STUDIES ON THE PATHOGENESIS OF OSTEOBLASTIC METASTASES BY PROSTATE CANCER

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INTRODUCTION

Malignancies have long been known to markedly alter skeletal and calcium homeostasis. By far the commonest lesion observed in association with most malignancies is skeletal resorption, occasionally accompanied by elevations in blood calcium (hypercalcemia). Osteolysis and hypercalcemia may occur either when the tumor has metastasized to bone, presumably by a paracrine mechanism, or in the absence of skeletal metastases, by an endocrine mechanism. Considerable effort has been expended over the years to attempt to discern the pathogenetic factors involved in these skeletal responses to cancer. This culminated in the isolation of a parathyroid hormone-like peptide (PLP) from cancers, such as renal cancer, which are commonly associated with osteolysis and hypercalcemia [1]. This material is now believed to be the major factor causing hypercalcemia of malignancy. In the human, 3 isoforms of this peptide may exist and this entity is believed now to be a member of the parathyroid hormone/PLP gene family. Currently it is less clear what pathogenetic moieties may act locally in the vicinity of a skeletal metastasis to cause osteolysis. Although prostaglandins of the E series and transforming growth factor alpha (TGFα) have been implicated in this regard, more evidence supports the role of peptide cytokines such as, interleukin-1 and tumor necrosis factor alpha (TNFα) in the local resorption induced by cancer metastases [2].

Although several cancers including thyroid, breast, kidney, lymphoma and lung have been associated with osteoblastic rather than osteolytic lesions, this skeletal reaction is an infrequent manifestation of cancer even in association with those particular malignancies. On the other hand, prostatic adenocarcinoma is unique with respect to the frequency of its association with osteoblastic lesions [3]. These seem to appear almost exclusively once the cancer has metastasized to bone, suggesting a paracrine mechanism in the genesis of these responses [4]. Although rare, lytic lesions may occur, and may sometimes accompany blastic lesions. Rarer still is the occasional development of an osteomalacic syndrome in association with prostatic cancer, possibly due to a factor which reduces both the renal phosphate threshold and the activity of the 25-hydroxyvitamin D 1α-hydroxylase enzyme in the kidney [5].

Most recent attempts to investigate the pathogenesis of the osteoblastic metastases of prostatic cancer were initiated by the demonstration of growth factor activity in extracts of prostatic tissue for osteoblastic cells grown in vitro [6,7]. This was subsequently followed by the demonstration that mRNA could be extracted from a prostatic cancer cell line (PC-3), injected into Xenopus oocytes, and direct the translation of a secreted protein which was mitogenic for osteoblastic cells [8]. Our
own studies on the pathogenesis of osteoblastic metastases of prostate cancer have also focused on identifying growth factors for cells of the osteoblast phenotype in prostatic cancer tissue.

STUDIES WITH HUMAN PROSTATIC TISSUES

Our overall aims have been to extract and characterize humoral factors from prostatic tissue which would stimulate the growth of osteoblasts and to examine the biological and biochemical characteristics of the prostate-derived growth factor(s).

In our initial studies human prostatic adenocarcinoma (CA) and human benign prostatic hyperplasia (BPH) tissues were obtained at surgery and kept frozen at 70°C until use. Tissues were dried, defatted and then extracted in neutral or acidic solutions. As indicator cells we employed primary cultures enriched with osteoblast-like cells, an osteoblast-derived osteosarcoma cell, UMR-108, and, as a control, primary cultures of fibroblasts. The presence and general characteristics of mitogenic activity in neutral extracts of prostatic tissue was first assessed in both CA and BPH extracts [9]. The time course of mitogenic activity of both CA and BPH extracts in primary cultures of fibroblasts, primary cultures enriched with osteoblast-like cells and in the UMR-108 osteosarcoma cells was found to be similar and to reach maximal stimulation by 24 h. Consequently, subsequent studies were performed after 22-24 h incubation of growth factors with these cells.

A variety of peptide growth factors have now been described [10,11] which may be either produced locally and act in an autocrine or paracrine fashion in skeletal tissue or which may reach the skeleton via the systemic circulation. We therefore, next examined the mitogenic effect of a variety of such growth factors in our indicator cells. This included insulin, insulin-like growth factor (IGF; a mixture of IGF-I and IGF-II), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), recombinant interleukin-1α and β (IL-1α and IL-1β) and transforming growth factor beta (TGF-β). All were found to be mitogenic in all three mesodermal cell types we studied. This contrasted with the behavior of peptides we have extracted from prostatic tissue which, as indicated subsequently, were preferentially active in osteoblasts. To determine whether our prostatic extracts contained unique mitogens we co-incubated the known growth factors and prostatic extracts with osteosarcoma cells and calvarial cells. When maximally-stimulating concentrations of CA extracts were co-incubated with known growth factors in cultures of cells of the osteoblast phenotype, enhanced activity was seen indicative of the distinct locus of action of these peptides. Consequently, unique mitogens appeared to exist within the prostatic extracts [12].

To identify the chemical characteristics of our prostatic mitogens we next investigated effects of proteolytic digestion and of acid stability. After tryptic digestion of both BPH and CA extracts, reductions in mitogenic activity of 55% to 85% were seen indicating the protein nature of the material [9]. Both CA and BPH extracts retained full mitogenic activity in all three indicator cells when extracted in acidic as well as in neutral medium, demonstrating the acid stability of the mitogens [12].

Further attempts at purification of BPH and CA acidic extracts were therefore pursued using reverse-phase high performance liquid chromatography (HPLC). Reverse-phase HPLC of extracts of BPH yielded mitogenic activity in all three indicator cells. However, regions in which mitogenic activity was demonstrable in primary cultures of osteoblastic cells and in osteosarcoma cells, but not in fibroblasts, could be observed in BPH tissue. Following rechromatography of these regions on reverse-phase HPLC, homogeneous UV peaks were obtained which retained bioactivity in osteoblastic cells but which failed to stimulate activity in fibroblasts. These peaks produced dose-dependent stimulation of [3H] thymidine incorporation and a dose-dependent increase in numbers of cells of the osteoblast phenotype. Additionally graded doses of this material increased alkaline phosphatase activity in the osteoblast-like cells. Reverse-phase HPLC of CA extracts also yielded activity which was mitogenic in the three indicator cells; however, a region of mitogenic activity selective for cells of the osteoblast phenotype was seen as well with this tissue (Fig. 1). Further chromatography of this region also yielded a homogeneous UV