Bone metastasis: Histological changes and pathophysiological mechanisms in osteolytic or osteosclerotic localizations. A review

Les métastases osseuses : aspects histologiques et mécanismes physiopathologiques dans les formes ostéolytiques ou ostéocondensantes. Une revue

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Summary The development of a bone metastasis involves interactions between the tumor cells, the bone marrow microenvironment and the bone cells themselves. A better understanding of the pathophysiological changes occurring in bone metastasis can be obtained from histopathological examination of invaded specimens. This review focuses on the main molecular mechanisms implied in the localization and growth of malignant cells in the bone marrow. The corresponding histologic developmental stages are illustrated both in osteolytic (or mixed metastasis) or in the osteosclerotic forms by histological analysis, immunohistochemistry and microcomputed tomographic analysis of bone samples. In both cases, the malignant cells find a "fertile soil" in the bone marrow microenvironment. They use the growth factors released by bone cells for the coupling between osteoclasts/osteoblasts to promote their own development. In turn, they elaborate a variety of cytokines that can promote osteoclastogenesis (PTHrP, IL-1, IL-6...) or on the contrary, other growth factors that can boost the osteoblastic activity (ET1, IGFs). A "vicious circle" occurs between the malignant cells and the bone cells leading to the radiological expression of the metastasis.

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Introduction

Relationships between bone remodeling and metastatic cells have been popularized by Sir Stephen Paget who noticed that some cancer cells had an increased propensity to migrate and expand in bone (cited by [1]). The “Seed and the soil” theory that he proposed in 1879 considers that some particular cancer cells (breast and prostate) possess characteristics that favor their anchorage in the bone marrow “which could not be explained by the vascular or lymphatic theory” which predominated at that time. The bone microenvironment represents a fertile soil capable of providing nutriments favoring the growth of metastatic cells (the seeds).

Bone is the host of the hematopoietic marrow and considerable interactions exist between bone and hematopoietic cells. Stromal cell is the osteoblast precursor (bone forming cell) and represents a major component of the hematopoietic stem cell niche [2]. Osteoclast (bone resorbing cell) derive by fusion of mononucleated precursors originating from a common ancestor with monocyte/macrophage/dendritic cell [3]. A large number of cytokines and growth factors are also shared with bone and hematopoietic cells. It is likely that metastatic cells, when localized inside the marrow spaces, will interfere and affect the subtle equilibriums controlling bone remodeling and hematopoiesis.

Bone remodeling

Bone remodeling is an adaptive mechanism that controls bone mass and microarchitecture throughout life. It is supported by the coordinated action of osteoblasts and osteoclasts in the time and space. Frost was the first to recognize that bone remodeling was due to basic multicellular units (BMU) in which osteoclasts resorb “old” bone packets (i.e., mechanically or metabolically unsatisfactory) to replace it by a new bone structure unit (BSU) [4,5]. Because bone cells differ in origin (osteoclasts: hematopoietic precursors; osteoblasts: stromal mesenchymal cells), a number of mechanisms have been described to explain bone remodeling.

Growth factors and the bone matrix

As described with a number of connective tissues, the bone matrix represents a reservoir of growth factors coupled to other non-collagenous proteins. Growth factors such as transforming growth factor β (TGF-β), insulin-like growth factors (IGFs-I and II) and bone morphogenetic proteins (BMPs) are synthesized and embedded by osteoblasts at the time of elaboration of the organic phase of the bone matrix (osteoid tissue) [6,7]. These factors are immobilized on other non-collagenic proteins that are anchored on the collagen fibers or the hydroxyapatite crystals of the mineral phase of the matrix. Fetuin (α2-HS glycoprotein) is synthesized by hepatocytes and deposited in large amounts in the calcified bone matrix where it can complex TGF-β [8]. Once “buried” in the bone matrix, these growth factors can remain inactive for years or even decades until the osteoclasts of a new BMU come and release them in the microenvironment during the resorption phase of the BMU. TGF-β, IGFs and BMPs are mitogenic and differentiation factors for stromal cells which can proliferate and differentiate locally into osteoblasts in the vicinity of bone resorption. In addition, TGF-β has been reported to have a negative effect on the differentiation of osteoclast precursors.

The RANK-RANKL-OPG system

Stromal cells and osteoblasts express the ligand for the receptor activator of nuclear factor κB (RANKL). RANKL is recognized by its receptor (RANK) which is expressed at the surface of mononucleated osteoclast precursors [3,9,10] (Fig. 1). When the two cells are in contact, stromal cells (or osteoblasts) elaborate monocyte colony stimulating factor (M-CSF), which induces osteoclast precursors to multiply and fuse to provide late plurinucleated cells. A soluble form of RANKL can also be secreted by osteoblasts and stromal cells. The osteoclast precursors possess molecular characteristics of osteoclasts (tartrate resistant acid phosphatase [TRACP], cathepsin K, metalloproteinases...) but they are not in contact which trabecular surfaces and lack the ruffled border (a specialized subpart of osteoclast membrane under which the bone matrix dissolution takes place).
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The coupling between bone formation and resorption is assumed at different levels. A. Osteoblasts incorporate growth factors in the latent and inactivated form inside the bone matrix at the time of formation of new bone. Several months/years later, osteoclasts release the activated form of these "buried" growth factors that activate the stromal cells and provoke their differentiation into osteoblasts. B. The RANK-RANKL-OPG System. 1) Stromal cells and osteoblasts express RANKL which is recognized by RANK at the surface of osteoblast precursors. The secretion of M-CSF is also necessary. 2) A soluble form of RANKL can also be secreted to activate osteoclastogenesis. 3) OPG is a decoy soluble receptor elaborated by stromal cells and osteoblasts and the ratio OPG/RANKL modulates osteoclastogenesis.

The Ephrin system

This system was recently found to allow a direct cooperation between osteoblasts (or their inactive form: the lining cells which are flat cells resident on to the quiescent bone surfaces) and osteoclasts. The system implies molecules expressed at the surface of osteoclasts and osteoblasts (or lining cells). The protein EphrinA2 (on osteoclasts) is recognized by EphA2 at the surface of lining cells: this inhibits the differentiation of new osteoblasts and increases the activity of osteoclasts at the onset of bone resorption. Conversely, EphrinB2 (on osteoclasts) when linked to EphB4, can stop the osteoclast action (thus inducing a signal for the end of the resorption period) and stimulates osteoblast recruitment and their activity [11].

Other molecules

Recently other molecules have been found to influence bone remodeling by controlling the osteoclast-osteoblast dialogue. Atp6vod2, an isoform of the vacuolar (H+) ATPase is expressed in osteoclasts; it appears necessary for allowing the fusion of osteoclast precursors and the secretion of a factor that inhibits the differentiation of osteoblast precursors into mature, matrix-secreting osteoblasts [12].

The metastatic cascade

From the primary tumor, malignant cells can acquire the capacity to metastasize due to an increased motility and invasiveness. This is associated with the capacity for tumor cells to destruct the basal lamina and detach from epithelial sheets. This mechanism is often referred as "epithelial mesenchymal transition" a phenomenon that mimics the gastrulation process observed at the early stages of embryonic development [13].

A number of important pathways or genes have been identified in the epithelial mesenchymal transition; among them: TWIST, Wnt/β-catenin, Notch and a variety of integrin/signaling [14]. Once the tumor cells were detached from the primary tumor, they enter blood or lymph circulation. This is facilitated by the fact that the primary tumor always induces neo-angiogenesis due to local hypoxia [15,16]. These neovessels have more permeable walls, a condition that favors the cell swarming (Fig. 2). When traveling into the circulation, cells can circulate into the sinusoid capillaries of the bone marrow. They have a particular histological constitution with large pores that are physiologically used by reticulocytes and other immature blood cells to join the circulation [17]. Malignant cells can adhere to the endothelium of the medullar sinus and extravase into the
Figure 2  The metastatic cascade leading to a bone metastasis. A. The primary tumor is associated with neoangiogenesis with abnormal vessels. B. The epithelio-mesenchymal transition allows malignant cells to extravase and circulate in the blood vessels. C. They can be distributed into the marrow circulation and (D) can leave it because the medullar sinusoids have holes between their endothelial cells. E. Once the metastatic cells are in the marrow spaces, interactions with the marrow and bone cells can occur to (F) alter the bone remodeling leading to the bone metastasis.

Cascade conduisant aux métastases osseuses. A. La tumeur primitive est associée à une néo-angiogenèse comportant des vaisseaux anormaux. B. La transition épithelio-mésenchymateuse permet aux cellules malignes de gagner la circulation sanguine. C. Les cellules se distribuent ainsi dans l’organisme et dans les cavités médullaires et (D) peuvent quitter la circulation car les capillaires sinusoides médullaires sont fenêtrés avec présence de trous entre les cellules endothéliales. E. Une fois que les cellules métastatiques sont dans les espaces médullaires, elles interagissent avec le microenvironnement médullaire et les cellules osseuses pour (F) alterer le remodelage osseux et conduire à une métastase osseuse.

As mentioned above, Sir Stephen Paget was the first to recognize that in some types of tumors such as breast or prostate cancer, bones and lymph nodes are interested in a manner that cannot be explained by a theory only based on embolization [1,18]. According to the “seed and soil” theory, these tumor cells (from breast or prostate adenocarcinomas) are “seeds” with particular properties that make them capable to localize in the bone marrow spaces. This particular microenvironment constitutes a “fertile soil” that will favor their growth and development [19]. Bone metastases are classically classified as: osteolytic, osteosclerotic or mixed, based on their appearance on X-ray images (Fig. 3). Osteolytic metastases are typical of breast, kidney and melanomas (Fig. 3A). Osteosclerotic tumors are most often observed in prostate cancers (Fig. 3C). Mixed lesions correspond to the association of osteolytic and osteosclerotic areas, giving a fuzzy aspect on X-ray images (Fig. 3E). However, in osteolytic metastases, where bone resorption is due to a considerable stimulation of osteoclastogenesis (and not by a direct action of the tumor cells which lack the machinery and enzyme equipment to resorb bone), one can always observe areas of
Mechanisms leading to the development of an osteolytic metastasis

As mentioned above, breast adenocarcinoma is the most frequent cause of the osteolytic metastases, although lung, kidney and thyroid are also frequently implied. Breast cancer is usually considered as the model for osteolytic metastases and animal models have been extensively studied (e.g. MDA-MB-231 cells in the mouse or the Walker carcinoma in the rat) [21]. The malignant cells of these tumors may present a number of characteristics that make them able to have a special tropism to the bone/bone marrow. These cells may express the α4β1 integrin which can bind its ligand, the VCAM-1 molecule expressed by the stromal cells of the bone marrow. They can also express the αvβ3 integrin whose ligand is osteopontin, a non-collagenic protein of the bone matrix. Osteopontin is a member of the small integrin-binding ligand, N-linked glycoprotein (SIBLING) family produced by osteoblasts, osteoclasts and osteocytes and localized in the bone matrix, stuck onto the hydroxyapatite crystals. Interestingly, breast tumor cells can also express this molecule which may increase their homing to the bone microenvironment. Also, the over-expression of the chemokine receptor CXCR4 favors the anchorage in the bone marrow (which produces high amounts of CXCL-12). Additional factors such as an increased motility of these cells (conferred by the expression of thymosine β5 and other stress proteins, e.g. Hsp 27) have also been advocated. The proto-oncogen C-Src has also been recognized to favor motility of these malignant cells, their growth and their bone homing [22]. Other factors have been recognized and are due the capacity of these cells to express bone specific transcription factors (Runx 2, MSX) or to synthesize specific noncollagenic proteins of the bone matrix.
Figure 4  Osteolytic metastasis. A. Dysregulation of the bone remodeling at early stages of marrow invasion. B. Immunohistochemical identification of a single malignant cell (from a breast cancer) identified by CK7 labeling (× 100). C. Dysregulation at an advanced stage. D. Immunohistochemistry of an advanced metastatic invasion from a breast adenocarcinoma (CK7 labeling, × 100). E. Bone resorption is due to osteoclasts occurring in the vicinity of malignant cells from breast adenocarcina cells (arrows) (HPS, × 400). F. MicroCT from an osteolytic metastasis showing disorganization of the trabecular microarchitecture increased eroded surfaces (arrow-heads) and foci of metaplastic reactive bone (arrows). G. Histochemical identification of osteoclasts and osteoclast-precursors by the TRACP method in the stroma of a kidney carcinoma (× 200). H. Metaplastic focus of woven bone anchored at the surface of preexisting trabeculae, arrows indicate eroded surfaces (Goldner’s trichrome, osteoid seams are in dark grey, × 100).
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When these malignant "seeds" have homed to the bone marrow (Fig. 4A), they can stay for an undetermined time without expansion and remain as dormant cells. They can be identified by immunohistochemistry with antibodies against cytokeratin (CK7, CK20, CK19) or epithelial membrane antigen which are not expressed by any normal cell in the bone marrow (Fig. 4B) [23]. The malignant cells can also release in the microenvironment a number of molecules that alter bone remodeling and will lead to the development of a bone metastasis. These are mainly growth factors and cytokines that favor osteoclastogenesis via the RANK-RANKL-OPG system: the parathyroid hormone related protein (PTHrP) and also IL-1, IL-6, IL-8, IL-11, TNF-α. PTHrP is the key factor in osteolytic bone metastasis, particularly in breast cancer. PTHrP upregulates the expression of RANKL on osteoblasts and stromal cells in the vicinity of the tumor cells. It was detected by immunohistochemistry in about 90% of bone metastases from breast cancer [24] but its expression in the primary tumor is not a predictive factor for bone metastasis. The final effect of PTHrP (and the other cytokines) is to promote osteoclastogenesis, due to the paracrine secretion of these cytokines (Fig. 4C-F). The increased osteoclastic activity, which develops only in the vicinity of the tumor, is responsible for the breakdown of bone trabeculae, and secondarily of cortical bone with extension in the soft tissues (Fig. 4D). Microarchitecture of the cancellous bone is altered and large trabecular areas can be destroyed (Fig. 4E) [20,25]. In advanced metastatic bone disease, hypercalcemia can reflect the release of large amount of calcium in the blood. Bone markers (such as carboxy-terminal collagen crosslinks CTX) can also be used to evaluate biologically the resorption of the organic phase of the bone matrix. However, during resorption of the bone matrix, large amounts of the deeply entrapped growth factors are released and activated in the microenvironment. TGF-β, IGF-I and II can promote the growth of the tumor cells locally. Breast cancer cells have receptors for TGF-β which can increase tumor invasiveness and progression via the Smad pathway [26]. Fragments of degraded collagen are also released from the eroded areas and the CTX peptide was shown to be a strong chemoattractant for recruiting locally new malignant cells. Taken together, it appears that a vicious circle occurs between bone remodeling and the tumor growth via the PTHrP/TGF-β axis (Fig. 4C): the more bone is resorbed by osteoclasts, the more the metastatic cells develop and stimulate osteoclastogenesis. Other vicious circles have been described to explain these interrelationships between malignant and bone cells: breast cancer often expresses the receptor for lysophosphatidic acid (LPA) and induces platelet aggregation. Platelets are rich in LPA which represents another growth factor favoring the tumor cell growth [27].

A side effect of the release of high amounts of TGF-β and IGFs is also to favor osteoblast stimulation in a paracrine way [28]. The histological consequence is the development of metastatic bone anchored onto the residual trabeculae (Fig. 4G). These foci of woven bone are incompletely mineralized; they are often observed in the vicinity of the eroded area and have a typical candelabra aspect with thin trabeculae containing large osteocytes [29]. They are responsible for the fuzzy aspect observed on the X-ray images and are also the cause of the uptake of radionuclide labeled bisphosphonates used in bone scans. The appearance of these metastatic foci is observed in almost osteolytic metastases, at least from a histological point of view. This phenomenon can be observed because the tumor cells do not secrete osteoblast inhibitors. On the contrary, myeloma (a hematological malignancy of the B-lymphocyte) is associated with pure osteolytic images because malignant plasma cells release strong inhibitors of the Wnt pathway, a key factor in the activation and function of osteoblasts. One should also notice that these secreted factors can diffuse through the vascular spaces of the cortices (Haversian canalis) to stimulate the proliferation of osteoprogenitor cells of the periosteum: the presence of woven bone in the periosteal areas is rather characteristic of a narrow metastasis [30].

Mechanisms leading to an osteosclerotic metastasis

In some rare cases, some malignant diseases are associated with marked osteosclerosis: this is sometimes reported for myelomas, thymomas, colorectal tumors... However, prostatic adenocarcinoma is the tumor which gives predominantly osteoblastic metastases [31]. In the USA, it is the second common cause of cancer leading to approximately 29,000 deaths/year [32] and approximately up to 90% of patients with prostate cancer will develop bone metastasis. Histopathological examination of bone specimens shows a large number of osteoblasts in the vicinity of the tumor cells. They are actively building trabeculae of woven bone that are anchored at the surface of pre-existing trabeculae of the patient and tend to fill the marrow cavity without extension in the soft tissues (periosteum and muscle fibers) (Fig. 5B). A number of factors have been identified that could explain why cells from prostate adenocarcinomas represent “good seeds” to develop in the bone marrow microenvironment. These cells frequently express the αvβ3 integrin which recognizes fibronectin, an adherence molecule present in large

Figure 5  Osteosclerosing metastasis. A. Dysregulation of bone remodeling at early stages of marrow invasion. B. Osteosclerosis at low magnification showing proliferation of metaplastic bone in the marrow spaces (Goldner’s trichrome, × 10). C. Bone remodeling at advanced stage. D. Metaplastic bone (woven bone) anchored at the surface of preexisting trabeculae (arrows), note the tubules of adenocarcinomatous cells (HPS, × 200). E. Immunohistochemical identification of cancer cells by PSA labeling in a case of prostatic carcinoma (arrows) (× 200). F. MicroCT from an osteosclerotic metastasis, prostate adenocarcinoma. G. TRAcP identification of osteoclasts in a prostate adenocarcinoma metastasis, metaplastic bone is arrowed (× 200). H. Increased osteoblastic surfaces (HPS staining) with metaplastic bone anchored on “old” trabeculae (HPS, × 400).
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Bone metastasis is a complex process involving interactions between tumor cells and the bone microenvironment. Various factors contribute to the development of bone metastases, including increased bone remodeling due to metastases. This process involves the release of cytokines and bone matrix degradation enzymes by tumor cells.

The bone microenvironment constitutes a "fertile soil" for these prostate cancer cells, allowing them to proliferate and grow in the bone matrix. Growth factors, such as IGF-I and II, FGF-1 and 2, and TGF-β, are important players in this process. These factors stimulate tumor cell proliferation and growth by activating various signaling pathways.

Increased bone remodeling due to metastases: a target for antiosteoclastic drugs

Bisphosphonates are specific blockers of osteoclast activity, which can be used to slow the progression of bone metastases. They inhibit osteoclast activity by inhibiting the release of cytokines and other regulatory factors. Bisphosphonates can be effective in treating bone metastases by reducing the rate of bone resorption and remodeling.

Importance in the vicious circles, lessons from animal models

Whatever the type of metastasis (osteolytic, mixed, or osteosclerotic), the vicious circle is induced by irritation of the malignant cells in the bone marrow. However, little attention has been paid to the development of bone turnover on dormant cancer cells hidden in marrow spaces. A few numbers of reports have shown that an increased bone remodeling, preexisting to the graft of malignant cells, leads to a tumor burden in myeloma and prostate cancer.

Ovariectomy or orchidectomy are two well defined conditions that increase bone remodeling (via inflammatory cytokines such as IL-6 and IL-7) after sex steroid deprivation. Recently, a calcium diet, inducing a secondary hyperparathyroidism and a high bone turnover has been found to increase the tumor growth.

inhibition of the farnesyl pyrophosphatase. This enzyme plays a key role in the cholesterol synthesis (mevalonate pathway) and is essential for the synthesis of small GTPase signaling proteins, that are vital for cell function and survival [48]. The use of bisphosphonates was found of considerable interest in osteolytic metastases; they can be used as an auxiliary treatment in patients with bone metastases in order to reduce bone pains, risk of fractures and hypercalcemia; thus improving the quality of life in the patients [49]. These agents prove also useful to delay the appearance of bone metastases [50]. In osteosclerosing metastases, these compounds act by blocking the resorption, which is always associated, and decrease the appearance of skeletal events [51]. In rats, the MatLyLu cell line (prostate cancer origin) induced osteolytic metastasis in about 15 days when injected locally. When rats are pretreated by zoledronate, the drug converts these osteolytic tumors into osteosclerotic ones by reducing the first steps of bone remodeling, thus avoiding bone fractures [52]. However, although bisphosphonates can have a direct effect on the malignant cells in vitro, this effect is not clearly evidenced in vivo since the high affinity of hydroxyapatite is capable to trap very rapidly the drug from the blood and extracellular fluids. A side effect of the bisphosphonate therapy is the occurrence of osteonecrosis of the jaw typically occurring after a dental extraction or some other gingival trauma: the wound fails to heal and infection with actinomycetes can occur [53]. Osteonecrosis is associated with the use of high dosages of bisphosphonates in cancer patients, especially when the drugs are received by perfusion; myeloma patients are more at risk than patients with bone metastases.

Other antiosteoclastic approaches are currently under clinical trials: Denosumab, a human monoclonal antibody against RANKL, being developed by the Amgen company, is under evaluation in bone metastasis [54]. Other drugs targeting the NFκB pathway may appear interesting in the development of new therapeutic strategies [55]. Because malignant cells release large amounts of free radicals, the use of scavenger molecule can also be advocated [56].

Conclusion

Malignant cells can develop in the bone marrow environment where they find a number of cytokines and factors that promote the tumor growth. In return, they can release a variety of cytokines which interfere with the cytokine network used by bone cells to ensure the physiological remodeling. Depending on the origin of the metastatic cells, a vicious circle occurs in the bone marrow microenvironment between malignant and bone cells. Breast and prostate cancers are especially osteophilic due to a number of molecular characteristics that confer to these cells the possibility to anchor in the bone marrow. Breast cancer cells are most often associated with osteolysis (with a reactive apposition of metaplastic bone) while prostate cancer generally induces osteosclerosis.

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