METASTASIS TO BONE: CAUSES, CONSEQUENCES AND THERAPEUTIC OPPORTUNITIES

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The most common human cancers — lung, breast and prostate — have a great avidity for bone, leading to painful and untreatable consequences. What makes some cancers, but not others, metastasize to bone, and how do they alter its physiology? Some of the molecular mechanisms that are responsible have recently been identified, and provide new molecular targets for drug development.

METASTASIS

LEUKOERYTHROBLASTIC ANAEMIA
A type of anaemia that is associated with cancers that involve the bone marrow, and that is accompanied by increased production of white blood cells.

Most patients with cancer die not because of the tumour in the primary site, but rather because it has spread to other sites. It is difficult to determine, however, precisely how frequently different tumours metastasize to bone. Patients with advanced breast and prostate cancers almost always develop bone metastases, and the chances are high that, in patients who are originally diagnosed with breast or prostate cancers, the bulk of the tumour burden at the time of death will be in bone. How long the patient lives with the tumour is likely to influence whether bone metastases will occur. For example, in patients who quickly succumb to cancer, due to an aggressively growing primary tumour, bone metastases will be relatively uncommon simply because they have not had time to develop. This does not mean that the tumour cells did not have the potential to grow in bone.

There are no reliable prevalence figures for people with bone metastases, but estimates can be made. Of the four million people who die in the United States each year, approximately one-quarter die from cancer, and 70% of these have either breast, lung or prostate cancer[1]. So, there are probably more than 350,000 people in the United States who die each year with bone metastases, and probably two to three times this number if patients in the European Union and Japan are also included. The number of bone metastases increases when we consider patients who are living with the condition, as patients with breast and prostate cancer frequently live longer than one year.

Bone metastases are infrequently silent — they are usually associated with severe bone pain, which can be intractable. The mechanisms responsible for bone pain are poorly understood[2], but seem to be a consequence of osteolysis (bone breakdown). There is evidence that bone-resorption inhibitors, such as osteoprotegrin (OPG) or bisphosphonates, might be used to alleviate bone pain[3] (BOX 1). Osteolysis is also accompanied by increased bone fragility; susceptibility to fracture is markedly increased, and pathological fractures frequently occur as a consequence of bone metastases. They often occur in load-bearing bones, and are a particular treatment problem when they are present in the neck or shaft of the femur, or in the pelvis. Other consequences of bone metastasis are LEUKOERYTHROBLASTIC ANAEMIA, bone deformity, hypercalcaemia, and nerve-compression syndromes such as spinal-cord compression (BOX 2).

It has been traditional to think of bone metastases as either osteolytic or osteoblastic (FIG. 1), with entirely different factors being responsible for each. From this viewpoint, osteolytic metastases are believed to be caused by osteoclast-activating factors — the most important of which might be parathyroid-hormone-related peptide (PTHrP) — and which are released by tumour cells in the bone microenvironment. Osteoblastic metastases, conversely, are believed to be caused by the cancer-cell production of factors that stimulate osteoblast proliferation, differentiation and bone formation. We now realize that osteolytic and

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Summary

- Common tumours, such as those of the breast, lung and prostate, frequently metastasize to bone, and in many patients with advanced disease the skeleton is the site of the most significant tumour burden.
- There are different patterns of bone effects in patients with cancer, ranging from purely or mostly destructive or osteolytic (breast cancer, myeloma), to mostly bone-forming or osteoblastic (prostate cancer).
- In the case of breast-cancer-causing osteolysis, the main mediator is parathyroid-hormone-related peptide (PTHrP), whereas, in osteoblastic lesions, known mediators include endothelin-1 and platelet-derived growth factor.
- In osteolytic metastasis, there is a 'vicious cycle' in the bone microenvironment, whereby bi-directional interactions between tumour cells and osteoclasts lead to both osteolysis and tumour growth.
- The molecular mechanisms that are responsible for this vicious cycle are now being clarified and involve tumour-cell production of PTHrP and bone-derived growth factors that are released as a consequence of increased bone resorption.
- Bisphosphonates interrupt the vicious cycle and cause not only a reduction in osteolytic bone lesions, but also decrease the tumour burden in bone.
- More-effective treatments for interruption of the vicious cycle are now being developed, including specifically neutralizing antibodies to PTHrP and more efficacious osteoclast inhibitors.

osteoblastic lesions are two extremes — morphological analysis has revealed that, in most patients, bone metastases have both osteolytic and osteoblastic elements.

Osteolytic and osteoblastic lesions

Although breast and prostate cancer are the two tumour types that most commonly metastasize to bone, the end result of metastasis by each is usually quite different. In breast cancer, bone metastases are predominantly osteolytic. Osteolysis is caused by osteoclast stimulation — not by the direct effects of cancer cells on bone[4]. Although the dominant lesion is lytic and destructive, there is usually also a local bone-formation response, which presumably represents an attempt at bone repair[5]. This increase in bone formation in patients with osteolytic lesions is reflected by increased levels of serum ALKALINE PHOSPHATASE — a marker of osteoblast (bone-forming cell) activity — and increased uptake of BONE-SCANNING AGENTS at the site of the lesion. However, despite this secondary increase in local bone formation, the predominant effect is osteolysis.

In prostate cancer, alternatively, bone metastases are frequently osteoblastic[6]. In these metastases, there is a profound local stimulation of osteoblasts adjacent to the metastatic tumour cells, as measured by alkaline phosphatase and OSTEOCALCIN levels. Up to 25% of patients with bone metastases from breast tumours, however, also have blastic lesions that are similar to those with metastatic prostate cancer, and some patients with prostate cancer have osteolytic lesions that are similar in nature to those seen in patients with metastatic breast cancer.

So the concept that there are basically two types of bone metastases is probably too simplistic. The processes of bone resorption and bone formation are almost always linked or coupled — although this coupling might be distorted in cancer. Recent observations indicate that there is a spectrum of bone metastasis. At one end, predominantly osteolytic lesions are associated with reduced osteoblast activity that is uncoupled from rates of bone resorption. Bone metastases that are predominantly osteoblastic, alternatively, also have resorptive components. There is much evidence that both resorption and formation are activated in most bone metastases. In addition to the increased uptake of bone-scanning agents at the metastatic site (a reflection of osteoblastic activity), serum markers of bone resorption, such as urinary hydroxyproline, deoxypyridyniline and pyridiniline crosslinks, are also frequently increased[7–13].

Researchers have followed the course of development of osteoblastic metastases from human breast cancer xenografts in nude mice[13]. This model allows sequential morphological observations of the metastases — information that is impossible to obtain from clinical studies. In this model, metastatic tumours are initially osteolytic — characterized by increases in osteoclast activity, which is associated with increased production of bone-resorption markers. This lytic activity is then followed by a wave of bone formation, and osteoclast activity is reduced. The precise molecular mechanisms that are responsible for this switch are not known. These studies, however, have important implications for therapeutic strategies. Treatments that are aimed at inhibiting bone resorption, such as bisphosphonates, might be effective not only in treating lesions that are primarily osteolytic, but also in treating osteoblastic metastases — if the osteoblastic response is dependent on previous osteoblast activity[12,14].

Some types of bone metastases are known to always be osteolytic. MYELOMA cells cause exclusively osteolytic bone lesions, and although myeloma is not strictly a metastasis, there are probably solid tumours that behave in a similar manner. There are also some osteoblastic lesions that seem to have little or no resorptive component. Charhon *et al.*[6] described some human cancers that cause osteoblastic metastases with

ALKALINE PHOSPHATASE
An enzyme that is found on the cell surface of osteoblasts, is involved in bone mineralization and is used as a serum marker of increased osteoblast activity.

BONE-SCANNING AGENTS
Agents, such as bisphosphonates, which localize to bone, that can be tagged with an isotope that allows their detection by imaging techniques. These are used in clinical medicine to detect sites of active bone turnover or bone disease.

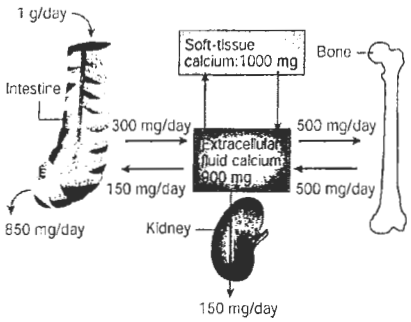
OSTEOCALCIN
A protein constituent of bone. Its function remains to be clarified, but circulating levels are used as a marker of osteoblast activity.

MYELOMA
A neoplastic disorder of the plasma cells that is associated with extensive bone destruction and production of large amounts of specific immunoglobulins.

Box 1 | Bisphosphonates

Bisphosphonates are a class of pyrophosphate analogues that bind with high affinity to mineralized bone surfaces and inhibit osteoclastic bone resorption. They are used extensively to treat patients with diseases of bone loss, such as osteoporosis and Paget's disease, and cancers that cause osteolysis. Common examples of bisphosphonates are pamidronate (Aredia), alendronate (Fosamax), zoledronate (Zometa) and clodronate (Bonefos). The bisphosphonate market for these diseases is over US $1.5 billion dollars annually. The newer bisphosphonates act by inhibiting specific enzymes in the mevalonate pathway of cholesterol biosynthesis in osteoclasts, which, in turn, leads to impaired prenylation of important small GTP-binding proteins such as RHO, and to subsequent changes in the cytoskeletal function that promotes osteoclast apoptosis.

Box 2 | **Hypercalcaemia**



Hypercalcaemia (increased blood-calcium concentration) is an important complication of osteolytic bone disease. It occurs relatively frequently in patients with extensive bone destruction, and is particularly common in breast, lung, renal, ovarian and pancreatic carcinomas, as well as in myeloma. It is distressing for the patient, and it must be recognized and treated vigorously.

Hypercalcaemia that occurs in most patients with cancer is due to the production of the peptide parathyroid hormone-related peptide (PTHrP) by the tumour[69–71]. PTHrP acts on PTH receptors to cause increased bone resorption and increased renal tubular calcium reabsorption[72]. Bone destruction is an important cause of hypercalcaemia, but the important contributing role of renal mechanisms has been under-appreciated. Hypercalcaemia occurs as a consequence of the combination of these effects and by overwhelming of the calcium homeostatic defence mechanisms. This can be appreciated when the calcium homeostasis for a normal adult in zero calcium balance is considered (see figure). The numbers are estimates of the amount of calcium that is exchanged between the extracellular fluid and gut, kidney and bone each day[73].

Bisphosphonates — a class of drugs that block bone resorption — have made an enormous difference in both the frequency and management of hypercalcaemia in patients with cancer. These drugs reduce the incidence of hypercalcaemia and, when it does occur, it is readily treated, at least initially[74]. Nearly all patients show a beneficial response.

However, we might have become complacent in the treatment of hypercalcaemia of malignancy[30]. Bisphosphonates might have only a transient beneficial effect because renal tubular calcium reabsorption is unaffected by bisphosphonates. This is not apparent in most patients because hypercalcaemia is usually a hallmark of extensive tumour burden and advanced disease, and many patients with hypercalcaemia die within one month of its onset. Neutralizing antibodies to PTHrP have been shown in preclinical studies to be effective in the treatment of hypercalcaemia[24].

no morphological evidence of a resorptive component. So, how do cancer cells metastasize to bone and, when they have reached their destination, how do they set up this cycle of bone formation and destruction?

Pathophysiology of bone metastasis

The initial steps in the development of bone metastases are similar to those of metastases to any other site. Primary tumour cells invade their surrounding normal tissue by producing proteolytic enzymes, which traverse the walls of small blood vessels in the normal tissue or of those induced by the tumour and enter the circulation[15]. They then travel to distant organ sites. These events have been described as inefficient, in that many cancer cells do not survive the normal protective host-surveillance mechanisms during these initial stages[16–18].

The cancer cells that do survive can enter the wide-channelled sinusoids of the bone-marrow cavity and are positioned to become bone metastases. Cancer cells must possess certain properties for this to occur. They must have the capacity to migrate across the sinusoidal wall, invade the marrow stroma, generate their own blood supply and travel to the endosteal bone surface (FIG. 2). At this site, they stimulate the activity of osteoclasts or osteoblasts, thereby determining whether the subsequent bone metastasis is osteolytic or osteoblastic. Each of these steps involves important molecular interactions between the tumour cells and the normal host cells, and each is a potential target for the development of drugs that are designed to abrogate the metastatic process.

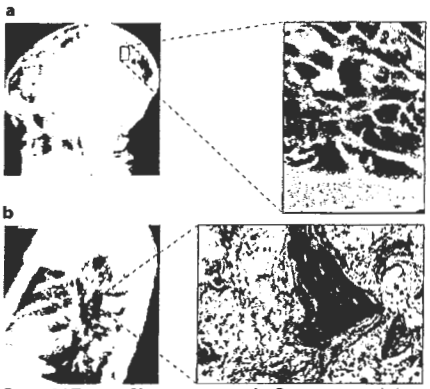


Figure 1 | **Types of bone metastasis.** Bone metastasis is often classified as either **a** | osteolytic or **b** | osteoblastic, and one of these effects is usually predominant. For example, metastases from breast and lung tumours are generally osteolytic, whereas metastases from prostate cancer are generally osteoblastic. However, most blastic metastases have a resorptive component, and most lytic lesions are accompanied by some attempt, albeit incomplete, of repair or bone formation. Reproduced with permission from REF. 75 (1998) Massachusetts Medical Society.

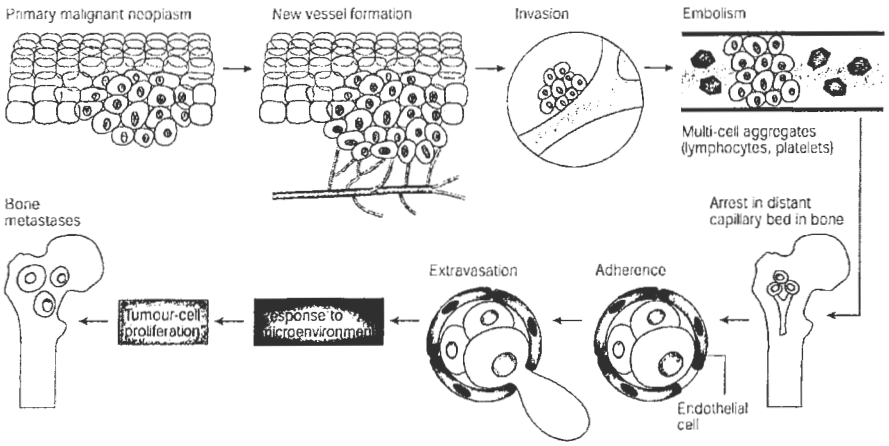


Figure 2 | **The steps involved in tumour-cell metastasis from a primary site to the skeleton.** Each of these steps represents a potential therapeutic target to reverse or prevent metastatic bone disease[76]. The primary malignant neoplasm promotes new blood-vessel formation, and these blood vessels carry the cancer cells to capillary beds in bone. Aggregates of tumour cells and other blood cells eventually form embolisms that arrest in distant capillaries in bone. These cancer cells can then adhere to the vascular endothelial cells to escape the blood vessels. As they enter the bone, they are exposed to factors of the microenvironment that support growth of metastases. Adapted from REF. 76.

Mechanisms of osteolytic metastasis

The mechanisms by which cancer cells cause osteolytic metastasis are gradually being unravelled. In metastatic human breast cancer, the peptide PTHrP is the main mediator of osteoclast activation, and human osteolytic breast cancer cells have been shown to express PTHrP *in vivo*. PTHrP expression is greater when the tumour cells are present at the metastatic bone site than when they are present in soft-tissue sites or in the breast[19,20]. Immunohistochemistry and *in situ* hybridization experiments have shown that breast cancer metastases in bone express higher levels of PTHrP than cells that have metastasized to soft tissue or that are present in the primary site. This indicates that PTHrP is a specific mediator of osteolysis in metastatic breast cancer, and it is likely to be the mediator of bone destruction in most other osteolytic cancers[21,22].

The role of PTHrP in inducing osteolysis is, however, complex. Henderson *et al.*[23] have recently published a clinical study showing that PTHrP expression by primary tumours is associated with a favourable outcome and less propensity to bone metastasis. Other preclinical and clinical data, however, associate PTHrP production with bone metastatic potential[19,20,24]. This could be due to the fact that tumour cells that express high levels of PTHrP are selected for their ability to metastasize to bone, or that the bone microenvironment increases expression of PTHrP from cancer cells that have spread there. Data from Henderson *et al.*[23] support the latter explanation. PTHrP that is expressed by prostate cancer cells has also been reported to have anabolic effects on bone[25]. It is possible that this tumour peptide could promote the osteoblastic metastases that are associated with prostate cancers, although there is no direct data to support this.

Increased expression of PTHrP is not the only phenotypic change that occurs in breast cancer cells that enter the bone microenvironment. Mutations in genes that encode mutant oestrogen receptors[26], interleukin (IL)-8 (REF. 27) and the receptor for PTH[28] have also been associated with bone metastasis. Tumour cells that reside in different metastatic sites might have subtle differences in phenotype that could affect not just the behaviour of the cells at that site, but also responses to therapy.

So, is PTHrP a viable therapeutic target for bone metastases? Osteolysis caused by human breast cancer metastases was shown to be blocked by neutralizing antibodies against PTHrP[24]. Furthermore, compounds that specifically decrease PTHrP expression have been shown to inhibit osteolysis caused by human breast cancer cells *in vivo*[29].

Blocking PTHrP might also have other clinical benefits; Ogata[30] has proposed that this peptide might also be associated with cachexia.

RANKL and osteolytic bone disease. PTHrP stimulates osteoclast activity by stimulating production of the cytokine RANKL (receptor activator of nuclear factor-κB ligand), which binds and activates its receptor, RANK, which is expressed by osteoclasts (FIG. 3). There is, however, a debate about the exact function of RANKL in the osteolytic bone activity that is associated with human solid cancers or myeloma. RANKL production by stromal cells is a final common mediator of osteoclast activity that is stimulated by several factors. Many researchers have reported that RANKL is expressed by tumour cells in the bone microenvironment, but it is not clear whether the production of RANKL by tumour cells

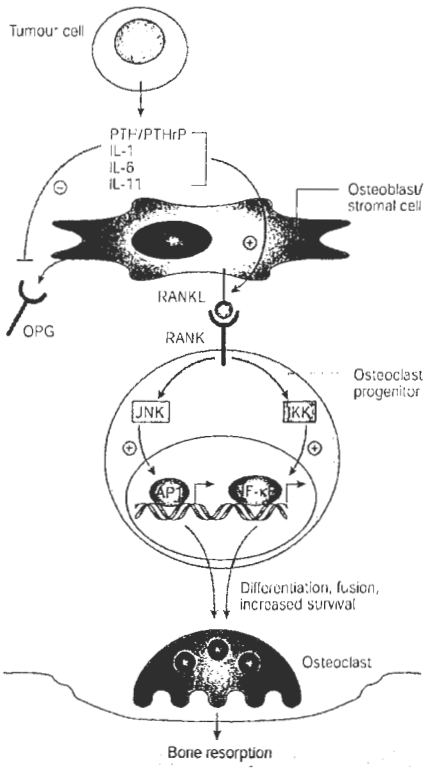


Figure 3 | **The RANK–RANKL system in osteolytic bone metastases.** Tumour production of factors such as parathyroid hormone (PTH) or PTH-related peptide (PTHrP), interleukin (IL)-1, IL-6 and IL-11 stimulate production of receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL) by osteoblasts and stromal cells. Some of these factors (for example, PTHrP) also decrease the production of osteoprotegerin (OPG) — a decoy receptor that prevents RANKL from binding to its receptor (RANK) on osteoclast progenitor cells. Signalling through RANK in osteoclast progenitors activates transcription factors such as AP1 (activated by JUN N-terminal kinase (JNK), or JUN) and NF-κB (activated by inhibitor of κB kinase (IKK)), leading to the differentiation of osteoclast progenitors into mature osteoclasts. These osteoclasts mediate bone resorption.

themselves is sufficient to activate osteolysis[31]. Some studies have shown that bone destruction is prevented by treatment with OPG — a soluble 'decoy receptor' for RANKL. OPG blocks the association of RANKL, as well as other ligands, with RANK. Almost all other mediators of osteoclastic bone resorption, however, also signal through RANKL, so results obtained from experiments that are designed to block RANKL activity do not reveal the importance of the tumour-specific production of RANKL. Moreover, there might be differential expression of RANKL by tumour cells that have metastasized to bone, compared with the same tumour cells at their primary site, although this has not yet been convincingly documented. Perhaps this will eventually be clarified in animal models in which the RANK–RANKL system is rendered ineffective specifically in osteoclastic cells by the use of tissue-specific promoters, or in which RANKL expression is specifically knocked-out in the tumour stroma.

CALVARIA
The skull bones. Rodent calvaria are frequently used in organ culture experiments to determine the effects of factors or compounds that stimulate or inhibit bone resorption or bone formation.

Mechanisms of osteoblastic metastasis

There is accumulating data to identify the factors that stimulate bone formation that is associated with metastatic tumours (BOX 3). One of the most well-studied mediators is the ubiquitous growth factor endothelin-1, which stimulates bone formation and osteoblast proliferation in bone organ cultures. Endothelin-1 is increased in the circulation of patients with osteoblastic metastases and prostate cancer[32] and is also expressed by breast cancer cell lines that cause osteoblastic metastases[33]. Osteoblast proliferation and bone metastasis have both been shown to be inhibited *in vivo* by endothelin-A-receptor antagonists[33,34]. Several other factors, as described below, have also been proposed to be potential mediators of osteoblastic metastasis that is associated with prostate cancer (FIG. 4).

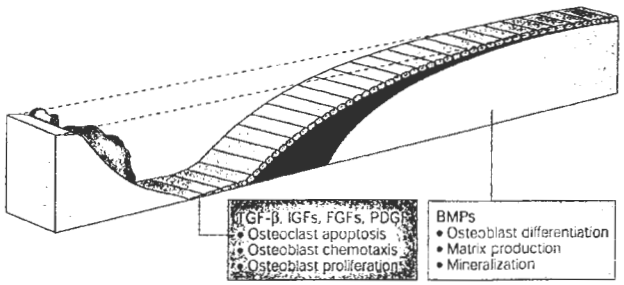
The transforming growth factor-β family. Several members of the transforming growth factor-β (TGF-β) family are powerful *in vivo* stimulators of new bone formation[35], and are candidate mediators of osteoblastic metastasis. TGF-β2 is expressed at high levels by PC3 human prostate cancer cells, and was originally isolated from the human prostate cancer cell line PC3 (REF. 36). TGF-β2 stimulates the proliferation of osteoblasts *in vitro*, as well as bone formation *in vivo*. Both normal, and neoplastic human and rat prostate tissues also express a variety of bone morphogenetic proteins (BMPs) — namely, *BMP2*, *BMP3*, *BMP4* and *BMP6* mRNA[37].

Proteases and their activators. There have been several reports that a mitogen for rat CALVARIAL osteoblastic cells has been purified from the conditioned media of PC3 cells, and that the sequences of the first ten amino acids were identical to that of the serine protease urokinase (uPA)[38]. Overexpression of uPA by rat prostate cancer cells has been shown to induce bone metastases *in vivo*[39], and an amino-terminal fragment of uPA has been shown to have mitogenic activity for osteoblasts[40]. The carboxy-terminal proteolytic domain might mediate tumour invasiveness or growth-factor activation. Proteases that activate growth factors such as TGF-β have been shown to have important functions in bone[41,42]. For example, the latent TGF-β binding protein, which functions to mask TGF-β activity, contains plasmin-sensitive cleavage sites. It is possible that, by activating plasmin, uPA prevents the sequestration of TGF-β.

Another proteolytic mechanism that might be important for metastasis of prostate cancer cells involves prostate-specific antigen (PSA) — a serine

Box 3 | **Bone growth factors and bone remodelling**

The cellular events that are responsible for bone formation (depicted by the increasing bone thickness) include osteoblast proliferation and differentiation, as well as osteoclast apoptosis. Osteoblast proliferation is driven by mitogenic factors, such as transforming growth factor-β (TGF-β), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs) and platelet-derived growth factor (PDGF). Osteoclast apoptosis is induced by TGF-β, but also by drugs such as oestrogen and bisphosphonates. During physiological bone remodelling, the release of TGF-β as a consequence of resorption might be the physiological inducer of osteoclast apoptosis and, therefore, be responsible for limiting osteoclast lifespan and impairing continued resorption. Osteoblast differentiation involves expression of the structural proteins of the bone matrix, such as type I collagen, as well as other bone proteins, including alkaline phosphatase and osteocalcin. The bone morphogenetic proteins (BMPs) act predominantly to stimulate osteoblast differentiation. Bone formation involves a cascade of events that, once triggered, continues until the osteoblasts undergo apoptosis, which might be regulated by ambient growth-factor concentrations and influenced by drugs such as corticosteroids and parathyroid hormone. In cancer patients who have osteoblastic metastases, any of these growth factors that are released by the tumour cells could be expected to ultimately cause osteoblast differentiation and subsequent bone formation.



protease that is overproduced by prostate cancer cells and is used as a marker of tumour burden. PSA can cleave PTHrP at the amino terminus[43,44], and could also potentially activate other growth factors that are produced by prostate carcinomas. It is not known whether it actually promotes tumour growth, but one possibility is that it could activate osteoblast-stimulating factors, such as insulin-like growth factor 1 (IGF1) and TGF-β by cleaving them from their binding proteins, or even by cleaving PTHrP to an anabolic fragment (see below).

Growth factors. Prostate cancer cells express large amounts of both acidic and basic fibroblast growth factors (FGFs)[45,46], which are potential mediators of osteoblast proliferation in patients with prostate cancer[47,48]. Both acidic (FGF1) and basic FGF (FGF2) stimulate bone formation *in vivo*[49,50]. Izbicka *et al.*[51] have shown that a human tumour cell line that produces an extended form of FGF2 activates osteoblasts and causes bone formation *in vivo*.

When the human breast cancer cell line MCF-7 is transfected stably with the ERBB2 (also known as HER2/Neu) proto-oncogene, it causes osteoblastic metastases in mice[13,52]. These tumour cells have been shown to produce the B isoform of platelet-derived growth factor (PDGF-BB). Conditioned media from these tumour cells promotes bone formation in bone organ cultures, but media from cells that are stably transfected with antisense oligonucleotides to PDGF-BB do not. This work indicates that PDGF-BB is a potential mediator of the osteoblastic response in some tumour types.

The bone microenvironment

Why do some cancers have such high avidity for bone — or any other specific metastatic site, for that matter? One reason might be that most circulating tumour cells pass through the bone marrow, as a consequence of its vascularity. However, there are other highly vascularized organs to which tumour cells rarely metastasize. It is, therefore, probable that the environment of bone provides a particularly fertile ground for the growth and aggressive behaviour of the tumour cells that reach it.

The concept that there is a relationship between the 'seed' (tumour cells) and the 'soil' (metastatic site) that determines a cancer's capacity to grow and thrive was first proposed by Stephen Paget more than 100 years ago[53]. In the case of bone metastasis by breast cancer cells, we now understand some of the molecular mechanisms that support this concept. As discussed above, breast cancer cells, when present in the bone microenvironment, overproduce PTHrP, and this leads to osteoclastic bone resorption[24]. Consequently, active growth factors are released from bone that cause proliferation of breast cancer cells. This stimulates further production of PTHrP, which, in turn, causes more bone loss[54]. Studies have also shown that IGF1, which is released during bone

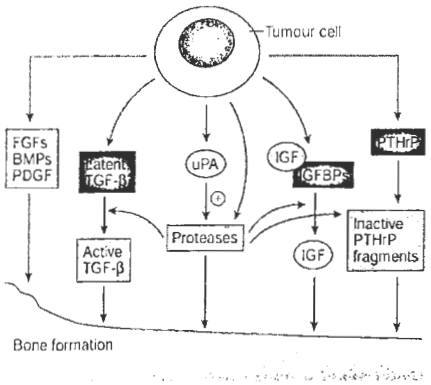


Figure 4 | **Model for osteoblastic bone metastases caused by prostate cancer.** The production of factors such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) by tumour cells can directly stimulate osteoblast activity and subsequent bone formation. Proteases, such as prostate-specific antigen, are induced by activators, such as urokinase (uPA). Proteases can activate latent TGF-β, release IGFs from inhibitory binding proteins (IGFBPs) and inactivate the osteolytic factor parathyroid-hormone-related peptide (PTHrP) to promote bone formation.

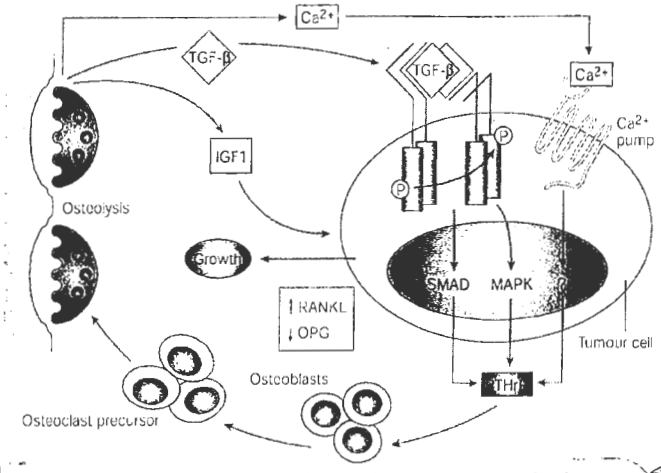


Figure 5 | **The 'vicious cycle' hypothesis of osteolytic metastases.** Interactions between tumour cells and osteoclasts cause not only osteoclast activation and subsequent bone destruction, but also aggressive growth and behaviour of the tumour cells. Production of the parathyroid-hormone-related peptide (PTHrP) by tumour cells activates osteoblasts to produce RANKL (receptor activator of nuclear factor-κB ligand) and downregulate osteoprotegerin (OPG). This activates osteoclast precursors, leading to osteolysis (see FIG. 3). Osteolysis leads to the release of bone-derived growth factors, including transforming growth factor-β (TGF-β) and insulin-like growth factor 1 (IGF1), and raises extracellular calcium (Ca^{2+}) concentrations. The growth factors bind to receptors on the tumour-cell surface and activate autophosphorylation (P) and signalling through pathways that involve SMAD (cytoplasmic mediators of most TGF-β signals) and mitogen-activated protein kinase (MAPK). Extracellular Ca^{2+} binds and activates a Ca^{2+} pump. Signalling through these pathways promotes tumour-cell proliferation and production of PTHrP. Other cytokines might also be involved, such as interleukin (IL)-1, IL-6, IL-11 and IL-18 (not shown). There are many therapeutic targets in this cycle, including PTHrP, the growth-factor-receptor interactions, and the TGF-β signal-transduction pathway. Bisphosphonates are on the market at present, whereas osteoprotegerin and PTHrP antibodies are in clinical trials.

resorption, can also induce tumour-cell proliferation under similar circumstances[55]. In this way, a 'vicious cycle' (FIG. 5) is set up between the tumour cells and bone: resorbed bone releases TGF-β and IGF1, thereby stimulating tumour-cell proliferation and further PTHrP release, which, in turn, causes more bone resorption, release of growth factors and subsequent release of PTHrP from the resorbed bone matrix of TGF-β and IGF1.

The evidence for this concept comes from a series of studies. First, when human breast cancer cells are inoculated into the left ventricle of nude mice, they cause osteolysis in distant skeletal sites that is abrogated by neutralizing antibodies to PTHrP[24]. PTHrP is also produced in greater amounts by breast cancer cells that have metastasized to bone than those that have metastasized to other sites[19,20]. TGF-β increases the production of PTHrP by breast cancer cells[54]. Breast cancer cells that are stably transfected with mutant TGF-β receptors that cannot respond to TGF-β do not produce PTHrP, and have markedly reduced osteolytic lesions following inoculation in nude mice[54]. IGF1 also promotes breast cancer cell proliferation[56,57], whereas mutant IGF1 receptors reduce the proliferation of breast cancer cells in bone[54].

Therapeutic approaches

The concept of the vicious cycle alters the approach to the treatment of bone metastases, because it means that osteolysis inhibitors might also decrease bone tumour burden. This concept has recently been supported by *in vivo* studies. Bisphosphonates, when administered to mice with osteolytic lesions following inoculation of MDA-MB-231 cells, have decreased bone lesions, as expected, but also a decrease in tumour burden[58]. Similarly, mice that are treated with neutralizing antibodies to PTHrP, as well as tumours that express mutant TGF-β receptors, experience a decrease in tumour burden[24,54]. There is a rationale for developing clinical inhibitors of the bone-resorption process, as so much data now indicates that osteolysis supports the growth and aggressive behaviour of metastatic cancers.

Diel *et al.*[59] and Powles[60] have provided clinical data to support the idea that osteolysis inhibitors also reduce tumour burden in bone. However, there is residual controversy over whether the same effects can be seen in soft-tissue metastases. The study by Diel and colleagues[59] indicates that osteolysis inhibitors do slow tumour growth in these sites, whereas the study by Powles[60] refutes this idea. One study has reported that bisphosphonates can actually promote soft-tissue metastasis[61]. This important issue will probably require much larger studies for it to be resolved definitively.

Preclinical data indicate that the bisphosphonates have no effect on soft-tissue metastases if they are administered after metastases are already established.

Table 1 | **Approaches to treating bone metastases**

Therapy	Mechanism	Stage of clinical development
Bisphosphonates	Block bone resorption; might block tumour-cell mitosis and stimulate tumour-cell apoptosis; alleviate bone pain	On the market
Osteoprotegerin	Prevents RANKL from binding its receptor and stimulating osteoclasts	Phase II
RANK-Fc	Prevents RANKL from binding its receptor and stimulating osteoclasts	Phase I
PTHrP antibodies	Neutralize PTHrP	Phase III
Vitamin-D analogues	Decrease PTHrP production	Phase III

PTHrP, parathyroid-hormone-related peptide; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand.

However, if they are given from the time of tumour-cell inoculation, they might increase soft-tissue metastases, presumably by rendering bone unsuitable as a site for metastatic growth. Although definitive recommendations cannot be made until clinical data are available, this data indicate that, although bisphosphonates can be safely administered when metastases are already present, they should be used prophylactically with great caution.

Newer bone-resorption inhibitors (TABLE 1) might be even more effective than bisphosphonates. These include direct inactivators of osteoclast activity. For example, OPG — the natural decoy receptor to RANK — is an extremely powerful and potent inhibitor of bone resorption[62]. RANK-Fc is a hybrid chimeric molecule that acts in an identical way to OPG[63]. These agents cause the most profound decreases in osteoclastic bone resorption, and can be expected to cause similar effects on tumour burden as do the bisphosphonates. In the case of osteoblastic metastasis, the early preclinical studies of Guise and colleagues[33] indicate that specific inhibitors of endothelin-1 signalling, namely antagonists of the endothelin-A receptor, have beneficial effects on both the bone lesions and the tumour burden.

Future research

Osteolysis in other cancers. So far, there has been little detailed examination of the factors that might be responsible for osteolysis in tumours other than breast cancer or myeloma. A series of cytokines, including RANKL and macrophage inflammatory protein-1α (MIP1α) have been proposed to promote osteolysis by melanoma cells. However, these are probably not the only cytokines that promote bone destruction that is associated with myeloma. IL-1, lymphotoxin, IL-6, hepatocyte growth factor and PTHrP might all have a subsidiary role[64]. In other cancers, factors such as IL-11, IL-18, TGF-α and even prostaglandins might be produced by tumours, or by host cells that are activated at the tumour site, and are involved in the pathophysiology of hypercalcaemia. There is data that inhibitors of prostaglandin synthesis might be effective at blocking tumour-induced osteolysis in some cases[65].

Will bisphosphonates be useful in treating bone metastases that are associated with tumours other than breast cancer, and will any form of bisphosphonate be satisfactory as a treatment for metastasis? These questions cannot be answered definitively at the present time, but it does seem likely that bisphosphonates will also be effective treatments for bone metastases from other cancer types. This is because the cellular mechanisms that are responsible for osteolysis are fundamentally identical, and should be blocked in a similar manner by similar agents.

There have been suggestions that some bisphosphonates induce tumour-cell apoptosis, and that this might be a mechanism for decreasing tumour burden in the metastatic site[66]. Evidence that bisphosphonates cause apoptosis comes from *in vitro* studies that involve relatively high concentrations of the drugs, but further studies are required to determine the direct effects of bisphophonates on cancer cells.

Osteolytic and osteoblastic factors. Many of the factors that promote osteoblastic metastasis remain to be identified. At present, most evidence supports a role for endothelin-1 in breast cancer metastasis, but there is less convincing data to support its involvement in prostate cancer metastasis — due, in part, to the relative absence of animal models of this disease. Many other factors — including PDGF, members of the TGF-β family, growth factors and proteolytic systems — are also involved, possibly all in the same tumour, but more work needs to be done to tie these mechanisms together. So far, TGF-β and IGF1 have been identified as the bone-derived factors that are involved in the vicious cycle of bone destruction and tumour growth. Other growth factors that are present in the bone microenvironment and released as a consequence of bone resorption, such as the PDGFs, BMPs, FGFs and possibly even extracellular calcium[67,68], might also be involved. It is, in fact, probable that the end result is a combined effect of several factors that act synergistically.

Tumour cells in the bone microenvironment have an altered phenotype, compared to the same cells in the primary site or other soft-tissue sites. The best evidence for this is in the increased expression of PTHrP in bone metastases. There are, however, other genes, including the PTH receptor and the oestrogen receptor, that have altered gene expression. TGF-β receptors might be particularly important, because the presence or abundance of TGF-β receptors might be the main influence on the aggressive behaviour of the tumour cell[55]. For example, one explanation for the dormancy that occurs in some tumours might be low expression levels of TGF-β receptors on tumour-cell surfaces during the dormant phase[55].

Mechanisms of abnormal coupling. Little is understood of the molecular mechanisms that are responsible for the balanced coupling between bone resorption and bone formation that occurs under normal physiological conditions. Furthermore, nothing is known of the mechanisms that are responsible for the distortion in this process that occurs in metastatic cancer growing in bone. Perhaps clarification of the aberrations in the coupling that is responsible for predominantly osteolytic or osteoblastic lesions will also shed light on the mechanisms that are responsible for normal bone remodelling.

Metastasis is the single most catastrophic complication of cancer, and understanding the biology of the process should provide not only greater insights into normal cell behaviour, but also lead to new therapies that are specifically designed to limit or prevent this cause of morbidity and mortality in patients with cancer. Our understanding of the cellular and molecular events is improving significantly, and the possibility of such therapy is becoming more realistic.

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