Review Article

The comparative biology of skeletal metastasis

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Abstract

Bone metastasis, a very common sequelae of cancer, is often associated with great morbidity. Understanding the biology of bone metastases may lead to therapeutic interventions to target the metastases. In addition to replacing bone marrow elements, the presence of tumour cells in bone modulates the normal bone remodelling process. Some tumours result in primarily osteolytic bone lesions, whereas others are associated with osteoblastic bone lesions. In either case, the resulting changes in the bone structure result in weakened bone that induces pain and is predisposed to fracture. The mechanisms through which cancer cells modulate bone remodelling are not clearly defined, but ongoing research using a variety of animal models will hopefully provide clues to prevent or slow the progress of bone metastases.

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Introduction

Bone is the third most common site for metastasis after lung and liver. Bone metastases are a major source of morbidity and mortality in patients with metastatic cancer. Reports on bone metastasis in veterinary patients are limited, and most of the clinical data and pathophysiologic mechanisms of bone metastasis are derived from human data or murine studies. Compared to primary bone tumours, such as osteosarcoma, that originate in the bone, bone metastases originate from a distal tumour site that eventually migrates to bone, although in some instances, a primary osteosarcoma itself may metastasize to other bone sites. In human beings, bone metastases account for over 99% of all tumours in bone, as opposed to only 1% for primary bone tumours.

The presence of tumour in the bone affects bone remodelling. The resultant phenotype depends upon the tumour type; osteolytic, osteoblastic or both types of induced changes may occur. For example, whereas both human breast and prostate carcinomas have a propensity to metastasize to bone, metastatic breast cancers usually result in osteolytic lesions and prostate cancer generally produces osteoblastic lesions (Reddi *et al.*, 2003). In dogs, metastatic tumours tend to colonize in highly vascularized regions of the skeleton, including the axial skeleton and proximal long bones in affected dogs (Durham & Dietze, 1986; Cooley & Waters, 1998). In humans, the most common locations of bone metastasis include the spine, ribs, pelvis, skull and proximal femur (Mirra *et al.*, 1989). In addition, the most common solid tumours involved with bone metastasis in humans originate from prostate, breast, lung, kidney or thyroid gland. This correlates with metastases in the dog, where Cooley reported mammary gland, prostate and urinary bladder tumours as the most frequent primary sites for bone metastasis (Cooley & Waters, 1998).

The metastatic cascade

The metastatic process is complex, and several barriers must be overcome before primary tumour cells

can spread and multiply at distal sites such as the bone. This complex pathway is referred to as the metastatic cascade. Briefly, tumour cells detach from the primary tumours and invade through their extracellular matrix by utilizing extracellular matrix-degrading enzymes. In addition, the adhesion properties of tumour cells may be altered, allowing for their migration and colonization at novel distal sites. Tumour cells then intravasate into the bloodstream, travel within the bloodstream to their target organ and extravasate into distal sites such as bone. Once at the target site, the tumour cells successfully reproduce into a tumour. A variety of growth factors, cytokines and extracellular–intracellular signalling mechanisms, many of which may be unique to their target site, may be altered to allow bone metastasis to occur. Furthermore, angiogenesis plays an important role in tumour proliferation and survival at distal sites. It is postulated that the bone microenvironment provides a favourable media for tumours involving the mammary or prostate gland, and communication between the tumour and stromal tissue may produce a vicious cycle of metastatic tumour development (Keller *et al.*, 2001; Cooper *et al.*, 2003). These properties of metastasis need to be investigated, because each step may be a potential target for the development of therapeutic strategies for minimizing and preventing the spread of primary tumours to distal sites such as bone.

Properties of bone metastases

There are only limited descriptions of metastasis to bone in veterinary patients (Cooley & Waters, 1998). Similar to humans, the primary tumours are typically epithelial in origin (Table 1). Mesenchymal tumours such as mesothelioma and aortic body tumours, however, have also been demonstrated to metastasize to bone. Of the animal tumours that metastasize to bone, prostate cancer has received the most attention. The dog is one of the few animal species that develops spontaneous prostate cancer and thus may serve as a valuable model for human disease. Human prostate cancer is typically considered to be osteoblastic, i.e. it produces bone. It has been demonstrated that canine prostate tissue implanted over the calvaria of mice induces abundant new woven bone formation on the adjacent periosteal surface. These results suggest that the canine prostate cancer cells act in a similar fashion to human prostate cancer cells.

Clinical significance of bone metastasis

The presence of bone metastases is often associated with poor survival time. The patient's survival is dependent on the degree of osseous involvement. In addition to adversely affecting survival time, bone metastases cause considerable morbidity. Bone metastases affect the patient through several mechanisms. They can induce pain due to microfracture and gross fracture. If the extent of metastatic volume is severe, myelophthisis may occur. Bone pain is poorly localized and often described as a deep ache accompanied by occasional sharp discomfort. The pain is not related to any event, but eventually becomes more prominent and may be aggravated

Table 1. Tumours metastatic to bone in dogs (a review of the literature)

Primary tumour	Reference
Chemodectoma	(Szczech et al., 1973)
Cutaneous squamous cell carcinoma	(Jonsson & Gustafsson, 1973)
Sebaceous gland carcinoma	(Case et al., 1969)
Mammary carcinoma	(Misdorp & den Herder, 1966)
Pheochromocytoma	(Stowater, 1979; Barthez et al., 1997)
Aortic body tumour	(Montgomery et al., 1980)
Urinary bladder carcinoma	(Cooley & Waters, 1998)
Prostate carcinoma	(Durham & Dietze, 1986; Lee-Parritz & Lamb, 1988; Cornell et al., 2000)
Transmissible venereal tumour	(Padovan et al., 1987)
Malignant mesothelioma	(Smith & Hill, 1989)
Renal cell carcinoma	(Arai et al., 1991)
Uveal melanoma	(Rovesti et al., 2001)
Nephroblastoma	(Terrell et al., 2000)

by movement. The origin of the bone pain is not proven; however, it may arise from stimulation of pain receptors in either the periosteum or endosteum. Mechanical stimulation can result from the growing tumour mass, formation of microfractures or development of pathologic fractures.

Treatment for bone metastasis is mainly palliative and is aimed at reducing pain and further bone destruction and improving quality of life. Owing to increased osteolytic activation of certain bone metastatic tumours, dogs may show signs of pain, hypercalcaemia, neurological signs with spinal metastasis and pathological fractures. In humans, bisphosphonates, which inhibit osteoclast-induced bone resorption, are commonly used to interfere with tumour-induced bone osteolysis (Green, 2002). These agents have been documented to reduce bone pain; however, there is no clear correlation between administration of bisphosphonate and tumour response. Other options include radioisotopes used either alone or with external beam radiotherapy to reduce the pain induced by skeletal metastases (Siegel & Cronin, 1997; Ramirez *et al.*, 1999; Serafini, 2001). These treatments, combined with surgery to improve bone stability, and analgesia can provide effective palliation of symptoms for the majority of patients (Gilson, 1998; Lester & Gaynor, 2000).

Recent advances in understanding the biology of osteoclasts has led to the development of novel compounds to inhibit bone resorption. Of particular note is the identification of a key osteoclastogenic factor, receptor activator of NFκB ligand (RANKL). In normal bone, osteoblastic cells regulate osteoclastogenesis and osteoclast activity by interacting with mononuclear haematopoietic osteoclast precursors (Roodman, 1996). The molecular mediator of this interaction was shown to be the osteoblast-expressed protein RANKL. Binding of RANKL to RANK on the osteoclast precursor initiates a cascade of intracellular signals that culminate in the acquisition and activation of the osteoclast phenotype (Lacey *et al.*, 1998; Yasuda *et al.*, 1998). The osteoblast also produces osteoprotegerin (OPG) that regulates excessive bone resorption by acting as a soluble decoy receptor for RANKL (Simonet *et al.*, 1997). Thus, OPG neutralizes the RANKL–RANK interaction, resulting in the inhibition of osteoclastogenesis. OPG is currently in human clinical trials to test its efficacy in bone metastasis.

Mediators of osteoblastic metastasis

A variety of factors may contribute to cancer-mediated bone mineralization. Prostate cancer cells produce a variety of factors that have direct or indirect osteogenic properties (Table 2) (Yoneda, 1998; Boyce *et al.*, 1999; Deftos, 2000). Some of these factors, such as bone morphogenetic proteins (BMPs) (Harris *et al.*, 1994; Autzen *et al.*, 1998; Hullinger *et al.*, 2000) and endothelin-1 (ET-1) (Nelson *et al.*, 1995), may directly stimulate differentiation of osteoblast precursors to mature mineral-producing osteoblasts. Other factors such as parathyroid hormone-related protein (PTHrP) may work through inhibition of osteoblast apoptosis (Karaplis & Vautour, 1997; Cornish *et al.*, 1999). Additionally, there are proteins that may work indirectly to enhance bone production, such as the serine proteases, prostate-specific antigen and urinary plasminogen activator, which can activate latent forms of osteogenic

Table 2. Osteoblastic factors produced by tumour cells

Factor	Reference
Bone morphogenetic proteins	(Bentley *et al.*, 1992; Hullinger *et al.*, 2000)
Endothelin-1	(Nelson *et al.*, 1995; Nelson & Carducci, 2000)
Insulin-like growth factors	(Perkel *et al.*, 1990; Pirtskhalaishvili & Nelson, 2000)
Interleukin-1 and interleukin-6	(Taguchi *et al.*, 1998; Le Brun *et al.*, 1999)
Osteoprotegerin	(Guise, 2000; Honore *et al.*, 2000)
Parathyroid hormone-related peptide	(Karaplis & Vautour, 1997; Cornish *et al.*, 1999)
Transforming growth factor-β	(Killian *et al.*, 1993)
Urinary plasminogen activator (urokinase)	(Goltzman *et al.*, 2000)

proteins, such as transforming growth factor-β (Killian *et al.*, 1993; Rabbani *et al.*, 1997). Finally, some molecules, such as OPG (Simonet *et al.*, 1997; Guise, 2000; Honore *et al.*, 2000) and ET-1 (in a dual role with its osteoblast-stimulating activity) (Chiao *et al.*, 2000), can enhance osteosclerosis through inhibiting osteoclastogenesis. Despite this gamut of putative mediators of prostate cancer-induced osteosclerosis, the investigators are unaware of *in vivo* studies that unequivocally demonstrate their role in this process. Other tumour types, such as osteosarcoma, also produce a variety of osteoblastic factors (Wlodarski & Reddi, 1987; Raval *et al.*, 1996; Laitinen *et al.*, 1998). It is most likely that several of these osteogenic factors work in concert to produce maximal bone production.

In one study, normal dog prostate tissue was implanted subcutaneously over mouse calvaria (LeRoy *et al.*, 2002). The prostate tissue remained viable and induced abundant new woven bone formation on the adjacent periosteal calvarial surface, and in some cases, new bone formation was also induced on the concave calvarial periosteum. These results are consistent with the pro-osteoblastic activity of prostate cancer and suggest that non-transformed prostate cells themselves are pro-osteoblastic. Intriguingly, osteoclast activity within the bone was also increased, suggesting that the overall bone remodelling is increased by the prostate cells.

Mediators of osteoclastic metastasis

A number of reports have shown that osteoclastic bone resorptive lesions are important to the development of bone metastases in several cancer types, including breast cancer, lung cancer and prostate cancer (Yoneda, 1998). These cancers may induce osteoclast activity through secretion of interleukin-1α, PTHrP or prostaglandin-E2 (PGE2) (Guise *et al.*, 1996; Mundy, 1997; Akatsu *et al.*, 1998). Tumour-mediated osteolysis in some instances, such as breast cancer, however, occurs indirectly through expression of molecules, such as PTHrP, that induce RANKL in osteoblasts (Roodman, 1999; Thomas *et al.*, 1999). This contrasts with the observations that giant-cell tumours directly

promote osteoclast activity via RANKL (Atkins *et al.*, 2001) and that prostate cancer cells directly induce osteoclastogenesis through RANKL (Zhang *et al.*, 2001a). Another factor that may play a role in tumour-induced osteoclastogenesis is human macrophage inflammatory protein-1α, which has been shown to be produced by myeloma cells (Han *et al.*, 2001). Because of the osteoclastic activity induced by many neoplasms, antiresorptive agents such as bisphosphonates or anti-PTHrP neutralizing antibody have been reported in breast cancer animal models to block tumour expansion in bone (Sasaki *et al.*, 1995; Alsina *et al.*, 1996). Furthermore, OPG has been shown to inhibit primary bone sarcoma-induced osteolysis and tumour-induced bone pain but not tumour burden in mice (Honore *et al.*, 2000). OPG, however, not only blocked osteolytic bone metastasis induced by human neuroblastoma NB-19 cells (Michigami *et al.*, 2001) but also reduced tumour burden in that model.

In vivo methods for research on bone metastasis

Advances in exploring the biology of prostate cancer skeletal metastasis have been hampered by the lack of good animal models. The ideal model to examine prostate cancer skeletal metastasis should include all aspects of the metastatic cascade, from primary tumour formation through overt metastases. Additionally, production of bone lesions that contain both osteoblastic and osteolytic components and, preferably, allowing for modelling the interaction between human tumour cells and human bone should be utilized in comparative models. An excellent review of human prostate cancer skeletal metastasis models has been recently published (Zhau *et al.*, 2000). We describe several novel models below.

One model recently developed for the comparative study of human prostate cancer is the severe combined immunodeficient (SCID) human system (Nemeth *et al.*, 1999). In this model (Fig. 1), macroscopic (1 cm) fragments of human fetal bones (femora and humeri) were implanted

subcutaneously into SCID mice. After implantation, the bone fragments remained anatomically intact with recognizable cortical and trabecular bone and bone marrow. After several weeks, human prostate cancer cells (from established prostate cancer cell lines or prostate cancer tumour xenografts) were injected into the mice, either via the tail vein or directly into the marrow compartment of the implanted bone. Large prostate cancer bone tumours (0.5–1.0 g) developed over the next 6–12 weeks in the implanted bone fragment. The tumours were composed of prostate cancer cells, bone cells and bone stroma/extracellular matrix. Histological examination demonstrated close interposition of all of these elements with each other, resulting in a complex tumour similar in histological appearance to clinical specimens of bone metastases. Depending on the cell line used, tumours were predominantly osteolytic, mixed osteolytic/osteoblastic or predominantly osteoblastic, as determined by radiographic and histological analysis. An advantage of this model is that human tumour cells are grown in human bone, as opposed to murine bone. A weakness of this model, however, is the use of fetal bone, which has a different composition than adult bone. To circumvent this weakness, human adult bone was implanted in mice to study the effects of osteoclast inhibitors on tumour growth in bone (Yonou *et al.*, 2003).

To provide an additional assessment of the metastatic cascade from the vascular system on through the target site, intracardiac injection of cancer cells can be performed (Yin *et al.*, 1999). Mice were re-anaesthetized and tumours were injected percutaneously into the left ventricle. After intracardiac injection, tumours typically developed in the long bones within a period of 4–8 weeks, depending on the cell line used. This model typically results in a take rate of 80–90%. The strength of this model is that it requires the cancer cells to extravasate from the blood vessel to the metastatic target site, which allows for good modelling of the later half of the metastatic cascade. A disadvantage is that the spontaneous release of metastatic cells from a primary tumour is not evaluated in this model.

One challenge with animal models is identifying locations of metastases at multiple sites and monitoring tumour growth in bone. Typically, measurements are made using radiographs. Radiographs, however, have several drawbacks, including: (1) lack of sensitivity, as over 50% of the bone mineral density has to change prior to it being detected as a change on radiographs; (2) they only provide a one-dimensional evaluation of the
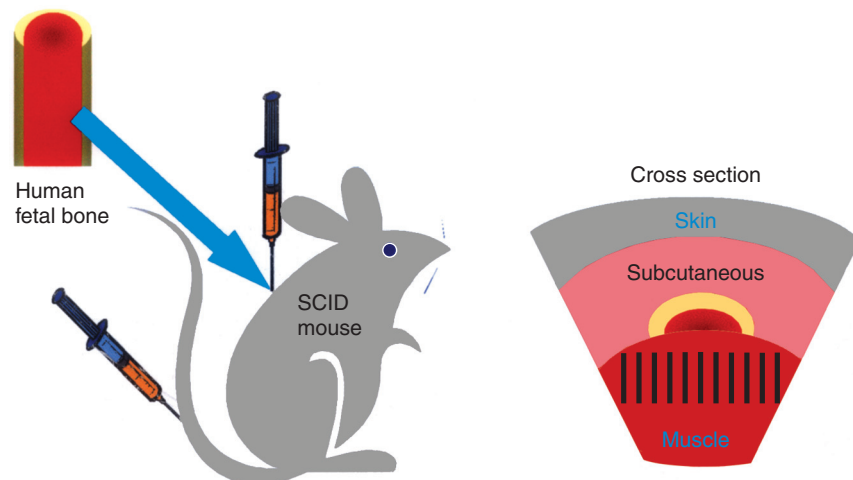
Figure 1. Schematic of the severe combined immunodeficient (SCID)-Hu model. Fetal long bone was longitudinally sectioned in half and implanted subcutaneously into the SCID mouse. The cross section demonstrates the implant location, with the open marrow section being placed adjacent to muscle and subcutaneous tissue and skin overlying the implant. Four weeks after implantation of bone, tumour cells were injected either directly into the marrow cavity or intravascularly.
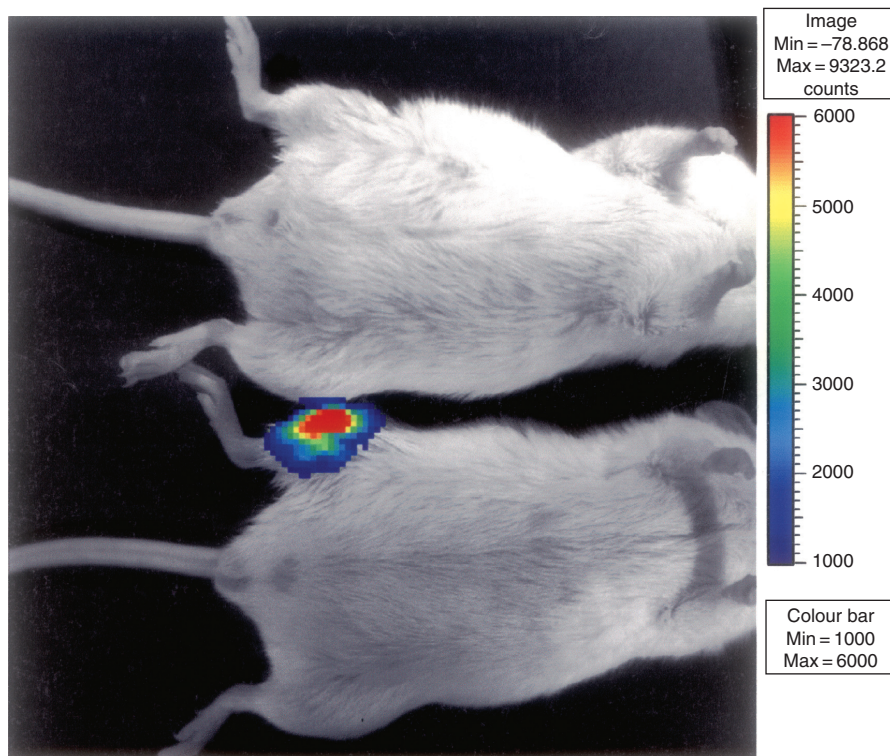
Figure 2. Luminescent imaging of prostate tumour in bone. A human prostate cancer cell line was engineered to express a luminescent signal (luciferase). The cancer cells were then injected into the tibia of mice and allowed to grow. After several weeks, the mice were subjected to imaging. The upper mouse was not injected with tumour cells. The lower mouse's right tibia was injected with tumour cells. Luciferase activity is indicated by the colours in the tibia. The scale indicates the degree of luminescent activity.

bone and do not allow quantitative determination of total tumour volume; and (3) they are an indirect measure of the tumour, as they only identify the effect of tumour on bone, not the tumour itself. Recent advances in fluorescent (Hoffman, 2001) and luminescent imaging (Zhang *et al.*, 2001b) have provided sophisticated and sensitive methods to identify and quantify tumour growth *in vivo* (Huang *et al.*, 2002). Briefly, cancer cells were engineered to express fluorescent or luminescent markers and then injected into mice through a variety of routes. This methodology can be used to identify tumour growth in bone (Fig. 2), thus providing a great opportunity to explore the biology of bone metastasis.

Conclusion

Bone metastases are a frequent and debilitating aspect of cancer. Advances in bone biology are leading to a better understanding of the patho-physiology of bone metastases. Furthermore, novel laboratory animal models and tumour-imaging technologies should facilitate defining the mechanisms of bone metastasis.

Acknowledgments

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