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Molecular pathogenesis and its therapeutic modalities of lung cancer metastasis to bone

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Abstract Bone metastasis is a critical problem of lung cancer patients. Reproducible animal models of lung cancer bone metastasis, like NK-cell depleted SCID mouse model with SCB-5 cells, are useful to explore the molecular mechanism and search of molecular targets. SBC-5 cells overexpressed PTHrP and that treatment with anti-PTHrP neutralizing antibody inhibited the production of bone metastases of SBC-5 cells in the NK-cell depleted SCID mouse model, indicating the critical role of PTHrP in bone metastasis in this model. In addition, we demonstrated that several compounds, including bisphosphonates and reveromycin A, potentially suppress osteoclast-activity were beneficial for the treatments of bone metastasis. Multi-modality therapy may be necessary for further augmenting the therapeutic efficacy against lung cancer bone metastasis.

Keywords Lung cancer · Bone metastasis · Osteoclasts · Molecular target therapy

Lung cancer is the major cause of malignancy-related death worldwide, and its incidence is rising in many countries. More than 50,000 new cases of lung cancer are detected annually in Japan, and the mortality rate of nearly 90% makes in the leading cause of cancer-related deaths [1]. The high mortality of this disease is predominantly due to the difficulty of early diagnosis and the highly metastatic potential of lung cancer. In many cases, the metastases to multiple organs are already developed by the time of the diagnosis. Over one third of patients with advanced lung cancer develop osteolytic bone metastasis, which cause pain, pathologic fractures, spinal cord compression, and hypercalcemia [2]. It leads to considerable reduction in quality of life in lung cancer patients. Currently, no curative therapy exists for bone metastasis, and clinical management is generally palliative. Therefore, the prevention and treatment of osteolytic bone metastasis are clinically important.

Bone metastasis is multi-step events regulated not only by cancer cells but also by host microenvironments. Of host microenvironmental cells, osteoclasts are suggested to play the critical roles. Osteoclasts cause bone resorption which supplies the space that cancer cells grow and leak out various growth factors from bone matrix [3, 4]. Therefore, osteoclasts are the ideal therapeutic target of osteolytic bone metastasis.

In order to further understand the molecular mechanism of bone metastasis, an animal model with human cancer cells is useful. In fact, bone metastasis models followed by intracardiac injection with melanoma [5, 6], breast cancer [7, 8], prostate cancer [9], and lung cancer [10] have been reported. We recently showed that depletion of natural killer (NK) cells in SCID mice markedly facilitate the formation of visceral metastasis (lung, liver, and kidney) by intravenously injected human lung cancer cell lines [11]. We further established the bone metastasis model using NK-cell depleted SCID mice [12].

1 Model of bone metastasis of human lung cancer

In order to facilitate the metastasis of human lung cancer cell lines, NK cells were depleted in SCID mice. For NK-cell

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depletion, anti-mouse IL-2 receptor β chain monoclonal Ab, TM-β1 (IgG2b) kindly supplied by Drs. M. Miyasaka and T. Tanaka (Osaka University, Osaka, Japan), was injected i.p. into SCID mice 2 days before tumor inoculation [11]. Though small cell carcinoma (SBC-5, SBC-3, SBC-3/ADM, H69, H69/VP) cells formed metastasis into multiple organs (liver, kidney, and lymph nodes), only SBC-5 cells reproducibly developed bone metastases [12]. Intravenously inoculated SBC-5 cells developed osteolytic bone metastases in all recipient mice by day 35 (Fig. 1). The mice with bone metastases showed neurological disorders (paraplesia or monoplesia of hind leg) and hypercalcemia, those are frequently observed lung cancer patients with bone metastases. Therefore, our bone metastasis model seems to reproduce the characteristics of bone metastasis of lung cancer in humans. Recently, a model for bone metastasis by human lung squamous cell carcinoma cells was reported [10]. In this model, tumor cells were inoculated into left ventricle of the heart and bone metastasis was developed in 75% recipient mice. The advantage of our model over others are (1) the incidence of bone metastases is 100%, (2) the procedure involved is easy, though NK-cell depletion in SCID mice is necessary to facilitate metastasis formation.

1.1 Molecular mechanism of bone metastasis

The mechanisms responsible for tumor growth in bone are complex and involve tumor stimulation of the osteoclast and the osteoblast as well as the response of the bone microenvironment. The balance of osteolytic and osteoblastic factors is thought to regulate the bone metastasis [13]. The molecules such as PDGF, IGFs, adrenomedullin, TGF-beta, FGFs, BMPs, ET-1, and VEGF are reported as osteoblastic factors which associated with osteoformation.

Osteoclasts originate from bone marrow stem cells, and play a crucial role in physiological and pathological bone resorption [14]. Several factors, including IL-1, IL-6, RANKL, macrophage inflammatory protein-1α and PTHrP, have been implicated in the differentiation/maturation of osteoclasts and bone destruction in malignant diseases [15, 16]. Of these factors, PTHrP has a 70% homology to the first 13 amino acids, of the N-terminal protein of PTH. PTHrP binds to the PTH receptor in the bone and kidney, stimulates osteoclast-mediated bone resorption and renal tubular calcium reabsorption, and hence induces hypercalcemia. Approximately 80% of hypercalcemic patients with solid tumors have detectable or increased plasma PTHrP concentrations [17]. In addition, PTHrP has been suggested to play a critical role in the production of bone metastasis. We previously reported that SBC-5 cells overexpressed PTHrP and that treatment with anti-PTHrP neutralizing antibody inhibited the production of bone metastases of SBC-5 cells in the NK-cell depleted SCID mouse model [18], indicating the critical role of PTHrP in bone metastasis in this model (Fig. 2).

1.2 Bisphosphonates as apoptosis-inducer of osteoclasts

Bisphosphonates are hydrolysis-resistant PP1 derivatives that have a high affinity for bone and inhibit osteoclastic bone resorption [19]. Bisphosphonates are clinically used for treatment of osteoporosis and hypercalcemia of malig-

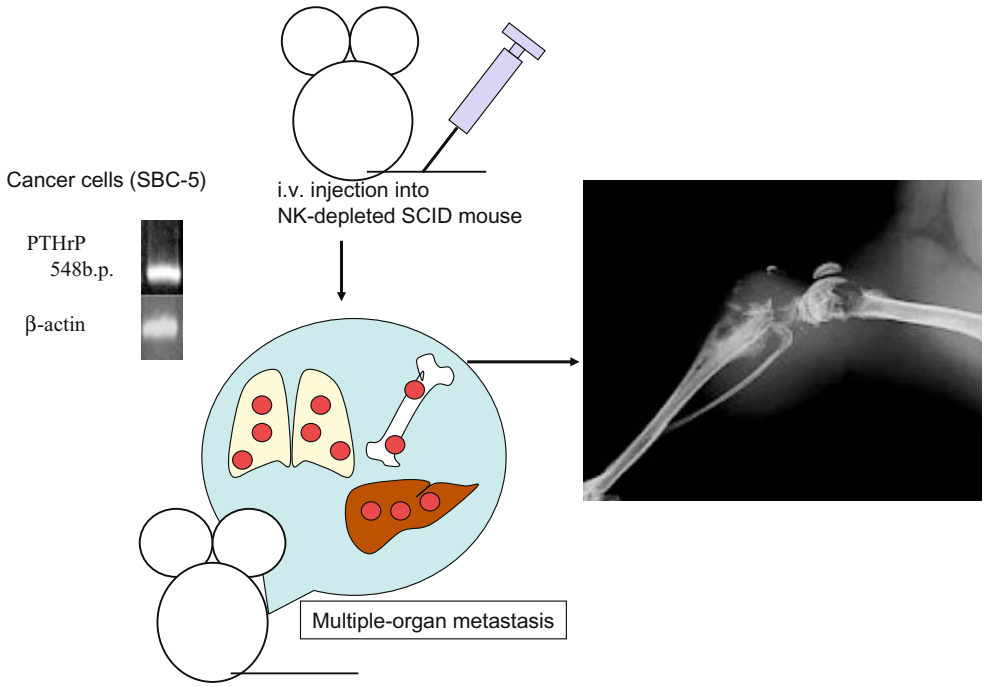

Fig. 1 Bone metastasis model by human lung cancer cell lines

nancy. We reported that a third generation bisphosphonate, minodronate (YM529), had the potential to inhibit bone metastasis of SBC-5 cells [20]. Similar to the results of this study, while YM529 did not have a direct inhibitory effect on SBC-5 cell proliferation, it reduced the number of osteoclasts in the bone lesions and inhibited the production of bone metastasis [20]. Another third generation bisphosphonate, zoledronate, has been approved and widely used in combination with chemotherapy for myeloma and solid tumors with bone metastasis [21].

1.3 Anti-osteoclastic activity of reveromycin A

Reveromycin A (RM-A) is an antibiotics that was discovered in the culture medium of Actinomycetes because of its inhibitory activity on epidermal growth factor (EGF)-dependent responses of mouse epidermal cells [22]. Then, RM-A was shown to have antitumor effect against a human ovarian carcinoma BG-1, which is known to be a transforming growth factor α (TGF-α)-secreting and estrogen receptor-expressing cell line [23]. RM-A targets isoleucyl-tRNA synthetase (IleRS) and inhibits its activity, and hence suppresses the growth and protein synthesis on yeast genetics. In addition, RM-A has a high potential to inhibit bone resorption by inducing osteoclast-apoptosis via suppression of IleRS in osteoclasts [24]. Therefore, RM-A seems to be a unique agent that has activity to both of the tumor cells and host microenvironmental cells (osteoclasts). We demonstrated that RM-A had the activity to suppress the production of bone metastasis by the inhibition of osteoclast activity (Fig. 3) [25], similar to bisphosphonates. The major difference between RM-A and bisphosphonates was the effect on tumor-cell derived PTHrP. RM-A, but not bisphosphonates, at non cytotoxic concentrations suppressed the production of PTHrP by SBC-5 cells at both protein and mRNA levels [25]. Together with the findings

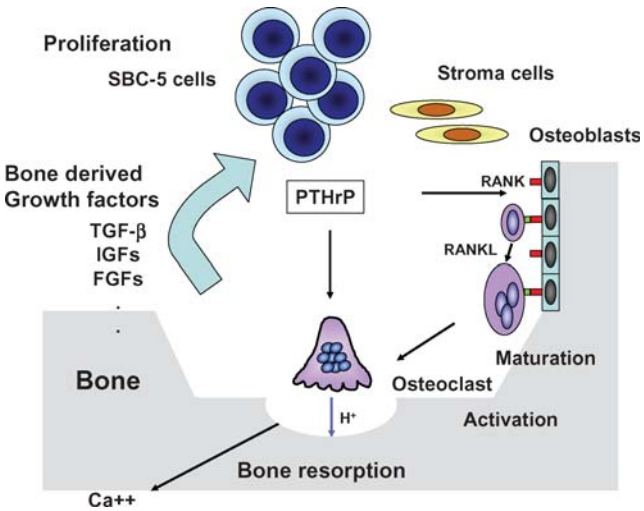

Fig. 2 Mechanism of osteolytic bone metastasis by SBC-5 cells

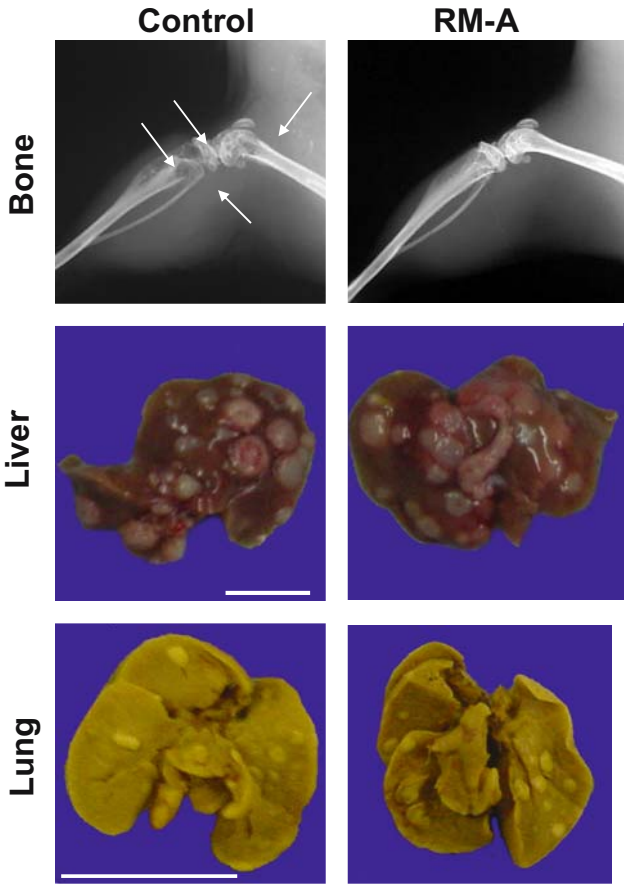

Fig. 3 Anti-metastatic effect of RM-A to the bone

that RM-A strongly inhibited the viability of mouse osteoclasts, RM-A has potential to suppress osteoclast activity by directly inducing apoptosis and indirectly inhibiting tumor-cell derived PTHrP production. Further experiments are warranted to clarify the mechanism by which RM-A inhibits PTHrP production.

1.4 Effect of angiogenic factors in bone metastasis

Angiogenesis is essential for the enlargement of the primary tumor and metastasis of a variety of cancers [26]. VEGF is an important regulator of tumor-angiogenesis, and its expression correlated directly with tumor vascular density and correlated inversely with the survival of patients with various solid tumors [26]. VEGF binds with a high affinity to two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), on endothelial cells. Of these two specific receptors, VEGFR-2 has the predominant role in transmitting VEGF-induced signaling responses and has been suggested as an antiangiogenic target [26]. The blocking of VEGF-VEGFR-2 signaling (by the treatment with a VEGFR-2 tyrosine kinase inhibitor, ZD6474) inhibited the bone metastasis and the visceral metastases

of SBC-5 cells, expressing VEGF, in NK-cell depleted SCID mice [27]. Therefore, VEGF is suggested to be one of therapeutic targets for bone metastasis, and the combined use of RM-A with the VEGFR-2 inhibitors may further augment therapeutic efficiency toward bone metastasis.

1.5 Future direction toward bone metastasis of lung cancer

Much attention has been paid to therapies targeting host-microenviromental factors (including angiogenesis and osteoclasts), in addition to those directly targeting cancer cells (including radiotherapy, chemotherapy and EGFR inhibitors). Since tumors consist of cancer cells and host stromal cells, dual targeting by these two modalities seems to be ideal. In fact, an anti-VEGF neutralizing antibody, bevacizumab, in combination with chemotherapy has been shown to augment the response rates of chemotherapy and prolong the survival in several solid tumors, such as colorectal cancer, breast cancer, and non-small cell lung cancer [28]. Both RM-A and bisphosphonates, such as YM529, seem to be useful drugs to target host osteoclasts. However, they did not have direct toxicity to human lung cancer cells. Therefore, combined use of additional therapies directly targeting tumor cells, such as chemotherapy and molecular targeted agents, may augment therapeutic efficacy to the bone metastasis. RM-A was originally reported to have activities toward both tumor cells and osteoclasts. However, anti-tumor effect was limited on a human ovarian carcinoma BG-1, which is known to be a transforming growth factor α (TGF-α)-secreting and estrogen receptor-expressing cell line [22]. Therefore, RM-A analogues that have higher cytotoxicity against cancer cells while maintaining their anti-osteoclast activity may be more beneficial for the treatment of bone metastases. Other strategy is intensive targeting of the osteoclasts. The combined use of RM-A with bisphosphonates may be useful for enhancing the therapeutic efficiency against bone metastasis.

2 Conclusion

Bone metastasis is a critical problem of lung cancer patients. The molecular pathogenesis is not fully understood. Reproducible animal models of lung cancer bone metastasis, like NK-cell depleted SCID mouse model with SBC-5 cells, are useful to explore the molecular mechanism and search of molecular targets. Several compounds, including bisphosphonates and RM-A, potentially suppress osteoclast-activity may be beneficial for the treatments of bone metastasis. Multi-modality therapy may be necessary for further augmenting the therapeutic efficacy against lung cancer bone metastasis.

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