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REVIEW ARTICLE

Pathogenesis of bone metastasis: a review

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BACKGROUND: Metastasic deposits from malignancies frequently lodge in the skeleton, including the jawbones.
METHOD: A review of the literature was performed in order to provide a coherent overview on the pathogenesis of bone metastasis.
RESULTS: Bone metastasis follows complex molecular interactions that enable tumor cells to detach from the primary site, invade the extracellular matrix, intravasate, extravasate, and proliferate within bone. They induce local bone changes that could manifest radiologically as either osteolytic or radiodense. In addition to the direct bone changes, malignancies can elaborate mediators that are released in circulation, leading to generalized osteopenia.
CONCLUSIONS: The spread of malignant neoplasms to bone is not a random process but rather a cascade of specific molecular events orchestrated through complex interactions between neoplastic cells and their environment.
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Keywords: bone metastasis; hypercalcemia of malignancy; jawbones in malignancy; osteoblastic metastasis; osteolytic metastasis

Introduction

Metastasis, the process by which a malignancy spreads from a primary to a distant site, is responsible for the majority of recorded cancer-related deaths. Over 400 000 patients are diagnosed with bone metastasis annually in the USA (1). Metastatic deposits of carcinomas are by far the most common type of malignant tumor-affecting bone (2). Certain malignancies exhibit osteotropism, or an extraordinary affinity to target and proliferate in bone, of which breast carcinoma is the most researched example. A comprehensive review of more than 500 cases of breast carcinoma, which affects one in 10 females in the USA, revealed that 69% had bone metastasis and bone was the most common site for the first distant relapse (3). The success of treatment of the primary malignancy far exceeds that currently achievable with metastatic disease. Detrimental as it may be, the process of metastasis is extremely inefficient and only a small percentage of tumor cells that leave the primary mass ultimately give rise to a metastatic deposit. The primary site of metastatic deposits to the jawbones in females is the breast followed by the adrenals, colorectum, female genital organs, and thyroid. For males it is the lung, followed by the prostate, kidney, bone, and adrenals (4). Blood flow has traditionally been accepted as the only determinant of the site of a metastatic deposit. Solid tumors metastasize most frequently to the vascular areas of the skeleton, and especially red bone marrow sites. This partially explains the propensity of jawbone metastases for the body of the mandible. However, the targeting of bone cannot be explained on hemodynamics alone. Highly vascular tissue-like skeletal muscle, despite receiving more than 25% of the cardiac output, rarely host metastatic disease (5).

The process of metastasis is not random, but rather a cascade of specific events that generally encompass detachment of tumor cells from the primary site, invasion into the surrounding tissue, intravasation, transport to the site of metastasis, extravasation, and bone degradation or bone formation.

Tumor cell detachment

Loss of cellular cohesion and detachment of neoplastic cells from the primary site at the advancing front of oral squamous cell carcinoma (Fig. 1) have been shown to correlate with the prognosis of the primary growth (6). Several reports have not only proven downregulation of cell adhesion molecules associated with these events (7–9) but also implicated E-cadherins as a major class of molecules involved (10, 11). The cadherins are a family of transmembrane glycoproteins that have an important role in morphogenesis and maintenance of a differentiated phenotype. Selectins belong to this group and also play a role in cellular adhesion in the metastatic cascade. E-cadherins mediate cell-to-cell binding and are anchored intracellularly to the actin cytoskeleton of cells via proteins referred to as β-catenins (12). Deregulation

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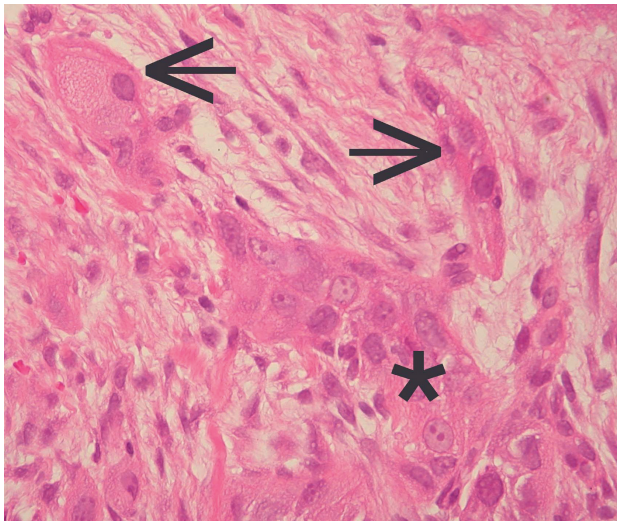

Figure 1 Detachment and infiltration of the stroma by small groups of neoplastic cells of a squamous cell carcinoma (arrows) from the main tumor mass (asterisk; hematoxylin and eosin stain, ×400).

of E-cadherin is associated with increased invasiveness of cells (7, 13) and the capacity of a neoplasm to metastasize is inversely related to the expression of E-cadherin (14, 15). Changes in β-catenin may also affect E-cadherin function with loss of cohesiveness when β-catenin expression is reduced. Collectively the loss of E-cadherin and β-catenin has correlated with reduced survival and poor prognosis in patients with malignancies (16, 17).

Tumor cell motility

Tumor cell motility is essential for migration of neoplastic cells from the primary tumor mass as well as at the site of metastatic growth. Several factors have been shown to enhance this function *in vitro*, including cytokines such as autotaxin (ATX) purified from melanoma cell lines (18) and hepatocyte growth factor (HGF) that induces cell motility through tyrosine kinase (19). HGF, also referred to as 'the scatter factor' has been shown to dissociate sheets of epithelium into individual cells (20, 21). Several other extracellular matrix proteins, such as laminin, fibronectin, and collagen IV are able to stimulate chemotaxis of cancer cells (22). The infiltration of a neoplastic cell through a three-dimensional matrix is achieved amongst other factors through actomyosin contractility. The signaling pathway for myosin phosphorylation in cell invasion is a topic of current research (23).

Interaction with the extracellular matrix

Several review articles have appeared over the past decade on the signaling network of tumor invasion, of which the most recent review the integrin system, the insulin-like growth factor system and the Rho family GTPases (24). Integrins are a class of receptors that mediate cell–matrix interactions. Adhesion of tumor cells to integrins allows them to invade locally and pass in or out of the vascular tree (22). Upregulation of integrin expression on malignant cells correlate directly with their metastatic potential (25–27). Bone is a rich source of laminin, collagen type I, osteopontin, and fibronectin and certain integrins have specific receptors for these molecules (28). The adhesive interaction between tumor cells and bone mediated by the integrin receptors enhance the bone-homing capacity of tumor cells and their ability to invade once present in bone.

For tumor cells to breach the extracellular matrix, they should be capable of producing enzymes. The presence of most of these enzymes correlates with a poor survival rate. Of the multitude of enzyme systems, metalloproteinases (MP) and the urokinase plasminogen activator system (uPA) have been studied in greatest detail. uPA is a multifunctional proteolytic enzyme, which is fundamental to the metastatic process (29–31). Many tumor cell types and stromal cells in bone express uPA and it is a major driving force in initiating MP activity in bone. The catalytic function of uPA is to convert plasminogen to plasmin. Plasmin activates MP that has a fundamental ability to cleave and denature fibrillary collagen (22). Zn^{2+} is essential for this activity. By degrading the matrix of bone, MPs have a secondary function in activating latent growth factors-like tumor growth factor (TGF)-β bound within the matrix of bone. Strong evidence is present in the literature linking MPs with tumor progression in bone (32).

Interaction with endothelium

Interaction with endothelial cells at the initial and later stages of the metastatic cascade is mandatory for the passage of tumor cells into vessels at the primary site and exit from vessels at the metastatic site. Several factors, including certain members of the immunoglobulin and selectin families, facilitate adherence of tumor cells to endothelium (33). Platelet-fibrin thrombi influence the arrest of tumor cells (Fig. 2) and platelet-derived lysophosphatidic acid is co-opted by aggressive breast and ovarian cancer cells as a tumor cell mitogen and promotor of osteolysis during bone metastasis (34). Chemotactic factors released by endothelial cells enhance tumor cell mobility (35). The expression of CD44 and VLA-4 by bone marrow endothelium (36) may play an important role in the bone-homing characteristics of multiple myeloma and prostate carcinoma cell lines (36, 37) both malignancies with an indisputable affinity for bone.

Direct effect on bone

The bone microenvironment is unique among metastatic target tissue because it is subjected to continuous remodeling under the influence circulating hormones and local bone-derived growth factors. The type of interaction between the bone microenvironment and the tumor cells can potentially give rise to osteolytic (bone resorbing) or osteoblastic (bone forming) metastasis. It is important to realize that the catabolic effects of

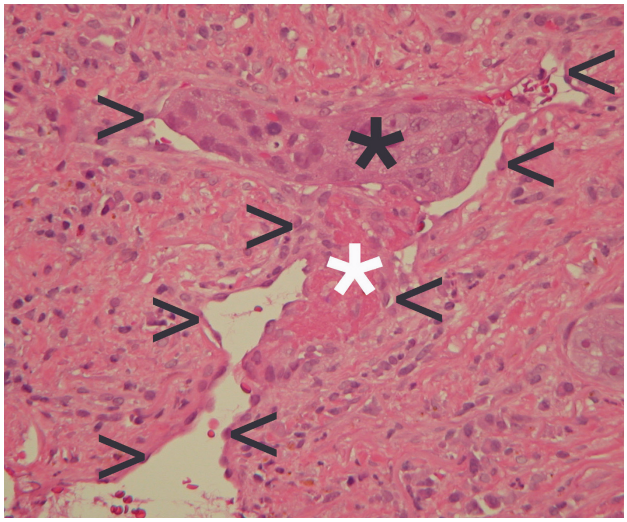

Figure 2 Arrest of a neoplastic deposit of a breast carcinoma (black asterisk) in a blood vessel (endothelial lining delineated by open arrows). Note the fibrin thrombus (white asterisk; hematoxylin and eosin stain, ×250).

radiotherapy and chemotherapy on bone homeostasis compound the skeletal morbidity of malignant disease.

Osteolytic metastasis

Osteolytic bone metastasis are characteristic for most malignancies (Fig. 3a). Breakdown of bone is the most common way in which neoplastic cells affect the skeleton. The close proximity of cancer cells to osteoclasts in bone metastases (38, 39) emphasizes their ability to stimulate bone resorption (Fig. 3b). Through the release of soluble mediators or via cell-to-cell contact, breast cancer cells may either stimulate the differentiation of osteoclast precursors or activate resident osteoclasts. This results in a measurable increase in osteoclasts on bone surfaces, which under normal physiologic circumstances are 0.11 osteoclasts per millimeter bone surface (±0.04%; 40). Mineralized bone matrix contains several growth factors, of which TGF-β and insulin-like growth factor (IGF) constitute the most important examples (41). During osteoclast-mediated bone resorption, the microenvironment is enriched with bone-derived growth factors that enhance tumor survival. Osteolytic metastasis is generally associated with collapse or fracture of weight-bearing bones, displacement of hematologic precursor cells, and hypercalcemia. Hypercalcemia resulting from bone resorbtion at the site(s) of the metastatic deposit has been referred to as local osteolytic hypercalcemia. Studies have demonstrated that patients suffering local osteolytic hypercalcemia do not present with increased circulating concentrations of the factors associated with humoral hypercalcemia of malignancy (42, 43). Local osteolytic hypercalcemia is the direct result of increased tumor-mediated osteoclast activity with bone destruction and release of calcium. If the tumor burden in bone is high enough, local tumor-produced factors

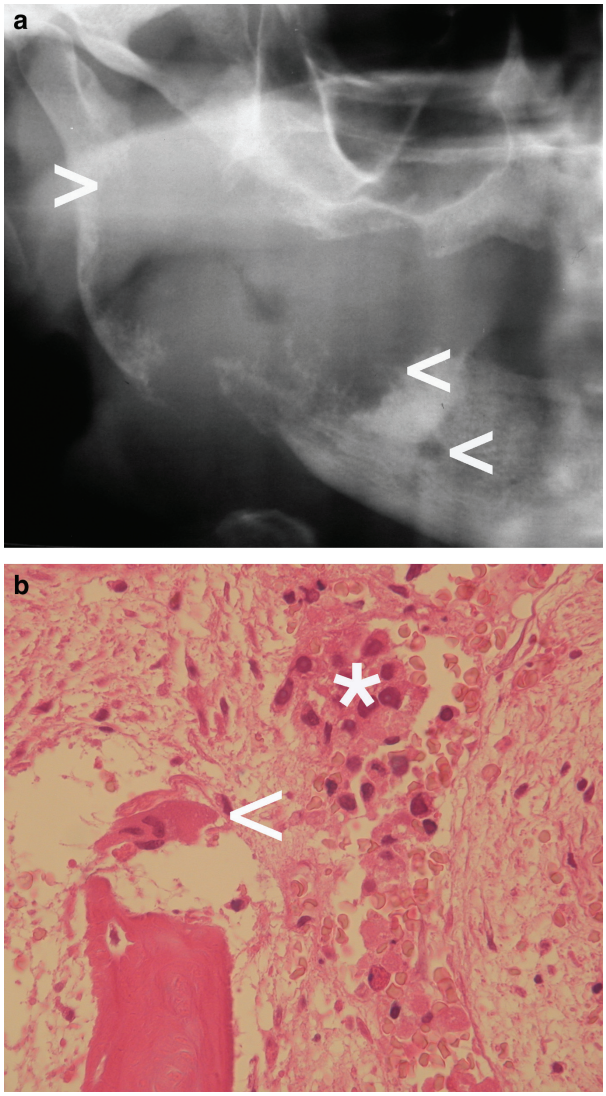

Figure 3 (a) Cropped panoramic radiograph of metastatic deposits of breast cancer in the mandible. Note the irregular destruction of bone (open arrows). (b) Biopsy of the lesion depicted in (a). Note the island of neoplastic cells (white asterisk) and the associated osteoclast (open arrow; hematoxylin and eosin stain, ×250).

may reach concentrations sufficient to enter the circulation and induce bone resorption distant from the metastatic site. This phenomenon partially explains the frequent occurrence of generalized osteopenia in the terminal cancer patient and was evident as early as 1889 when Stephen Paget noted, 'a general degradation of bones sometimes occurs in carcinoma of the breasts, yet without any distinct deposition of cancer within them' (44).

The most elaborately studied chemical mediators of bone resorption are parathyroid hormone-related protein (PTHrP) and TGF-β. The secretion of an immunologically distinct factor with PTH-like biologic activity, better known as PTHrP, has been identified in several neoplasms, including renal carcinoma, squamous cell carcinoma, and carcinoma of the breast. PTHrP has been detected by immunohistochemistry

and *in situ* hybridization in 92% of breast carcinoma metastasis compared with only 17% in metastasis to non-bone sites (45, 46). Several other studies on breast carcinoma have proven a constant relationship between PTHrP positivity and the development of bone metastasis and hypercalcemia (47, 48). The majority of hypercalcemic patients with solid tumors and Hodgkin's lymphoma have detectable plasma PTHrP concentrations.

The PTHrP may also mediate lactation-associated bone loss as it is expressed in lactating mammary tissue. PTHrP increases renal tubular absorption of calcium, reduces renal phosphorus uptake, and increases osteoclastic bone resorption. It differs from the action of PTH by decreasing serum concentrations of $1,25(OH)_2$ Vit D, an important distinction from patients with primary hyperparathyroidism where the opposite applies. Hypercalcemia of malignancy is usually associated with suppressed PTH concentrations and ectopic production of PTH by neoplasms remains a rare event. Hypercalcemia of malignancy differs from hypercalcemia secondary to hyperparathyroidism by the uncoupling of bone formation from bone resorption, two processes that are generally linked. In hyperparathyroidism there is a measurable increase in both osteoclasts and osteoblasts, whereas in patients with hypercalcemia of malignancy only osteoclasts increase. Serum osteocalcin concentrations are significantly lower in patients with bone metastases. PTHrP is thought to be responsible for these biochemical changes. Cytokines such as interleukin (IL)-18 appear to be involved in the early stages of breast cancer metastasis and initiate the process of bone resorption. PTHrP expression is induced later to stimulate a vicious cycle of bone destruction (49). Bisphosphonates have become the most useful antiresorptive agent for the treatment of malignancy-induced hypercalcemia. They are highly effective inhibitors of bone resorption that selectively inhibit osteoclasts (50), thereby preventing many of the debilitating effects of malignant disease on the skeleton. The suppression of bone remodeling by bisphosphonates; however, lead to serious complications in dental practice of which the poor-healing capacity of bone with subsequent extensive bony sequestration in osteomyelitis is only one example.

The TGF-β is highly expressed by differentiated osteoblasts and osteoclasts, it is contained in bone matrix and released in active form during osteoclastic bone resorption. Although TGF-β is implicated in osteoblastic metastases, it could under certain circumstances play a role in osteolytic metastases. Amongst its many attributes, TGF-β induces PTHrP activity (51), acts as chemoattractant for breast cancer cells (52, 53), inhibits growth of some neoplastic cell lines while in others growth is stimulated (22). The growth factors other than TGF-β that are present in mineralized bone matrix and that are released during bone breakdown include IGF-I and IGF-II, fibroblast growth factors (FGF-1 and -2), bone morphogenetic protein (BMP), and platelet-derived growth factor.

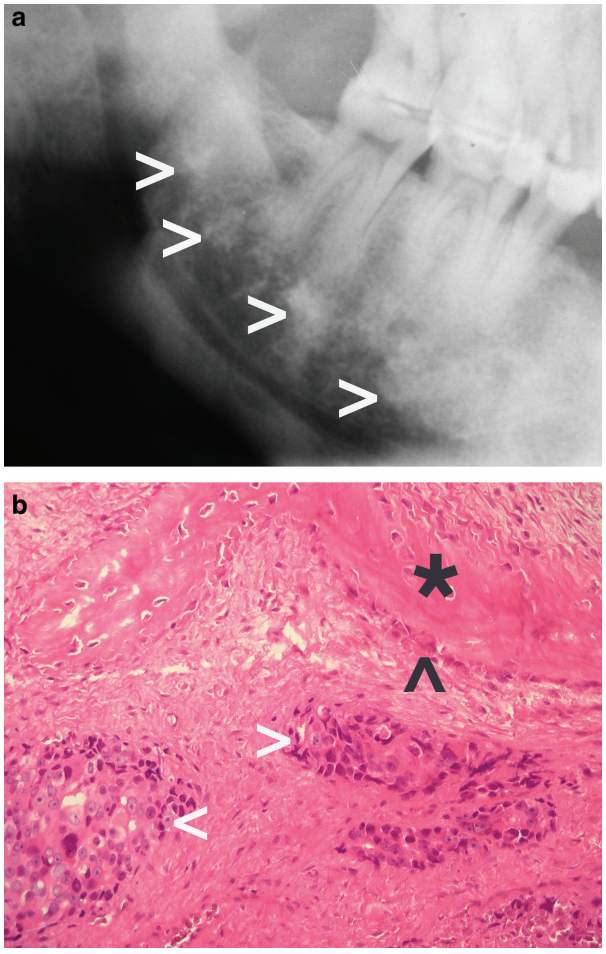

Figure 4 (a) Cropped panoramic radiograph of the corpus of the mandible in a patient with carcinoma of the prostate. Note the irregular radiolucent lesions (open arrows). (b) Biopsy of lesion in (a). Note the malignant infiltrate (white arrow heads) in close approximation to a focus of woven bone (black asterisk) lined by osteoblasts (hematoxylin and eosin stain, ×400).

Osteoblastic metastasis

Neoplastic cells in bone may under certain circumstances induce osteoblastic metastasis which present radiologically as radiodense (Fig. 4a). They are microscopically characterized by new bone formation (Fig. 4b). This is pathognomonic for carcinoma of the prostate and less frequent in other malignancies. Unlike osteolytic metastasis this type of bone involvement can potentially cause hypocalcemia (54, 55). Pathologic fractures may occur within osteoblastic metastasis due to the intrinsic low strength of the newly formed woven bone. Unlike osteoclastic metastasis, the osteoblastic type lacks an appropriate animal model and scientific information is therefore limited to studies of human prostate cancer.

Several mediators have been implicated in the osteoblastic response of bone to neoplastic deposits. During normal bone formation TGF-β is secreted by osteoblasts in latent biologic inactive form and incorporated in bone extracellular matrix. Osteoblasts not only produce

TGF-β, but they also possess high-affinity receptors for it, providing the capacity for autocrine stimulation of osteoblastic replication. Several agents including proteases such as plasmin or cathepsin D and an acid environment can activate latent TGF-β (56, 57). Prostate cancer cells have the capacity to synthesize TGF-β. Amongst its many actions, it stimulates the production of matrix proteins and inhibits their degradation by decreasing the synthesis of matrix-degrading enzymes and increasing the synthesis of protease inhibitors (58). TGF-β promotes cells of the osteoblast lineage and the formation of new bone (59).

The IGF system has an anabolic effect on bone, is complex and consists of two ligands, IGF-I and IGF-II, two receptors, and six binding proteins (IGFBPs). This system promotes mitogenic stimulation of osteoblasts, increases bone matrix apposition rates and decreases the degradation of collagen and the expression of interstitial collagenase. This suggests roles in the preservation of the bone matrix and maintenance of a critical skeletal bone mass. The regulation of this system is complex (60, 61). IGFs are potent mitogens for prostate cancer cells (62). Significant positive correlation exists between serum concentration of IGFBP-2 and the prostate-specific antigen (PSA) as well as between IGFBP-2 and tumor stage in patients with prostate cancer (63). Prostate cancer cells produce several proteases, such as PSA, urokinase type plasminogen activator, and cathepsin D, which may be responsible for dissociating IGF-I and IGF-II from respective binding proteins to result in not only enhanced tumor growth, but also mitogenic effects on osteoblasts.

Of all the proteases, PSA concentrations have been shown to correlate significantly with the presence of bone metastasis (64). Immunoreactive PSA has been demonstrated in a significant percentage of breast cancers (65) although it was once believed to be a product of prostate epithelium only. This finding is interesting, as metastatic breast carcinoma is one of the few other malignancies capable of presenting with osteoblastic skeletal metastasis. PSA has been shown to proteolyze IGFBP-3 in several fragments some of which retained the ability to bind IGF whereas others had kallikrein-like or chymotryptic-like enzymatic activity (66). PSA stimulate osteoblast proliferation at low concentrations probably through activation of TGF-β (67). Furthermore, there is evidence that PSA inactivates the biologic effects of PTHrP to facilitate cAMP production in osteoblasts (68).

Both acidic (FGF-1) and basic (FGF-2) FGFs are expressed in prostate cancer cells (69). They stimulate replication of osteoblasts (but do not increase their functions) and play a role in bone repair where cell division is required. FGF-2 suppresses the formation of osteoclasts. When injected adjacent to mouse calvaria they exhibit a potent bone-forming response (70).

Several types of BMP have been described and the list is increasing. Normal prostatic tissue expresses BMP-4 and certain prostate cancer cell lines express BMP-2 and BMP-3 in larger amounts. BMP stimulates the formation of ectopic bone when injected intraperitoneally or subcutaneously (71). This is the result of their stimulation of replication and differentiation of cells of the osteoblastic lineage and, in contrast to TGF-β, enhances the expression of differentiated osteoblasts (63, 72). BMP-3 decreases osteoclastic resorption and is chemotactic for monocytes.

The mean plasma concentrations of endothelin (ET)-1 in men with advanced hormone refractory prostate cancer with metastasis to bone were found to be significantly higher than prostate cancer patients without metastasis or normal controls. ET-1 is a potent vasoconstrictor produced by prostatic epithelium and concentrated in seminal fluid (73). Osteoblasts have high-affinity receptors for ET-1 (74). ET-1 decreases osteoclast motility and bone resorption (75) and stimulates BMP-induced bone formation (76). IL-6 facilitates ET-1 production in a human breast cancer cell line, a phenomenon thought to be of great significance in osteoblastic breast carcinoma metastasis (77). Mice inoculated with a breast cancer cell line and treated with selective ET-1 receptor antagonists had significantly fewer osteoblastic bone metastasis and less tumor burden than untreated mice (78).

Conclusion

Bone is the most common site for distant relapse of most cancers. Although the process of tumor spread is poorly understood mainly due to the unavailability of an appropriate animal model, recent developments have provided insight into aspects of the complex cascade of specific events required too establish a metastatic deposit in bone. As knowledge unfolds, cancer adjuvant therapy will be directed increasingly toward the prevention of the devastation caused by bony metastases. As in the case of bisphosphonate therapy, the adjuvant regimes will decrease the morbidity of disseminated malignant disease, at the expense of bone homeostasis. Amongst other skeletal complications, it is certain to impact on bone healing and the process of osteomyelitis.

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