PREVENTION OF BONE METASTASIS BY A NEW ANGIOGENESIS INHIBITOR

++Iida, K; *Wakabayashi, H; **Hiraki, Y; ***Mitsui, K; ***Kondo, J; *Morita, K; *Seto, M; *Sonoda, J; *Uchida, A
+++Orthopaedic Surgery, Mie University Faculty of Medicine, Tsu-City, Mie, Japan. 2-174, Edobashi, Tsu-City, Mie, Japan. +81-59-231-5022, Fax: +81-59-231-5211, k-iida@clin.med.mie-u.ac.jp

Relevance to Musculoskeletal Condition
This study presents suppression of bone metastasis by a new angiogenesis inhibitor derived from cartilage.

Introduction
Hyaline cartilage is avascular and resistant to neoplastic invasion, suggesting the presence of antitumor factors. We have already reported that Chondromodulin-I (ChM-I) purified from fetal bovine cartilage, acts as an inhibitory factor for vascular endothelial cell proliferation. Angiogenesis seems to play a key role in the development and growth of bone metastasis. In this study, we investigated the effect of Chondromodulin-I on bone metastasis in an animal model.

Material and Method
An animal model of extraskeletal bone metastasis
It is difficult to evaluate precisely the development of metastasis in many bones of an individual animal for bone metastasis. We have already reported a new animal model that developed metastasis in a bone transplanted subcutaneously (44th annual meeting). In this extraskeletal model, it is easy to evaluate bone metastasis and convenient to administer anti-cancer drug topically.

Effect of rhChM-I on bone metastasis
The femur and tibia were removed from female C57BL/6 mice, and these bones were transplanted in the dorsal subcutis of other female C57BL/6 mice aged 6 weeks. At 28 days after bone transplantation, B16 melanoma cells (1x10⁶) suspended in 0.1ml of PBS were injected into the left heart ventricle of the mice by use of a 29-gauge needle under the anesthesia with pentobarbital (day 0).
(First Experiment)
From day 0, a daily topical administration of rhChM-I (20 and 200µg/0.2ml PBS) and PBS only (control group) was given to 4 mice per group around transplanted bones for 28 days. All mice were sacrificed on day 28, and incidence of bone metastasis was evaluated in the extraskeletal bone (transplanted subcutaneously) macroscopically and microscopically.
(Second Experiment)
From day 0, a daily intraperitoneal administration of rhChM-I (200µg/0.2ml PBS) and PBS only (control group) was given to 4 mice per group for 28 days. All mice were sacrificed on day 28, and incidence of bone metastasis was evaluated in the extraskeletal bone macroscopically and microscopically.

Cell proliferation assay in vitro
On day 0, suspending B16 cells (5.0 x 10³ cells/ml) in RPMI-1640 + 10% FBS were poured 50µl each into the wells of a 96-well culture plate and cultured in an incubator at 37 °C for 24 hour. On day 1, the culture medium was added by 50µl RPMI-1640 containing various concentrations (200µg/ml ~ 200g/ml) of rhChM-I. In the control group, 50µl RPMI-1640 only was added to the culture medium. On day 5, 10µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (MTT 5mg/ml) was added and incubation was performed for 4 hour at 37 °C. Then medium was washed out with PBS. Formazan formed was extracted with 100µl of acid-isopropanol and measured at a wavelength of 540 nm.

Results
Effect of rhChM-I on bone metastasis
(First Experiment)
No significant difference were found between control groups and groups treated with rhChM-I 20µg. In the mice treated with rhChM-I 200µg, bone metastasis developed in only 25% of the extraskeletal bone transplanted subcutaneously, whereas in the control mice, bone metastasis developed in 100% (Table 1).
(Second Experiment)
Metastasis in the extraskeletal bone was found in only 25% of the mice treated with rhChM-I 200µg intraperitoneally. In the control mice, bone metastasis developed in 50% of the extraskeletal bone (Table 2). Thus, rhChM-I topical and intraperitoneal administration inhibited bone metastasis in this animal model at dose of 200µg. Body weight of the mice treated with rhChM-I did not decrease throughout the experimental period when compared to controls. Adverse effects to lung, liver, kidney were not found histologically.

Cell proliferation assay in vitro
rhChM-I demonstrated an inhibitory effect on the growth of B16 melanoma cells at a concentration of 10 ~ 100 µg/ml.

Discussion
Metastasis is the process by which a tumor cell leaves the primary tumor, travels to a distant site via the circulatory system, and establishes secondary tumor. Angiogenesis seems to play a key role in the development of metastasis. Therefore, it is possible for inhibition of angiogenesis to suppress bone metastasis. ChM-I inhibited DNA synthesis and proliferation of vascular endothelial cells as well as tube morphogenesis in vitro. Inhibition of angiogenesis at secondary sites prevents the proliferation of tumor cells, subsequently suppresses metastasis. This study demonstrated inhibitory effect of ChM-I to bone metastasis.

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<th>untreated</th>
<th>rh ChM-I (200µg)</th>
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<tr>
<td>Extraskeletal bone</td>
<td>4/4 bones (100%)</td>
<td>1/4 bones (25%)</td>
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Table 1. Incidence of bone metastasis treated rhChM-I by topical injection (First Experiment)

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<tr>
<th></th>
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<th>rhChM-I (200µg)</th>
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<tr>
<td>Extraskeletal bone</td>
<td>2/4 bones (50%)</td>
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Table 2. Incidence of bone metastasis treated rhChM-I by intraperitoneal injection (Second Experiment)

**Institute for Frontier Medical Sciences, Kyoto University, Kyoto-City, Kyoto,Japan

***Research Center, Mitsubishi Co.,Ltd., Yokohama-City, Kanagawa, Japan.