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Invited Review

Arterial microvascularization and breast cancer colonization in bone

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Summary. Bone is one of the most preferential target organs of cancer metastases. Breast, prostate and lung cancers have a special predilection for colonization in bone. In an animal model in which inoculation of cancer cells into the left cardiac ventricle selectively develops osteolytic bone metastases but rarely forms metastases in non-bone organs, the pattern of breast cancer colonization in bone was studied radiologically and histologically. Colonization of cancer cells in bone was found to initiate and develop along with or at the terminal end of the major arteries running into bone. It should, therefore, be re-recognized that the anatomical vasculature still remains as a critical factor which influences cancer colonization in bone in addition to cellular and molecular properties of the bone microenvironment and metastatic cancer cells.

Key words: Breast cancer, Bone metastasis, Osteolysis, Osteoclast, Hemodynamics

1. Colonization of cancer cells in bone

Bone is one of the most preferential target organs of cancer dissemination in patients with hematologic malignancies and carcinomas of the breast, prostate and lung (Mundy and Yoneda, 1995, 1996). Despite the fact that bone metastases occur primarily in the hematopoietic (red) marrow through arterial routes (Berrettoni and Carter, 1986), arterial blood supply to the red marrow is very low (0.4 ml/min/g organ weight) compared to other organs which are also preferential sites of cancer spread, such as lung (13.6 ml/min/g) and liver (0.82 ml/min/g) (Weiss et al., 1980). Hence, the hemodynamic theory, in which Ewing (1928) proposes that the frequency of cancer metastases in different organs is dependent on the numbers of cancer cells delivered to them in their arterial blood, appears unlikely to be the mechanism underlying the preferential spread of cancer cells to bone. On the other hand, the seed and soil theory, which is another classical long-lasting concept proposed by Paget (1889) reasonably accounts for the preference of cancer dissemination to bone and thus has been widely accepted. Abundant storage of a variety of growth factors in bone (Hauschka et al., 1986) constitutes a fertile microenvironment which facilitates the colonization of metastatic cancer cells (Yoneda et al., 1995; Yoneda, 1996). In addition, it seems likely that carcinomas of lung, breast an prostate which frequently spread to bone instinctively posses or acquire the capacity to migrate either actively (automotility) or passively (chemotactic attraction), arrest (adherence), survive (escape from immune cell attack) and proliferate in the bone microenvironment. These host-cancer cell interactions play a principal contributional role in the establishment and progression of cancer colonization in bone. Thus, current studies of bone metastasis are directed toward determination of cellular and molecular events which are involved in the mediation of the interactions between the bone microenvironment and metastatic cancer cells. Is there then no room to consider an involvement of hemodynamic factors in cancer metastasis to bone which accomodates more than 2.5 liters of blood every minute (Arguello and Cohen, 1996)? This is the issue to be discussed in this short review.

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2. An animal model of experimental bone metastasis

We have reported a unique animal model in which inoculation of cancer cells into the left cardiac ventricle in young female nude mice selectively develops osteolytic bone metastases 4 to 5 weeks after cell inoculation (Nakai et al., 1992; Sasaki et al., 1995; Mbalaviele et al., 1996). Not all cancer cells inoculated into the heart formed ostolytic bone metastases, thus demonstrating cell-selectivity of this model. Prostate cancer cells such as PC-3, LNCap and PA III developed bone metastases but did not form osteoblastic lesions.

They formed osteolytic lesions in this model (unpublished data). A human neuroblastoma cell line (SK-N-SH) developed mixed osteolytic and osteoblastic lesions (Yoneda et al., 1994). Of note, cancer cells very rarely spread to non-bone tissues including lung, liver, kidney and brain in this model (Mbalaviele et al., 1996). Although the reasons for this bone-selectivity are not known, the observation suggests that arterial hemodynamics have little to do with bone-preferential dissemination of cancer cells in this model. Using this animal model, we studied a human estrogen-independent breast cancer cell line MDA-MB-231 for its capacity to develop osteolytic bone metastases radiologically and histologically, and tested various agents which suppress bone metastases (Sasaki et al., 1995; Guise et al., 1996; Mbalaviele et al., 1996). Bone metastatic capacity of estrogen-dependent human breast cancer cells such as MCF-7 and T-47D cannot be studied in this model, because estrogen levels in female nude mice are not sufficient to support the growth of these breast cancer cells. Moreover, supplementation of estrogen to promote their growth causes sclerosis of bones which in turn interferes with osteolysis and misleads interpretation of histological pictures.

3. Radiological and histological pattern of cancer colonization in bone

As shown in Figure 1, the radiograph displays multiple osteolytic lesions formed in the epiphysis, diaphysis and periosteal surface of the left femur and tibia 4 weeks after the inoculation of MDA-MB-231 cells. This radiologic pattern of bone metastasis resembles that seen in patients with breast cancer except for metastases at the periosteal surface. Periosteal cancer metastases occasionally occur in lung cancer. Histological examination revealed that MDA-MB-231 cells colonized proximal and distal sides of the growth

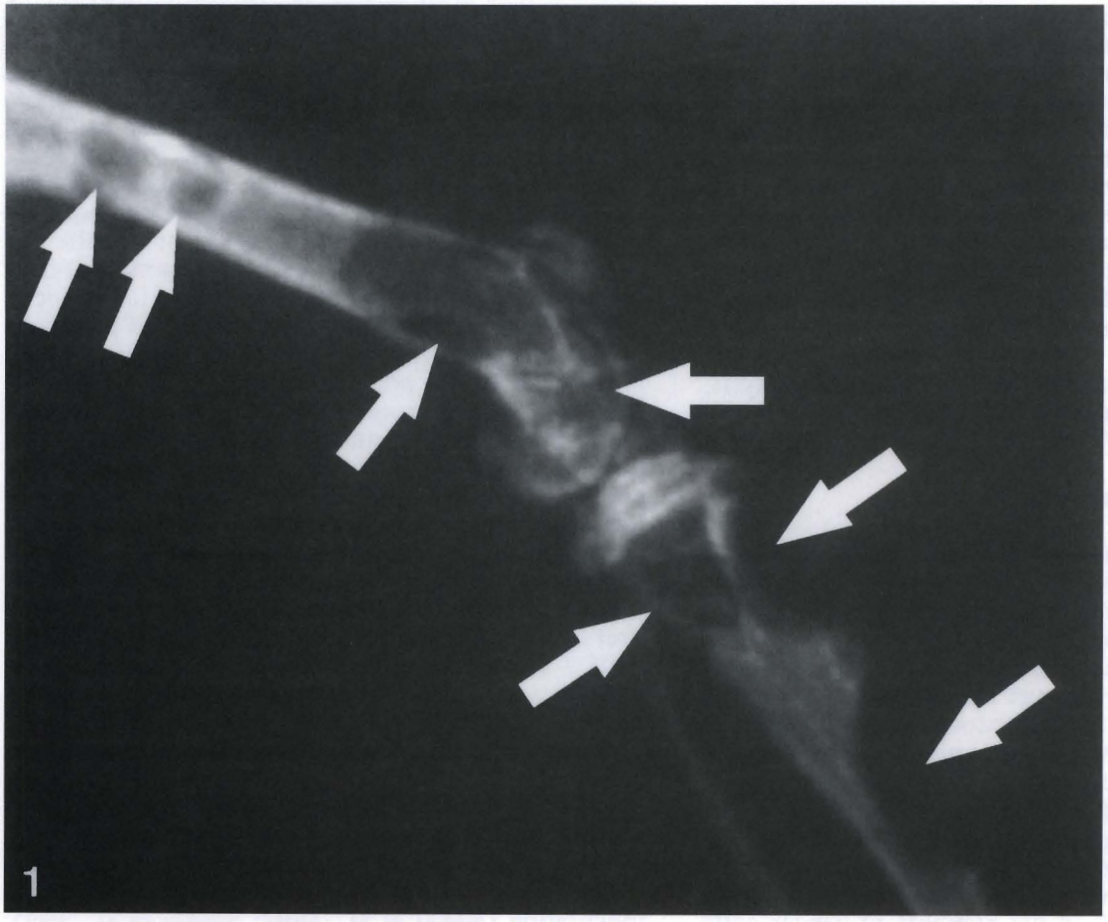
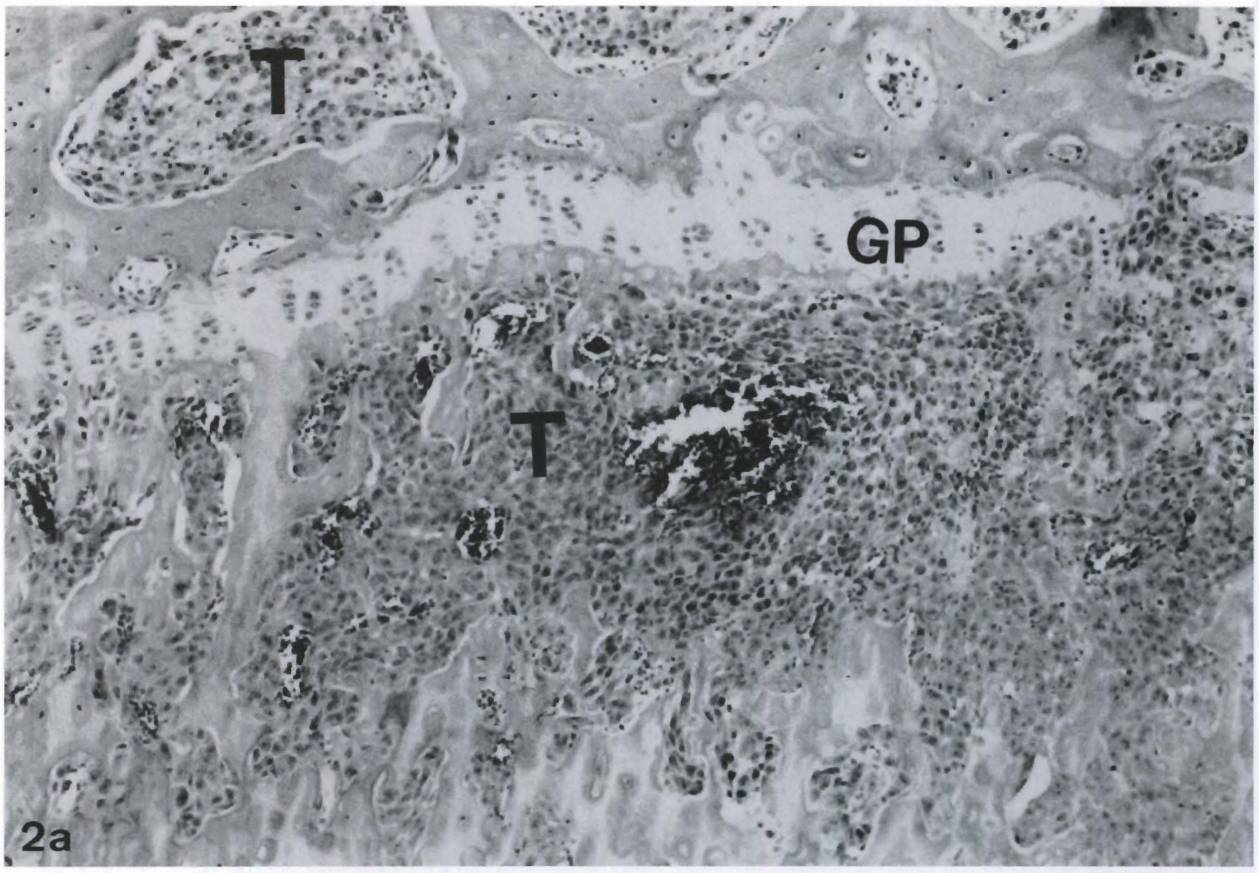


Fig. 1. Representative osteolytic bone metastases in the left leg of female nude mice. MDA-MB-231 cells ($1x10^5$/animal) are inoculated into the left cardiac ventricle of 4-week-old animals. Four weeks later animals were examined radiologically for osteolytic lesions as described previously (Mbalaviele et al., 1996). Note multiple radiolucent lesions in epiphysis, metaphysis, diaphysis and periosteal surface in the femur and tibia (arrows).

Fig. 2. MDA-MB-231 cell colonization in **a**, epiphysis; **b**, marrow sinus (arrows) and; **c**, periosteal bone surface. Mice were sacrificed three weeks after cell inoculation and bones were processed for conventional histological examination. T: MDA-MB-231 tumor; GP: growth cartilage plate; M: muscle. White arrows in C show osteoclasts. H.-E. Staining. a, x 100; b, x 40; c x 200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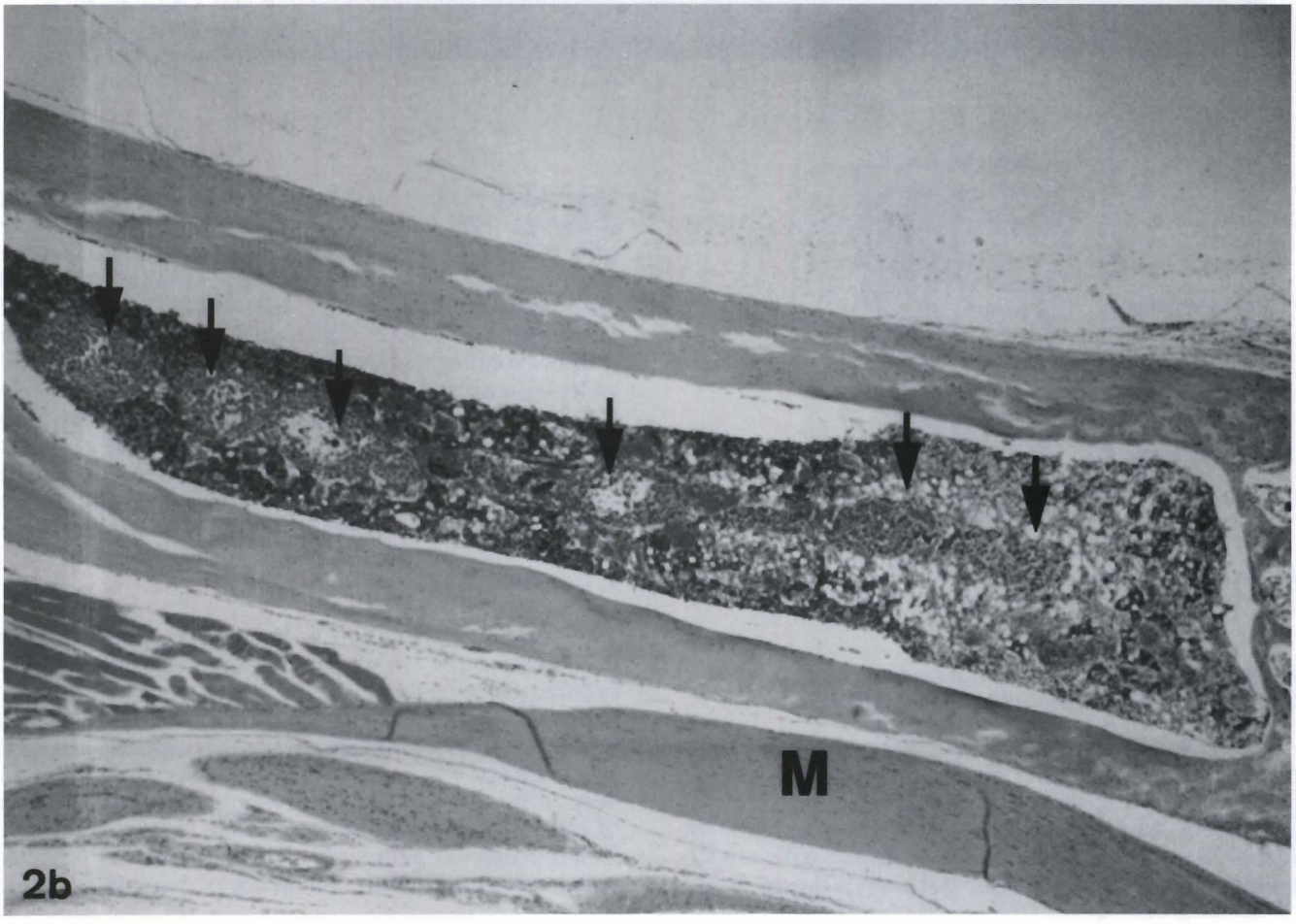
T
2a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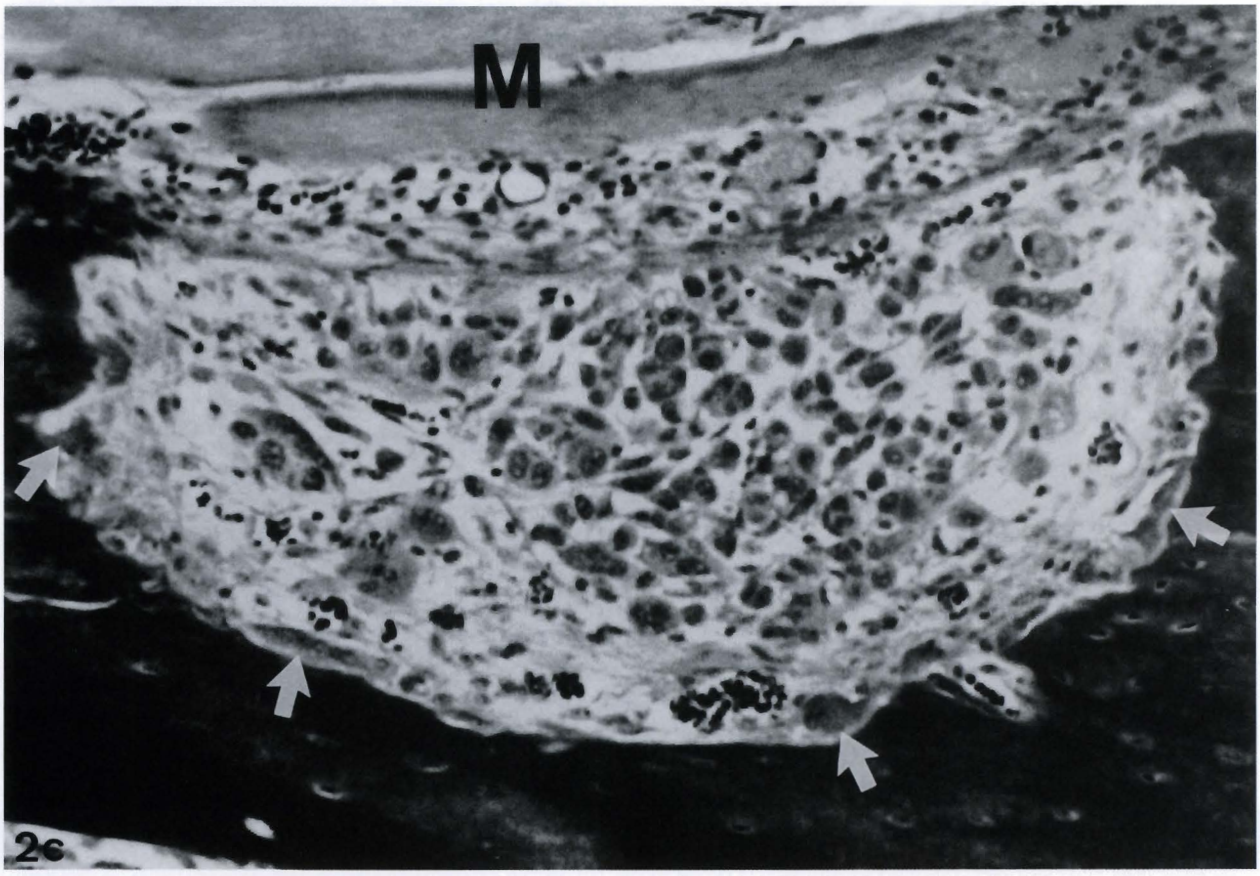

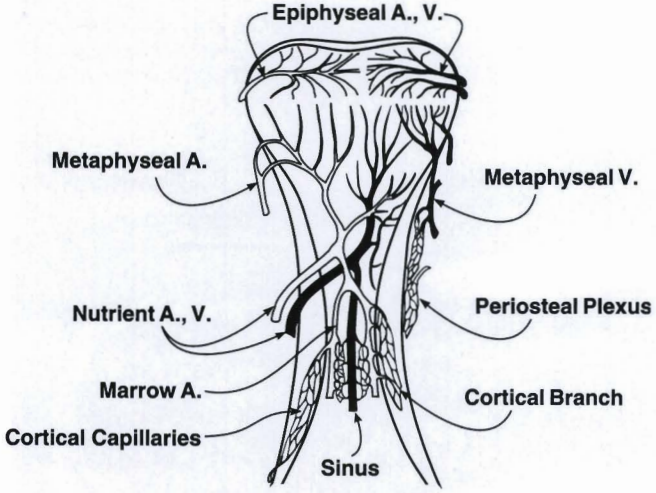

Fig. 3. Bone vasculature. Modification of the Figure shown in Jee, 1988. Open lines indicate arteries and closed lines indicate veins.

cartilage plate (Fig. 2A), marrow sinus (Fig. 2B) and periosteal bone surface (Fig. 2c) accompanied by aggressive osteoclastic bone resorption (Fig. 2c). We found that colonization by MDA-MB-231 cells already occurred only 7 days after cell inoculation in the epiphyseal plate. Consistent with the previous result (DeBruyn, 1981). Figure 2B appears to show that cancer cells in the marrow sinus cannot freely migrate into the marrow cavity. Not unexpectedly, these radiological and histological patterns of MDA-MB-231 breast cancer cell colonization in bone coincide with the vasculature of the arteries running into bone, including epiphyseal and metaphyseal arteries, nutrient artery communicating into the sinus and cortical capillaries (Fig. 3). Thus, although the arterial blood flow may not be critical while cancer cells journey from the primary site to the bone, their colonization in bone is primarily directed based on the anatomical arterial vasculature.

4. Conclusion

Cancer dissemination to bone consists of multiple sequential steps which include diverse and complex cellular and molecular events. These steps can broadly be divided into two major steps, i.e., the general steps which occur before breast cancer cells reach bone, and specific steps which occur during cancer colonization in bone. Currently, efforts are focused on an identification of molecular and cellular components which play a role in these steps to gain a clue for the design of pharmacological agents for the treatment of bone metastases.

Because bone metastases frequently occur in breast cancer and cause deleterious complications which spoil the quality of life in breast cancer patients, pursuit of this goal is very important and should be achieved. Here, it is shown that cancer cell dissemination in bone is also dependent on the intra-osseal arterial micro-vasculature, suggesting that the hemodynamics are still a critical factor which modulate cancer housing in bone. How useful this informations for the development of the therapeutic strategy for bone metastases is not known at the present time. Perhaps, this information may help clinicians detect micrometastases in bones in patients with breast cancer at early stages, leading to early diagnosis and timely initiation of treatment. It is also possible that selective delivery of vasoconstrictors to bone might impair cancer cell colonization in bone.

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