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Instructional lecture

Metastatic bone disease: pathogenesis and new strategies for treatment

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Abstract Bone is the third leading site of metastatic disease, after the lung and liver. Pain, pathological fractures, neurological deficits, and forced immobilization significantly decrease the quality of life of patients with bone metastasis. The development of metastasis, from the migration of malignant cells from the primary tumor to their proliferation at a distant site, involves a series of sequential steps: angiogenesis, matrix degradation, cell motility, cell attachment, and cellular proliferation. A better understanding of the pathogenesis of metastasis may be expected to lead to the development of new treatment modalities for bone metastasis. Currently, antiangiogenic agents, matrix metalloproteinase (MMP) inhibitors, and hyperthermia are some of the newer therapeutic modalities that seem to hold promise for the treatment of metastatic bone disease.

Key words Metastatic bone disease · Pathological fracture · Angiogenesis · Hyperthermia

Metastatic bone tumors are the most common neoplasms of the bone. Although the precise incidence of bone metastasis in Japan has not been determined, more than one-third of the 500,000 patients with newly diagnosed invasive cancer annually have bone-seeking malignant tumors. It is estimated that more than 150,000 patients with bone metastasis suffer from pain and paralysis in Japan. Orthopedic surgeons must recognize the presence of the significantly large number of patients with metastatic bone disease, as compared with the approximately 90,000 patients annually undergoing total joint arthroplasty, which is currently the most popular surgery in Japan.

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Bone is the third leading site of metastatic disease, after the lung and liver. Bone metastases are most frequently associated with tumors originating in the breast, lung, prostate, kidney, or thyroid gland. Nearly half of patients with breast and prostate cancer eventually develop bone metastasis.[4,5] Pain, pathological fractures, neurological deficits, and forced immobilization significantly decrease the quality of life of these patients. Advances in chemotherapy and radiotherapy have allowed cancer patients to live longer, and are also expected to meet the challenges in the treatment of metastatic bone disease.[14,17,19,20] To plan treatment and develop new treatment modalities for metastatic bone tumors, we must first understand the pathogenesis of metastasis.

Pathogenesis

The development of metastasis, beginning with the migration of malignant cells from the primary tumor, to their proliferation at a distant site, involves a series of sequential steps: angiogenesis, matrix degradation, cell motility, cell attachment, and cellular proliferation (Fig. 1).[3,4] Angiogenesis is an essential process for the growth of the primary tumor. The rich neovascularization increases the chances for the tumor cells to reach the blood stream and colonize secondary sites.[11] Cellular attachment to other cells and/or matrix protein is another important process. Lysis of matrix proteins by specific proteinases is required for invasion of blood vessels by the tumor cells and extravasation.[13] Before extravasation, selective adhesion of the tumor cells to specific sites on the endothelial luminal surface occurs at sites of organ homing. After extravasation, the tumor cells grow selectively only in organs that provide the appropriate growth factors or extracellular matrix environment. Growth factors released from the bone marrow that stimulate metastatic malignant cells arising

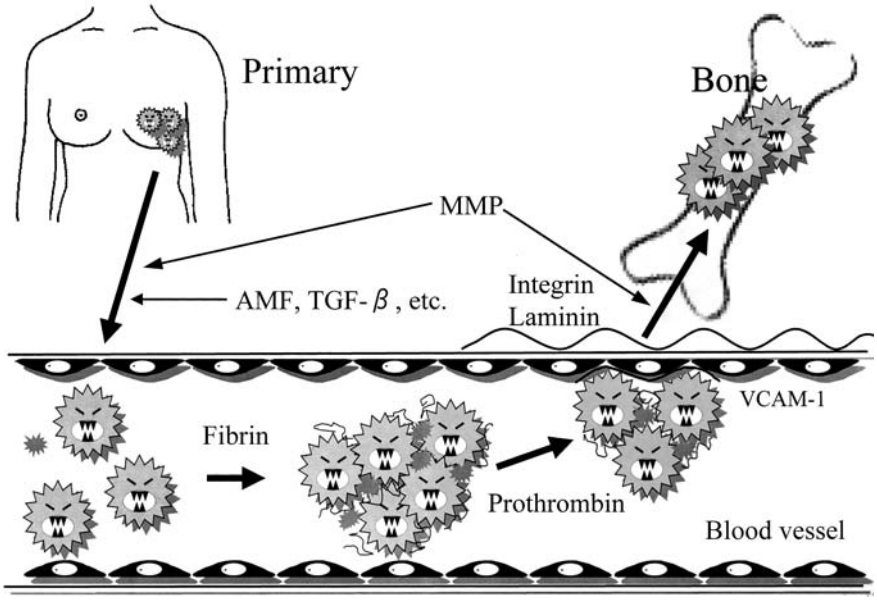


Fig. 1. Diagrammatic representation of the cellular basis of metastasis. *MMP*, matrix metalloproteinase; *AMF*, autocrine motility factor; *TGF*-β, tumor growth factor-β; *VCAM*, vascular cell adhesion molecule

from breast cancer to proliferate in the bone and bone marrow have been identified.[4,21] Regulation of these steps involved in the development of metastasis, including angiogenesis, cell attachment, invasion, and release of growth factors, may be expected to allow more effective treatment modalities to be developed for metastatic bone disease.

Invasiveness is correlated with the capacity of malignant cells for metastasis. In general, highly invasive tumors have the greatest propensity to metastasize, since tumor cells have to disseminate from the site of the primary tumor; invade the surrounding matrix, blood vessels, and lymphatics; become trapped by and invade the blood vessel wall and be carried to a distant organ by blood flow; and finally proliferate at a secondary site before the process of metastasis can be completed.

To evaluate the relationship between the invasiveness of tumor cells and their capacity to metastasize, we have developed a modified method of the Boyden chamber assay for measurement of tumor cell invasiveness and established a highly invasive cell line (unpublished results). First, we coated the upper side of the filter with the cellular matrix; the cells were cultured on this filter and allowed to invade the lower side of the filter to cause progressive degeneration of the matrix. Only the cells that reached the lower surface of the filter were collected and cultured, and they were then made to invade the matrix again.

A highly invasive cell line of MDA-MB-231, a human breast cancer cell line, was established by multiple repetitions of the above steps. This cell line was inoculated into the left ventricle of a nude mouse, and its capacity to metastasize to the bone was evaluated. This highly invasive cell line was found to be more invasive than the parental cell line by the Boyden chamber assay. The number of bone metastases was significantly higher following inoculation of the highly invasive cell line into the left ventricle of nude mice than following inoculation of the parental cell line. Moreover, severe bone metastases causing pathological fractures were only observed in the nude mice inoculated with the highly invasive cell line. Thus, the highly invasive cell line established from MDA-MB-231 was thought to have a great capacity to metastasize to the bone (unpublished).

Thus, invasiveness is an important factor in bone metastasis, and matrix metalloproteinases (MMPs) have been shown to play a major role.[10] Targeting MMP activity could be one of the strategies for the control of bone metastasis.[13] An experiment involving inoculation of an MMP inhibitor into nude mice to prevent human breast cancer cells from metastasizing to the bone suggested that the clinical application of an MMP inhibitor is effective for controlling bone metastasis. However, the efficacy appeared to wane as the MMP inhibitor was administered in the late stage of metastasis, and the optimal time of administration of an MMP inhibitor in the clinical setting remains to be determined. It is quite possible that it might be more effective to prescribe the MMP inhibitor in the early stage after resection surgery of the primary tumor, rather than after bone metastasis has been diagnosed. Moreover, 20 different kinds of MMPs have been discovered to date, which have various roles under normal physiological conditions. It is important, therefore, to identify the specific MMP that participates in bone metastasis and to develop selective MMP inhibitors for effective prevention of bone metastasis with minimal side effects.[12] Although MMP inhibi-

tors have not yet been developed for clinical use, they are believed to be potentially useful for preventive therapy against bone metastases in cases of breast cancer.

Specific interests in clinical aspects

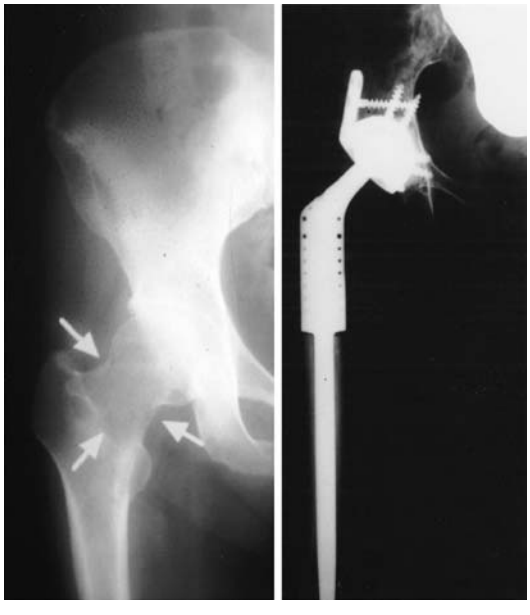
In a patient with a documented history of previous primary malignancy and localized bone pain, metastasis is the main differential diagnostic possibility that must be considered. The suspicion should be even stronger if multiple sites in the skeleton are involved. The detection of a solitary metastatic bone lesion in cases without a previously documented primary cancer poses a difficult diagnostic challenge. In such a case, the lung and kidney should be investigated first as the primary site, because more than 70% of patients in whom detection of bone metastasis precedes that of the primary malignancy have lung or kidney cancer.

Conventional roentgenography and radionuclide bone scans are the two major modalities considered useful for evaluating patients with suspected skeletal metastasis. Bone scanning is a highly sensitive imaging procedure used for the early detection of skeletal metastases, but it does not provide any additional information regarding the extent of the tumor over that provided by computed tomography (CT) and magnetic resonance imaging (MRI). Imaging procedures are very useful for the accurate diagnosis and staging of metastasis, and for avoiding the morbidity and mortality associated with inappropriate aggressive treatment.[5,22]

Improvements in the oncologic management strategies of advanced cancer have resulted in increased survival of cancer patients. This has encouraged more aggressive treatment of patients with pathological fractures.[1,6] The current philosophy is that even in cases of end-stage cancer, if the patient's general condition permits it, a more aggressive surgical management program may provide significant benefits in terms of pain relief, early ambulation, and ease of administration of nursing care.[2] Even joint replacement has been performed in case of metastatic bone disease (Fig. 2).

Following internal fixation, tumor cells may disseminate both locally and through the circulation, but there is no clinical evidence to suggest that such spread is of any clinical importance.[7] It was once believed that pathological fractures due to metastasis do not have the potential to heal, but we have encountered a significant number of cases in which fracture healing was observed. Particularly in cases of breast cancer, prostate cancer, and myeloma, bony consolidation can be obtained by rigid internal fixation conducted for a pathologic fracture, under effective chemotherapy or radiotherapy (Fig. 3). In such cases, we are wary of applying devices augmented by methyl methacrylate cement.

It has yet to be determined unequivocally whether radical resection of a solitary metastatic osseous lesion can result in cure. Nonetheless, such surgery may reduce the systemic effects of the tumor burden, in addition to conferring pain relief. Prosthetic replacement after aggressive surgical debulking may also be an option in selected patients.[16]



a,b

Fig. 2. After resection of the destroyed proximal fragment of the femur secondary to metastatic gastric cancer, a constrained hip prosthesis was inserted. **a**, pre-operation; **b**, postoperation

New strategies for treatment

Antiangiogenesis

The metastasis-inhibiting effect of a new polyamine synthesis inhibitor, chondromodulin-I (ChM-I), purified from fetal bovine cartilage and alginate, as an angiogenesis-inhibiting factor was investigated experimentally. Inhibitors of the polyamine biosynthetic pathway have received considerable attention because of their potential usefulness as antitumor agents in the treatment of cancer. Their use is based on the observation of the greatly increased production of polyamines, putrescine, spermidine, and spermine in various types of cancer. MGBCP [methylglyoxal bis(cyclopentylamidinohydrazone)], which was synthesized as a multienzyme inhibitor of the polyamine-synthesizing pathway and is a potent inhibitor of three polyamine-synthesizing enzymes, *S*-adenosylmethionine decarboxylase, spermidine syn-

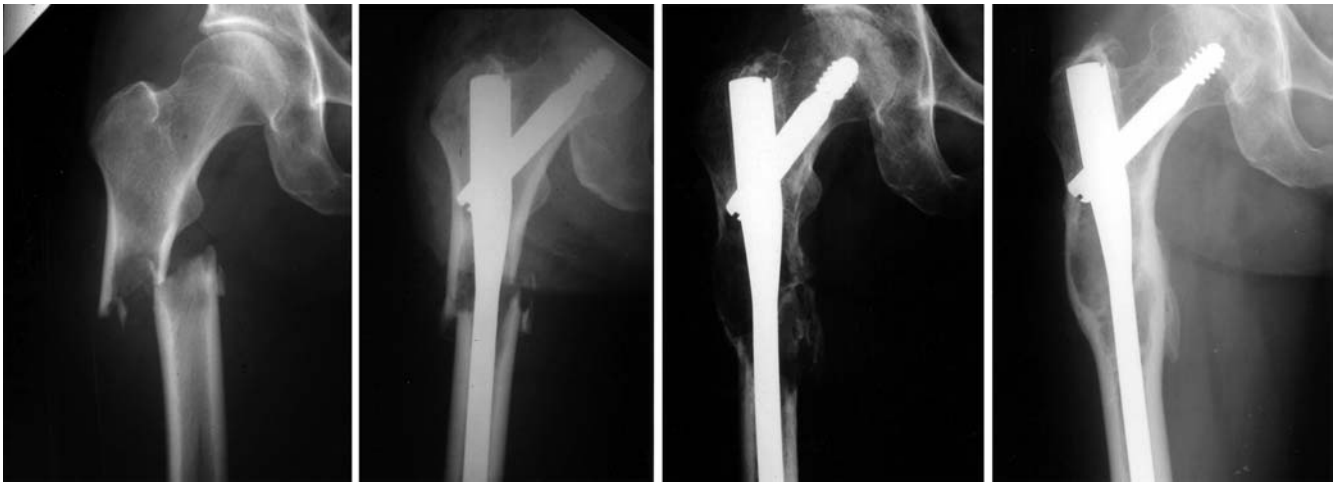


Fig. 3. **A** Internal fixation with a nail-plate apparatus was performed for destruction secondary to myeloma, with pathologic fracture. **B** Bony union was confirmed by x-ray after 1 year

thase, and spermine synthase, is believed to have a strong inhibitory effect against polyamine synthesis.[18]

Angiogenic molecules, such as fibroblast growth factor-2 (FGF-2), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF), have been reported to be present in cartilage. On the other hand, cartilage is known to be uniquely avascular among the mesenchymal tissues and exhibits resistance to vascular invasion. It is thought that antiangiogenesis agents exist in cartilage, and ChM-I has been purified from fetal bovine cartilage. ChM-I is a 25-kDa glycoprotein with 121 amino acid residues and is encoded as the C-terminal portion of a larger precursor (335 amino acids) molecule. Purified ChM-I has been shown to inhibit DNA synthesis, proliferation of vascular endothelial cells, and tube morphogenesis in vitro.[8]

Alginate hydrogels have been used in many biomedical applications, such as dental impressions and wound dressing, and they have no reported adverse effects. Alginate is the main component of biofilm, which seems to mediate antibiotic resistance in diffuse panbronchiolitis and metal-related osteomyelitis, by preventing vascular invasion and thereby causing decreases in the local concentrations of antibiotics. These results strongly suggest that alginate hydrogels may possess antiangiogenic efficacy. We have already shown in studies that alginate inhibits new formation of vessels in extraskeletal bone in vivo, and also growth of human vascular endothelial cells (HUVEC) in vitro (unpublished).

These antiangiogenic agents were administered by continuous intraperitoneal injection for 28 days in our original bone metastasis animal model, in which metastases of extraskeletally implanted bones and orthotopic bones were found after injection into the left ventricle of B16 melanoma. Following treatment with MGBCP, it was found that the metastatic rate to the extraskeletal bones was reduced to 16.5% and that to the bones of the hind limbs was reduced to 60% of the corresponding rates in controls. The metastatic rates after treatment with ChM-I or aliginate were found to be approximately half of those in controls (Table 1). No adverse effects were found, either macroscopically or microscopically.[18] These results suggest that antiangiogenic agents may have the potential to regulate metastasis to the bone (Fig. 4).

Table 1. Inhibition of bone metastasis

Group	Extraskeletal bone	Hind limbs
MGBCP/untreated	16.5%	60% (n = 14, 15)
rhChM-I/untreated	50%	50% (n = 4)
Alginate/untreated	25%	66.7% (n = 11)

The femur and tibia were removed from female C57BL/6 mice, and the bones were transplanted into the dorsal subcutis of other female C57BL/6 mice. After 4 weeks, 1×10^5 cells of B16 melanoma were injected into the left heart ventricle of the mice. Methylglyoxal bis(cyclopentylamidinohydrazone) (MGBCP), recombinant human chondromodulin-I (rhChM-I), and alginate were administered intraperitoneally for 28 days after the inoculation of B16 melanoma cells. After 28 days, all mice were sacrificed, and the incidence of bone metastasis was evaluated macroscopically and radiologically in the hind limbs and extraskeletally implanted bones. Incidence of bone metastasis = bone metastases in treated group/bone metastases in untreated group × 100

Hyperthermia

As a less invasive treatment modality and for the local control of metastatic tumor, surgical intervention supported by hyperthermia is considered promising. We have developed a ferromagnetic thermoseed of bone cement and a metallic intramedullary nail thermoseed for local hyperthermia for the management of deep-

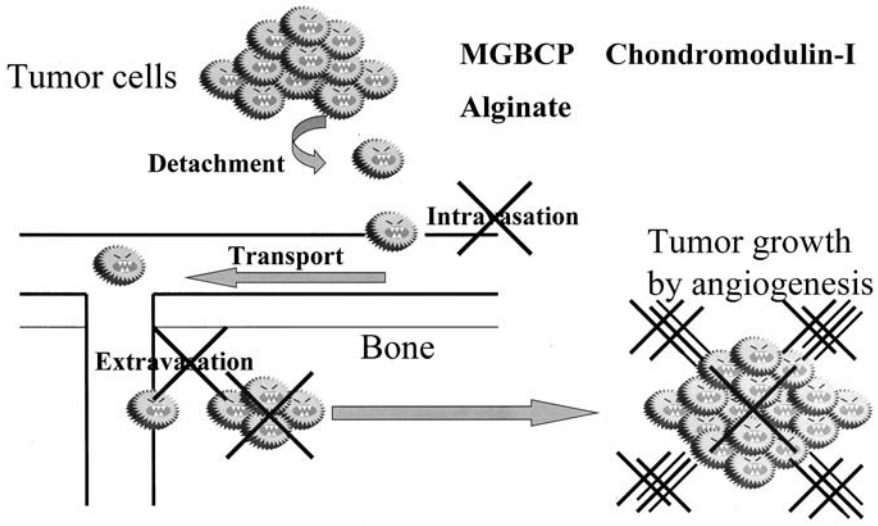


Fig. 4. Diagrammatic representation of the cellular basis of metastasis inhibition by antiangiogenic agents. *MGBCP*, methylglyoxal bis(cyclopentylamidinohydrazone)

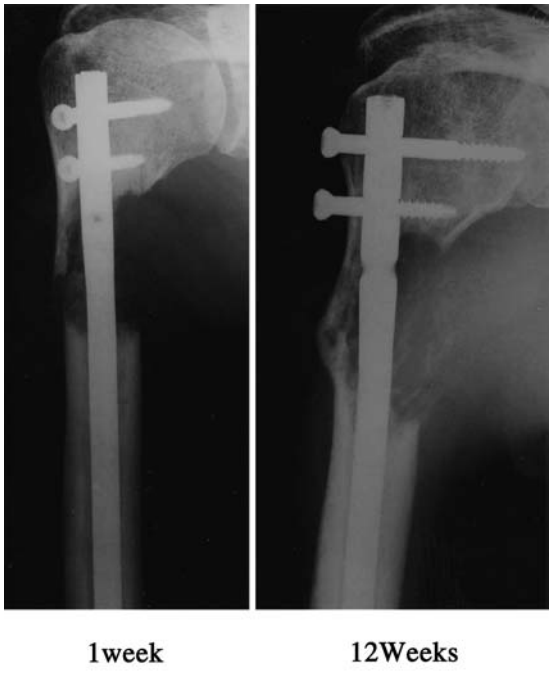


Fig. 5. This patient, a 75-year-old-man who had liver cancer with a metastatic tumor in the right humerus, presented with severe pain at the site of metastasis due to impending bone fracture. An intramedullary nail made of titanium was inserted into the right humerus. Ferromagnetic hyperthermia sessions were administered 10 times, starting a week after the operation. On the 12th week after the operation, bone formation was confirmed by x-ray

seated tumors, especially of the bone, and have been involved in related experimental studies for several years now. In our system, it is very easy to increase the temperature of the thermoseeds in the bone beyond 50°C by adjusting the volume of the magnetite and the intensity of the magnetic field. We have also demonstrated that this ferromagnetic hyperthermia system prevents local bone destruction caused by tumors in experimental animals.[9,15] Clinical application of this system for the treatment of metastatic bone disease was started about 2 years ago, based on the abovementioned experimental results.

Briefly, a titanium alloy intramedullary nail is inserted for a pathological fracture or an impending fracture. The product (output of 4 kW, fixed frequency of 1.5 MHz) manufactured by Yamamoto BINITA (Osaka, Japan) is used. The cylinder-form coil is installed and the focus part is arranged on the inside. An exchange magnetic field is generated in the cylinder-form coil, and heat is generated in the intramedullary nail. The hyperthermia treatment is generally started about a week after the operation. Each hyperthermia session lasts for about 10 min, and the sessions are conducted about twice a week.

We have already administered this treatment in five patients with bone metastasis involving the humerus in two cases, the femur in two cases, and the tibia in one case. The primary tumor arose from the lung in two cases, the liver in one case, the kidney in one case, and a peripheral nerve sheath in one case. In four of the five patients, local control of the bone metastases was confirmed on plain radiographs, which showed new bone formation at the metastatic sites and arrested progression of the destructive changes (Fig. 5).

There were no critical side effects. All the patients reported feeling heat in the limb during hyperthermia, but this complaint disappeared immediately as the switch of the equipment was turned off.

Conclusion

In the management of metastatic bone disease, it is important to understand the pathogenesis of metastasis,

which, beginning from the migration of malignant cells from the primary tumor to proliferation of these cells at a distant site, involves several sequential steps: angiogenesis, matrix degradation, cell motility, cell attachment, and cellular proliferation, all of which are important factors in the development of bone metastasis. Strategies targeting inhibition of these processes may be expected to be potentially useful for the prevention of bone metastasis. The current philosophy is that even in patients with end-stage cancer, a more aggressive surgical program of fracture management may provide significant benefits.

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