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Original Paper

Cellular and Molecular Mechanisms of Breast and Prostate Cancer Metastasis to Bone

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BONE AS A TARGET ORGAN FOR BREAST AND PROSTATE CANCER DISSEMINATION

IT HAS been well recognised that bone is one of the most common sites of cancer spread. Walther [1], who autopsied as many as 3000 patients who had died of cancer between 1927 and 1941, reported that bone was the third most preferential organ of distant metastasis of cancer, after lung and liver (Table 1). In the same study, it was found that prostate and breast cancer were the two most common cancers which disseminate to bone (Table 2). Furthermore, bone was demonstrated to be the first and second most common site of distant metastasis in prostate and breast cancer, respectively (Table 3). Because the study was conducted before anticancer agents emerged, it is likely that the data are a true reflection of the natural pattern of organ dissemination of prostate and breast cancer. These results then raise the question of why prostate and breast cancer have this predilection for spreading to bone.

Generally, cancer metastasis to distant organs is probably modulated by the inherent metastatic ability of cancer cells and the host environment encountered by the cancer cells. Cancer cells may eventually acquire additional abilities necessary for the advancement of metastases, under the influence of the host environment and, conversely, the host environment may also be changed due to the presence of metastatic cancer cells. These interactions between cancer cells and the host environment are critical to the progression of cancer metastases to target organs (Figure 1). Thus, understanding the bone microenvironment and determination of the metastatic properties of prostate and breast cancer cells, at cellular and molecular levels, are important to define the mechanisms of preferential metastasis of these cancers to bone and consequently to develop therapeutic strategies. Pharmacological agents which inhibit prostate and breast cancer cells or disrupt their interaction with the bone environment should be beneficial for the treatment of prostate and breast cancer patients with bone metastases. Bisphosphonates are such drugs that specifically inhibit host-derived osteoclasts, thereby disrupting the communication between metastatic breast cancer cells and bone. Later, experimental evidence which indicates that these approaches are feasible for the treatment of bone metastasis of breast cancer will be described.

UNIQUENESS OF THE STRUCTURE AND CELLULARITY OF BONE

Haemodynamic theory and bone metastasis

The haemodynamic theory was proposed by Ewing in 1928 [2]. He proposed that the development of metastases in a given organ was dependent on the blood volume flowing into that organ. The theory has been pertinent in some cases of colon cancer metastasis. However, considering low blood flow in the red marrow in bone compared with that in other preferential target organs of breast and prostate cancer, such as lung and liver [3], the high frequency of breast and prostate cancer metastasis to bone cannot be explained simply by the haemodynamic theory. Presumably, any circulating cancer cells may be able to migrate into bone through the blood stream but may not be able to survive in bone. Perhaps breast and prostate cancer possess inherent capacities which not only direct them to bone, but also enable them to survive, proliferate and colonise in bone. One important goal of research in this area is to identify these capacities at the molecular level.

'Seed and soil' theory

Compared with other common target organs for cancer dissemination, bone has several unique features in its morphological structure and cellularity. It has a hard calcified matrix which shows relatively low cellularity and metabolic activity. Of note, however, is that the calcified matrix stores varieties of osteoblast-derived growth factors [4] which may serve as essential nutrients for cancer cells which localise in bone. These growth factors are probably continuously released into the bone marrow cavity as a consequence of osteoclastic bone resorption during physiological bone remodeling. Thus, bone provides a fertile microenvironment which facilitates colonisation of metastatic cancer cells. In this regard, bone is a representative target organ for cancer metastasis which validates the 'seed and soil' theory proposed by Paget more than a century ago [5]. This appears

to be one major reason why breast and prostate cancer frequently colonise bone. However, it should be noted that this concept does not explain why only certain types of cancers preferentially spread to bone and why others rarely do.

Within bone, is the multicellular and highly-metabolic bone marrow. Haematopoietic stem cells, which give rise to all blood cell elements and osteoclasts, as well as mesenchymal stem cells which differentiate into osteoblasts, chondrocytes, adipocytes and stromal cells, are relevant to the topic discussed here. Of particular importance is the presence of bone-resorbing osteoclasts and bone-forming osteoblasts. As discussed above, osteoblasts produce and store diverse growth factors in the hard calcified matrix. It seems likely that without osteoclastic bone resorption, growth factors stored in the calcified matrix are less available to metastatic cancer cells. Furthermore, although still controversial, clinical and experimental evidence has shown [6, 7] that metastatic cancer cells by themselves are unable to destroy the hard calcified matrix and thus are dependent on osteoclasts for the progression of osteolytic metastasis. It is, therefore, likely that osteoclastic bone resorption is essential for cancer cells to obtain sufficient nutrients and space for their growth in the hard calcified tissue.

Table 1. *Distant metastasis of cancers* [1]

Metastatic site	Patients with metastasis % (total 2974)
Lungs	55
Liver	43
Bone	23
Kidneys	9
Adrenals	8
CNS	6
Spleen	4

Table 2. *Cancers metastatic to bone* [1]

Primary site	No. of autopsy	Patients with bone metastasis %
Prostate	113	66
Breast	186	64
Thyroid	104	38
Kidney	97	33
Lungs	286	29
Testis	22	26
Liver	54	24

Table 3. *Distant metastasis of breast and prostate cancer* [1]

Metastatic site	Patients with metastasis % Breast (186)	Prostate (113)
Bone	64	66
Lungs	84	63
Liver	48	15
Kidneys	12	6
Adrenals	11	6
Thyroid	10	

Numbers in parentheses represent number of patients studied.

PATTERN OF BONE METASTASIS

There are two patterns by which cancer cells affect bone: osteolytic and osteoblastic (osteosclerotic). Breast cancer predominantly develops osteolytic bone metastases and prostate cancer predominantly develops osteoblastic bone metastases. In either case, cancer cells colonising bone will interact with both osteoclasts and osteoblasts in the bone marrow cavity. These cellular communications contribute to the pattern of progression of bone metastasis. In the case of breast cancer, osteoclasts are the major interacting host cells and in the case of prostate cancer, osteoblasts are the primary partner (Figure 2). These interactions are probably mediated through soluble factors and/or cell–cell contact. Therefore, identification of these soluble factors and cell adhesion molecules (CAM) involved in this partnership is important for understanding the pathophysiology of bone metastasis and in the design of therapeutic agents for the treatment of bone metastases. From a pathophysiological point of view, one critical characteristic which facilitates breast and prostate cancer colonisation in bone may be the production of soluble factors and/or expression of CAMS which mediate these cellular interactions. 'Bone-seeking' breast and prostate cancer may inherently possess this capacity.

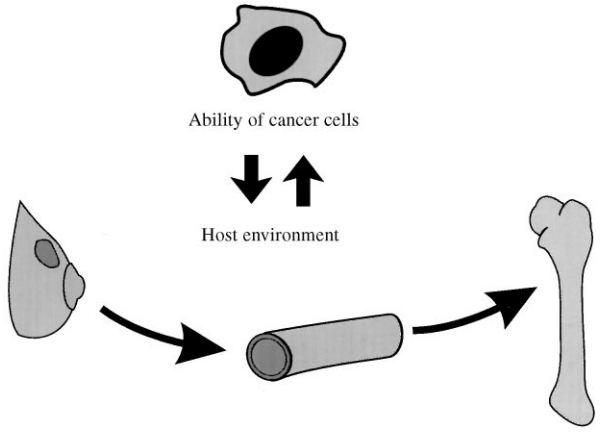

Figure 1. Interaction between cancer cell and the host environment during distant metastasis.

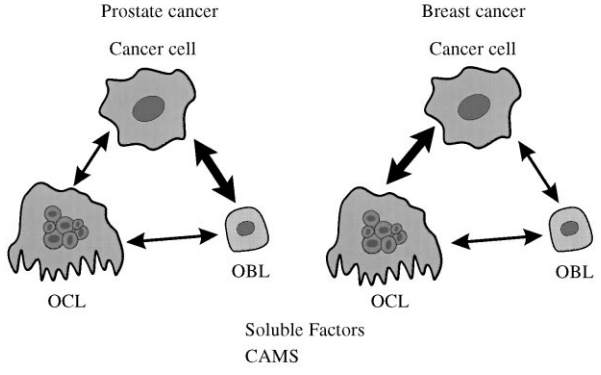

Figure 2. Interactions between cancer cells, osteoblasts (OBL) and osteoclasts (OCL) in bone metastasis of prostate and breast cancer. In prostate cancer metastasis to bone, in which osteosclerosis predominantly develops, osteoblasts may communicate with prostate cancer cells more closely than osteoclasts, whereas in bone metastasis of breast cancer, which predominantly cause osteolysis, osteoclasts may be a major player interacting with metastatic breast cancer cells.

BONE METASTASIS OF PROSTATE CANCER

As shown in Table 1, Walther [1] detected bone metastases in more than 60% of prostate cancer patients at autopsy. The characteristic feature of bone metastasis of prostate cancer is the predominant osteoblastic reaction, i.e., bone formation. In one clinical study, it was reported that 108 of the 122 prostate cancer patients had osteoblastic bone metastases [8] and similar results have been reported by several studies [for review, 9]. Compared with osteolytic metastasis developed in breast cancer, complications associated with osteoblastic metastases are less symptomatic. Hypocalcaemia is occasionally seen in prostate cancer patients with osteoblastic metastases.

Production of osteoblast-stimulating factors by prostate cancer

Because prostate cancer predominantly develops osteoblastic bone metastases, it is probable that prostate cancer cells produce a factor(s) which stimulates osteoblastic bone formation. This idea was first suggested by Gutman and associates in 1936 [10]. They determined serum and bone phosphatase activity in patients with prostate cancer and found increased phosphatase activity at the site of new bone formation in these patients. From this finding, these authors proposed that a chemical factor(s) which increased bone formation was produced by prostate cancer. Since then, many groups have described osteoblast-stimulating factors produced by prostate cancer cells. Some of these factors are listed in Table 4. Serine-proteases such as prostate specific antigens (PSA) and urokinase may not have direct stimulatory effects on osteoblasts, but rather work through activation of latent forms of transforming growth factor β (TGFβ) or interference with the binding of insulin-like growth factor-binding proteins (IGFBF) to IGFs [9]. Identification of bone morphogenetic protein (BMP) type IB receptors (BMPIBR) in prostate cancer cells [11] indicates that BMPs are not only paracrine factors for osteoblasts, but also autocrine factors for prostate cancer cells.

Autocrine/paracrine factors which stimulate prostate cancer

Primary prostate cancers grow at a relatively slow rate. However, once they spread to bone, their growth is often accelerated, suggesting that the bone microenvironment may provide a proliferation-stimulating factor(s) for metastatic prostate cancer cells. Based on this hypothesis, Gleave and associates [12] found several growth factors produced by bone marrow fibroblasts and Rossi and Zetter [13] identified transferrin in the bone marrow as a mitogen for metastatic prostate cancer cells. Smith and colleagues [14] demonstrated that spermine, which is abundantly expressed in the prostate, inhibits prostate cancer cell proliferation, suggesting that endogenous polyamine may be a negative regulator of prostate cancer growth and responsible for the slow growth rate of primary prostate cancer. The same group also showed that thymosin β15 promotes motility of prostate cancer cells and suggested that prostate cancers which express thymosin β15 may show increased metastatic property [15]. Endothelin -1 is produced by prostatic epithelium and the prostate gland expresses high-affinity endothelin -1 receptors. Nelson and colleagues [16] reported that endothelin -1 may play a role in the osteoblastic metastasis of prostate cancer. Some of the other factors which may stimulate prostate cancer are listed in Table 5.

Table 4. Osteoblast-stimulating factors produced by prostate cancer

Factor	Reference
Bone phosphatase-elevating-factor	10
Prostatic osteoblastic factor	29
Osteoblast-stimulating factor	30
Osteoblast mitogenic factor	31
Bone morphogenetic proteins	11, 32, 33
Serine-proteases	
PSA	9, 34
Urokinase	9, 35
Endothelin-1	16

Table 5. Autocrine/paracrine prostate cancer-stimulating factor

Factor	Reference
Bone fibroblast-derived factor	12
Transferrin	13
Calcitonin	36
Neurotensin	37
Spermine	14
Thymosin β15	15

Cross-talk between metastatic prostate cancer cells, osteoblasts and osteoclasts

Although the predominant pattern of prostate cancer metastasis to bone is osteoblastic, careful and extensive histological examination of metastatic bone lesions often reveals the presence of osteoclastic bone destruction in conjunction with osteosclerosis [9]. Consistent with this finding, there is a classical but still valid theory that every primary or metastatic cancer in bone begins with osteolysis, introduced by Roland [17]. In one experimental study, it was found that pre-treatment with dichloramethylene bisphosphonate, which is a specific inhibitor of osteoclasts, suppressed the development of both osteolytic and osteoblastic lesions by PA III rat prostate adenocarcinoma [18]. It is possible that osteolysis might provide space, nutrients and calcium that are essential for the subsequent osteoblastic reaction [17]. At cellular levels, these observations may indicate that metastatic prostate cancer cells first interact with osteoclasts to initiate osteolysis and subsequently or concurrently communicate with osteoblasts to develop osteosclerosis (Figure 3). Soluble molecules or CAMs which mediate this cellular network need to be identified.

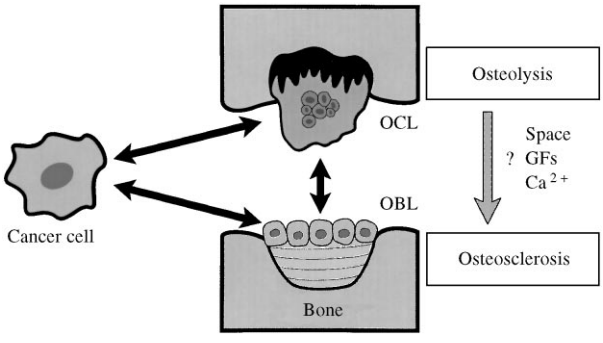

Figure 3. Relationship between osteoclastic osteolysis and osteoblastic osteosclerosis in bone metastasis of prostate cancer. Oseteolysis may provide space, calcium and bone-derived growth factors that are essential for the growth and differentiation of osteoblasts (OBL) OCL, osteoclast, leading to the osteosclerosis.

BONE METASTASIS OF BREAST CANCER

Breast cancer is another representative cancer which has a predilection for colonising bone [19]. Distinct from prostate cancer, breast cancer predominantly causes osteolytic bone metastases, which is accompanied by deleterious complications including severe bone pain, hypercalcaemia, pathological fractures and neural compression syndrome. Thus, bone metastasis is one of the major causes of morbidity in patients with breast cancer.

Animal model of bone metastasis of breast cancer

To study breast cancer metastasis to bone, we have used female nude mice in which an oestrogen-independent human breast cancer cell line MDA-MB-231 (MDA-231) was injected into the left cardiac ventricle. The details of this animal model of bone metastasis have been described [20, 21]. Nude mice inoculated with MDA-231 cells demonstrate radiographically well-defined osteolytic bone metastases with increased numbers of osteoclasts, mostly at the distal femur and proximal tibia 3–4 weeks after inoculation. Some mice infrequently manifest unilateral hindleg paralysis, indicating the presence of vertebral metastases. One notable feature of this model is that MDA-231 cells very rarely spread to the calvariae. We have used this animal model to study the effects of stimulation and inhibition of bone resorption on metastasis to bone, since, as discussed above, it seems likely that bone provides a fertile environment for metastatic cancer cells by releasing growth factors through osteoclastic bone resorption during bone remodelling.

Effects of stimulation of bone resorption on bone metastasis. Because the calvariae is an uncommon site of MDA-231 metastasis, we determined whether stimulation of local bone resorption on the calvariae induces MDA-231 metastasis to that site. Calvarial bone resorption was stimulated by repeated subcutaneous injections of recombinant human interleukin-1β (rhIL-1β) for 3 days according to the method described by Boyce and associates [22]. A day after the final injection of rhIL-1β, mice were inoculated with MDA-231 cells and monitored weekly by X-ray for the development of bone metastases. Four weeks later, we observed discernible tumour formation on the calvariae of rhIL-1β-treated mice and radiological examination revealed multiple osteolytic lesions on the calvariae of these mice. Control mice receiving vehicle showed no tumour formation and bone metastases on the calvariae. Because rhIL-1β was given locally on the calvariae, MDA-231 metastasis to other bones was not affected.

Effects of inhibition of bone resorption on metastasis. We used the bisphosphonate, risedronate, to inhibit bone resorption. Risedronate was given either (1) after osteolytic bone metastases were established; (2) simultaneously with MDA-231 cell inoculation; or (3) before MDA-231 cell inoculation. In either treatment, inhibition of osteoclastic bone resorption by risedronate resulted in a suppression of both the progression of established osteolytic bone metastases and the development of new osteolytic bone metastases [23]. Furthermore, risedronate selectively impaired the growth of MDA-231 breast cancer in bone. Although not significant, the growth of MDA-231 cells in soft tissue appeared to be increased in risedronate-treated animals.

These data demonstrate that stimulation of bone resorption can induce bone metastases to uncommon sites and that inhibition of bone resorption inhibits bone metastases. The result suggests that osteoclastic bone resorption provides space and nutrients necessary for colonisation of metastatic breast cancer cells in bone and strongly supports the notion that bone is a fertile 'soil' for metastatic breast cancer cells.

Metastatic ability of breast cancer cells

Fertility of the bone microenvironment does not necessarily explain why breast and prostate cancer preferentially colonise bone and why some types of cancers rarely spread to bone. It is most likely that breast and prostate cancer possess characteristics which allow them to spread selectively to bone and enable them to survive, proliferate and progress in collaboration with osteoclasts and osteoblasts. One major goal to be accomplished is to identify these characteristics at molecular levels.

Parathyroid hormone-related protein (PTHrP). PTHrP was originally identified in several human cancers including breast cancer, associated with humoral hypercalcaemia of malignancy. PTHrP is a powerful stimulator of osteoclastic bone resorption. Recent clinical studies have demonstrated that PTHrP expression is elevated in breast cancers metastasised to bone compared with primary breast tumours and breast cancers which spread to non-osseus organs. These results indicate a specific role of PTHrP in bone metastasis of breast cancer. We found that expression of PTHrP in MDA-231 human breast cancer cells increased osteolytic bone metastases and that neutralising antibodies to PTHrP markedly suppressed them [24]. PTHrP has also been detected in human prostate cancer [25].

PTHrP production by MDA-231 cells is stimulated by TGFβ, which is abundant in bone [4]. Expression of dominant negative TGFβ type II receptors in MDA-231 cells, markedly suppressed the development of osteolytic bone metastases. It has, therefore, been suggested that there is a 'vicious cycle' between bone-derived TGFβ and breast cancer-derived PTHrP in the progression of osteolytic bone metastases.

Matrix-degrading proteolytic enzymes. Cancer cells invade surrounding tissues to enlarge the tumour mass by secreting proteolytic enzymes such as aspartic, cysteine and serine proteases and matrix-degrading metalloproteinases (MMPs) [26]. The MMPs are a family of Zn^{2+} dependent endopeptidases. In stable conditions, MMPs are present in latent forms and need to be cleaved by proteolysis to exert their activity. Increased plasma levels of MMPs have been correlated with invasion and metastasis in patients with breast cancers [26]. Further studies have demonstrated that activation rather than expression of latent forms of MMPs was more closely associated with the malignant behaviour of breast cancer cells [26]. We found that MDA-231 human breast cancer cells produce both MMP-2 (gelatinase A) and MMP-9 (gelatinase B) in latent forms. However, when these cells were cultured on ECM (extracellular matrix), derived from osteoblasts and basement membrane ECM matrigel, expression of active forms of MMP-2 and -9 was induced [27].

Tissue inhibitors of matrix metalloproteinases (TIMPs). Cancer invasiveness and metastatic capacity are controlled not only by MMPs, but also by their corresponding natural ubiquitous inhibitors, TIMPs. Since cancer cells secrete TIMPs as well as MMPs, it appears that TIMPs function as metastasis-suppressor proteins and that the metastatic potential of cancer cells depends on the balance between MMP and TIMP production [28]. Overexpression of the *TIMP-2* gene has been shown to result in inhibition of invasion

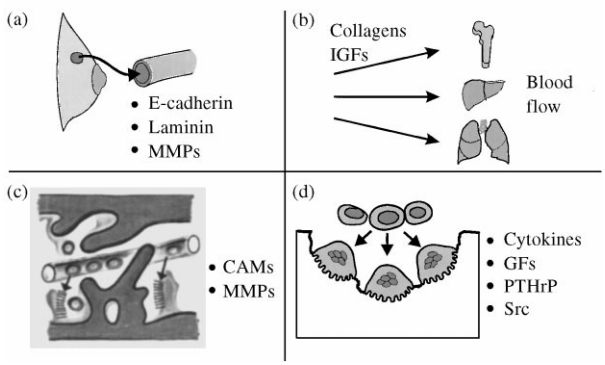

Figure 4. Molecules involved in the common (a), (b) and specific (c), (d) steps of breast cancer metastasis to bone.

and metastasis *in vivo* and injections of recombinant TIMP-2 have impaired metastasis development.

We have shown that overexpression of TIMP-2 in MDA-231 human breast cancer cells markedly inhibits osteolytic bone metastases and prolongs survival of tumour-bearing animals [27]. Moreover, combination of TIMP-2 expression with the bisphosphonate, ibandronate, more profoundly suppressed MDA-231 metastasis to bone than each treatment alone. Thus, it is suggested that inhibition of bone resorption, which consequently disrupts the interaction of metastatic breast cancer cells with the bone environment, and interference with breast cancer cell's characteristics are more efficient and selective interventions for the treatment of osteolytic bone metastasis of breast cancer.

Unidentified characteristics. It is probable that breast and prostate cancer cells that are metastatic to bone possess yet-unidentified characteristics. As an attempt to identify such properties, we recently established several subclones of MDA-231 cells by repeated *in vivo* selection in target organs. One subclone spreads exclusively to bone and another clone metastasises only to brain. We are currently studying these subclones for expression of distinct genes or proteins which may contribute to the predilection of breast cancer cells for bone.

CONCLUSION

Cancer dissemination to distant organs consists of multiple and complex sequential steps. Breast and prostate cancer metastasis to bone can broadly be divided into two major steps, that is, the common steps which occur before cancer cells arrive in bone and thus are probably shared with metastases to other organs; and the specific steps which occur where cancer cells colonise bone (Figure 4). In both common and specific steps, a variety of cellular and molecular events are involved. Each of these events is a target in the design of therapeutic interventions for the treatment of bone metastasis. Thus, our efforts should be directed toward identification of these events.

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