increased PCNA expression. This is explained by the biological aggressiveness and invasive potential of tumours in the high proliferation group.

Since the prognostic value of PCNA expression is still inconclusive, we suggest that prospectively collected data with larger series of patients should be accumulated before assigning PCNA an important role as a biological prognostic factor in ovarian cancer.


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# Hypercalcaemia in Small Cell Lung Cancer: Report of a Case Associated with Parathyroid Hormone-related Protein (PTHrP)

**R. Stuart-Harris, V. Ahern, J.A. Danks, H. Gurney and T.J. Martin**

**Although hypercalcaemia is frequently associated with malignancy, it is very rare in small cell lung cancer despite the high incidence of lytic bone metastases. We report a patient with extensive small cell cancer who presented with hypercalcaemia. Investigations suggested parathyroid hormone (PTH) receptor stimulation, although the serum PTH level was not elevated. PTH related protein (PTHrP) was localised in a biopsy specimen from the tumour. Although hypercalcaemia is rare in small cell lung cancer, when hypercalcaemia does occur, PTHrP may be a causal factor.**

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## INTRODUCTION

HYPERCALCAEMIA IS a frequent complication of many solid tumours, especially renal cancer, breast cancer and non-small cell lung cancer. Overall, approximately 12% of patients [1] with primary lung cancer develop tumour-induced hypercalcaemia (TIH). TIH is particularly common in squamous cell carcinoma of the lung, where up to 25% of patients develop hypercalcaemia at some time during the course of their illness. TIH is principally induced by increased bone resorption or reduced renal calcium excretion, but more than one mechanism may occur in any one patient. Although bone metastases are frequently present, it is now known that hormonal factors are important in the majority of cases of TIH, whether or not bone metastases are present. Parathyroid hormone (PTH) related protein (PTHrP) has recently been implicated as one of the main humoral factors causing TIH [2].

Small cell lung cancer (SCLC) is a common malignancy with a propensity to early dissemination especially to bone, and is also commonly associated with ectopic hormone production. By the use of specific immunohistochemical staining, some SCLC biopsy specimens have been shown to contain PTHrP [3]. However, hypercalcaemia is a rare complication.

We report a patient with extensive SCLC who presented with TIH. Biochemical investigation suggested parathyroid hormone (PTH) receptor stimulation and the PTHrP antigen was localised in a biopsy specimen of the tumour.

## PATIENT AND METHODS

In July 1989, a previously well 67-year-old man presented to Westmead Hospital with a 4 month history of exertional dyspnoea, cough, and hoarse voice. In the previous 4 weeks he had developed nausea and anorexia and his weight had decreased by 15 kg. He had smoked 20 cigarettes a day since late teenage years but there was no history of exposure to occupational carcinogens. On examination, he was dehydrated and profoundly lethargic. On auscultation, air entry to the lungs was reduced bilaterally. However, there was no clinical evidence of metastatic disease.

A chest X-ray demonstrated an ill-defined opacity in the left upper and mid zones with associated volume loss. At bronchoscopy there was paralysis of the left vocal cord and a tumour was obstructing the left upper lobe bronchus. Endoscopic bronchial biopsy failed to provide a definitive diagnosis but fine needle aspiration of the left lung lesion demonstrated SCLC. Abnormal serum biochemical results were: calcium 3.68 mmol/l, (normal 2.13–2.63; corrected to a serum albumin of 40 g/l, 3.66) and gamma-glutamyl transferase 81 μ/l (normal 8–43). There was evidence of thrombocytopenia (platelet count $90 \times 10^9$/l) and a bone marrow trephine confirmed infiltration by SCLC. No further investigations were undertaken due to the poor condition of the patient. Plasma PTHrP was not measured as no suitable plasma assay existed at the time.

A diuresis with 1 l of normal saline every 4 h was commenced with correction of hypercalcaemia by the sixth day of admission. Chemotherapy was commenced on the fourth day of admission and comprised carboplatin 100 mg/m$^2$ and VP-16 120 mg/m$^2$, each given intravenously on 3 consecutive days. The day after chemotherapy was commenced, the patient developed epistaxes and investigations demonstrated a platelet count of $23 \times 10^9$/l, a thromboplastin time of 33 s (normal < 16 s) and a D-dimer in the range of 2000–4000 ng/ml (normal < 50–200 ng/ml), consistent with a diagnosis of disseminated intravascular coagulation. Despite intensive support with intravenous fluids and transfusions of platelets and fresh frozen plasma, the patient died 6 days after admission.

Post mortem examination confirmed a small cell cancer of the left upper lobe bronchus abutting the pulmonary artery, trachea and aorta. Histologically, there were extensive areas of necrosis and haemorrhagic infarction. Metastatic disease, similar in appearance to the primary lung tumour, was found in the liver and diffusely involving the bone marrow. However, both parathyroid glands were normal.

For immunohistochemical staining for PTHrP, tissue was taken at autopsy and fixed in neutral-buffered formalin without delay. After the blocks were processed and paraffin-embedded, 5 μm sections were cut and placed on slides pretreated with 2% 3-aminopropyltriethoxysilane (AES).

Serial sections were used for PTH immunohistochemistry [3]. Positive and negative controls were included in each assay. The immunohistochemically positive controls consisted of sections of normal skin in which the spinous keratinocyte layer contained the PTHrP antigen [3]. Negative method controls and tests for antibody specificity included the substitution of non-immune rabbit serum for the primary anti-serum and an unrelated immune rabbit serum and overnight pre-absorption of human PTHrP (1–16) anti-serum with PTHrP (1–34). If the positive and negative controls did not give the expected results, the experiment was discarded.

## RESULTS

At presentation, the calculated renal tubular threshold for phosphate was low (0.66 mmol/l, normal 0.8–1.3) but the calculated renal tubular threshold for calcium was normal (1.76 mmol/l, normal 1.7–2.2). Serum PTH was not elevated (0.1 ng/ml, normal < 0.4). Nephrogenous cyclic AMP was elevated at 2.3 nmol/100ml GFR (normal < 1.9). These results were obtained according to the methods described elsewhere [4–7].

PTHrP antigen was localised to the cells within the small cell carcinoma (Fig. 1). The immunoperoxidase studies showed specific staining within the cytoplasm of the tumour cells. Some background staining was seen in the sections where non-immune rabbit serum was substituted for the primary anti-serum (Fig. 2). This was attributed to autolysis of the autopsy material between the time of death and that of fixation. However, more intense staining was observed in the section stained with the primary anti-serum.

## DISCUSSION

Hypercalcaemia occurs when an imbalance in the homeostatic mechanisms for normocalcaemia occurs, either due to increased bone resorption, reduced renal excretion of calcium, or a combination of both. In malignancy even when bone metastases are present, bone resorption probably occurs from the stimulation of resorption by cytokines, such as tumour necrosis factor, interleukin 1, transforming growth factor alpha, or other mediators such as prostaglandins. The humoral mechanisms for hypercalcaemia operate when bone metastases are absent, and the existence of a PTH-like substance produced by tumours was first proposed by Albright in 1941 [8].

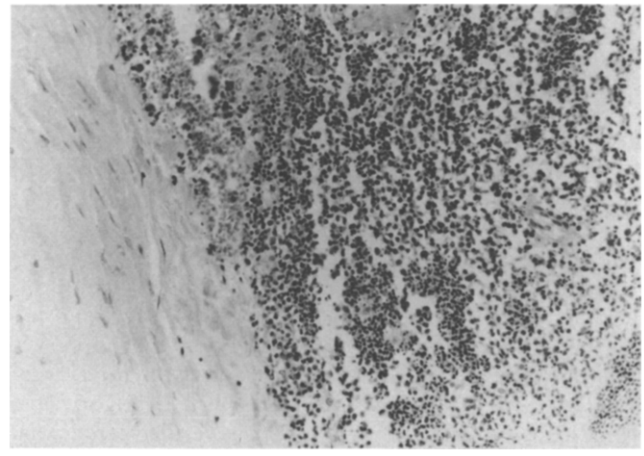

**Fig. 1. Small cell carcinoma of the lung, showing positive staining for parathyroid hormone-related protein in the tumour. PTHrP (1–16) immunoperoxidase × 50.**

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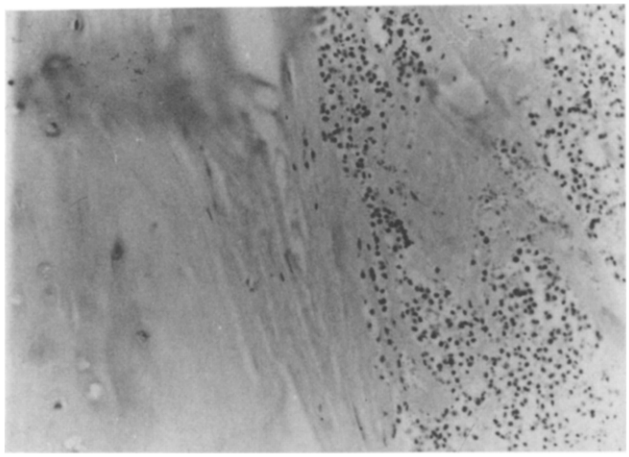

**Fig. 2. A non-immune control of Fig. 1 showing an absence of staining for PTHrP (1–16). PTHrP (1–16) immunoperoxidase × 50.**

PTHrP is a recently identified protein, detected in normal tissues such as skin, kidney, lactating breast tissue, pancreas, lung, testis and placenta [3]. The function of PTHrP in normal tissues is unknown but current evidence supports a paracrine or autocrine role in calcium homeostasis [2, 7]. Both PTH and PTHrP act via PTH receptors on target cells to cause osteolysis, raised nephrogenous cAMP, increased renal tubular resorption of calcium, and renal phosphate loss [7, 9, 10]. Bone and kidney are the major target organs of circulating PTHrP [11].

PTHrP has been shown to be an important factor in the genesis of TIH. Danks *et al.* have used immunohistology to localise PTHrP in a wide variety of tumours including breast cancer, squamous cell carcinoma, renal cortical cancer, neuroendocrine tumours, medullary thyroid carcinoma, osteogenic sarcoma, melanoma and SCLC [3].

To date, only 16 cases of hypercalcaemia associated with SCLC [12–22] have been reported, (see Table 1). As SCLC commonly produces a variety of structurally unrelated peptides including ACTH, ADH and calcitonin, a humoral factor was considered likely to be causal in most cases of hypercalcaemia. Of the 16 cases reported previously, PTH levels were assayed in only 10 and in the only one of these in which the PTH level was elevated, convincing evidence was presented that the tumour did indeed produce PTH. The cause of the hypercalcaemia in the remaining patients must remain speculative.

*Table* 1. *Previous reports of hypercalcaemia in SCLC*

| Ref. | Patients' age (years) | Bone/bone marrow involvement | PTH level |
|---|---|---|---|
| 12 | 53 | Yes | — |
| 13 | 64 | No | — |
| 14 | 53 | — | — |
| 15 | — | — | — |
| 16 | 46 | Yes | Normal |
| 17 | 38 | Yes | Normal |
| 18 | 56 | Yes | Normal |
| | 43 | No | Normal |
| | 54 | Yes | Normal |
| | 53 | No | — |
| | 71 | Yes | Normal |
| | 64 | Yes | Normal |
| 19 | 58 | No | Normal |
| 20 | — | — | Normal |
| 21 | — | — | Normal |
| 22 | 73 | Yes | Elevated |

— = not known

In the patient reported here, the low tubular phosphate threshold, elevated nephrogenous cAMP production and normal serum PTH level are consistent with PTHrP secretion. PTHrP was demonstrated in the tumour by immunohistochemical staining.

In a prior series of tumour biopsies stained for PTHrP [3], three SCLC tumours demonstrated positive staining, although in no case was this associated with hypercalcaemia. When hypercalcaemia occurs in association with tumours which stain positively for PTHrP, this may reflect sufficient tumour bulk, capable of producing a sufficient quantity of PTHrP to over-ride normal calcium homeostasis. Alternatively, cancer cells may be able to process the PTHrP to sufficient amounts of an active, secreted form.

Although SCLC commonly metastasises to bone, hypercalcaemia is rare. In the future, patients with SCLC and other rapidly doubling tumours should be investigated for evidence of PTHrP production in order to establish both the incidence and magnitude of PTHrP production by these tumours. The recent introduction of a sensitive assay for PTHrP in plasma should facilitate this task [23].


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# C-*erb*B-2/HER-2 Protein in Human Intracranial Tumours

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**Normal and neoplastic human intracranial tissues were examined by immunohistochemistry for c-*erb*B-2/HER-2 protein expression. Positive staining was observed in 1/41 gliomas, 1/2 medulloblastomas, 1/1 germinoma, 11/16 meningiomas, 1/3 anaplastic meningiomas and 11/19 metastatic brain carcinomas. No positive staining was observed in normal intracranial tissues. Thus, the expression of the c-*erb*B-2/HER-2 protein is limited to intracranial tumour tissues, principally meningiomas and metastatic carcinomas to the brain.**
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## INTRODUCTION

THE PROTOONCOGENE c-*erb*B-2/HER-2 coding for a 185 kDa transmembrane receptor protein with structural similarities to epidermal growth factor receptor and endowed with tyrosine kinase activity, is overexpressed and amplified in various human tumours, most commonly in breast tumours [1]. Recently, a ligand for this receptor has been described [2].

In human intracranial tumours the c-*erb*B-2/HER-2 protein has been detected in meningiomas [3,4] whilst in gliomas conflicting results have emerged [3–7]. This study is an attempt to clarify this question, and a variety of human intracranial tumours were examined immunohistochemically with an anti-c-*erb*B-2 monoclonal antibody to identify tumours expressing this protein.

## MATERIALS AND METHODS

Tumour samples from 84 human intracranial tumours of various histological types were obtained during surgery at the Department of Neurosurgery, University Hospital, Trondheim, Norway in the period 1986–1992. Normal and non-neoplastic intracranial tissues (brain and meningeal tissues from various pathological conditions including haemorrhages, infection, radiation and tumour infiltration) were obtained during surgery at the Department of Neurosurgery or during autopsy at the Department of Pathology, University Hospital, Trondheim, Norway. The tissue samples were immediately put in liquid nitrogen and stored frozen until analysis. In the immunohistochemical analyses the NCL-CB11 anti-c-*erb*B-2 monoclonal antibody (Novocastra Lab. Ltd., Newcastle upon Tyne, U.K.) [8] and an avidin-biotin immunoperoxidase technique (Vectastain ABC kit, Vector Lab., Burlingame, California, U.S.A.) were used. Frozen sections were fixed in acetone and incubated overnight at 4°C with the primary antibody at a 1:40 dilution. Frozen cell pellets of the SK-BR3 breast cancer cell line served as positive controls in each experiment [8]. In the negative controls the primary antibody was omitted or an irrelevant mouse monoclonal antibody of the same isotype was used. The specificity of staining was tested by pre-incubation of the primary antibody with protein extract of SK-BR3 cells. The intensity of positive staining was estimated as negative, weak, moderate or strong. The proportion of positive staining tumour cells and the proportion with strong intensity were estimated as $< 25$, 25–50 and $> 50\%$. The kappa statistic was used to test the intraobserver reproducibility of the immunohistochemistry [9].

## RESULTS

The staining results are summarised in Tables 1 and 2. Positive staining was localised to the plasma membrane and to cytoplasm. Membrane staining was most prominent in the metastatic adenocarcinomas whereas granular cytoplasmic staining was most common in the other tumours. The positive staining intensity varied from tumour to tumour and within the same tumour but was, in general, moderate. In the majority of positive tumours more than 50% of the tumour cells were

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