論　文　内　容　要　旨

miR-125b accumulated in bone matrix suppresses osteolytic bone metastasis

（骨基質由来 miR-125b は溶骨性骨転移を抑制する）

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Background: MicroRNAs (miRNAs) are involved in RNA silencing and post-transcriptional regulation of gene expression. Growing evidence demonstrates that miRNAs play pivotal roles in the skeletal system. Matrix vesicles (MVs) budding from osteoblasts accumulate in the immature bone matrix osteoid and involved in the initiation of bone mineralization. Previously we showed that miRNAs as cargos of matrix vesicles (MVs) accumulated in bone matrix. Of these, miR-125b inhibits osteoclast formation during bone metabolism by downregulating its target *Prdm1*, a transcription repressor. Downstream targets of PRDM1, such as IRF8 and MAFB (anti-osteocastogenesis factors), were subsequently upregulated, resulting in decreased osteoclast formation. Transgenic (Tg) mice overexpressing miR-125b under the control of the human osteocalcin promoter exhibited high bone mass with a decreased number of osteoclasts. Cancer bone metastasis causes osteolytic lesions with an increased number of active osteoclasts. In silico studies showed miR-125b may target multiple genes involved in the survival and function of breast and prostate cancer cells that frequently metastasize to bone.

Hypothesis: Taken all together, it is suggested that miR-125b accumulated in bone matrix may abrogate osteolytic bone metastasis through its inhibitory effect on bone resorption.

Materials and methods: We used the mouse osteoblast MC3T3-E1 cells to obtain MVs. qRT-PCR was performed to determine miR-125b and U6 (internal control) levels. The human prostate cancer PC-3, human breast cancer MCF-7 and murine mammary carcinoma Py8119 cells were subjected to either MTT assay, EdU incorporation, Wound healing migration assay, or Transwell invasion assay to determine the effects of MVs and/or miR-125b mimic on cancer cell activities. To confirm the effects of miR-125b on bone metastasis, Py8119 cells tagged with luciferase were injected into wild-type (WT) and Tg mice via the caudal artery. Osteolytic metastasis was evaluated by in vivo bioluminescence imaging, micro-CT (μCT) and histological analysis. These mice were also compared using Kaplan-Meier survival analysis.

Results and conclusion: MTT revealed a decrease in proliferation of PC-3, MCF-7 and Py8119 cells, when treated with MVs. Treatment of PC-3 cells and MCF-7 cells with MVs decreased either their

migration and invasion. Because levels of miR-125b in cancer cell lines including Py8119 were extremely lower than those in MC3T3-E1 cells and MVs, Py cells were transfected with miR-125b mimic. EdU incorporation and transwell migration assay revealed that the synthetic miR-125b mimicked the inhibitory effect of MVs. In vivo study revealed that bioluminescence signals were detected as early as day 17 post-injection in WT mice but not in Tg mice. The signals at 21 days post-injection were significantly lower in Tg than WT mice. Multiple bone morphometric parameters determined by μCT analysis of the distal end of femurs indicated more severe defects in cortical and trabecular bones in WT mice versus Tg mice. Metastatic lesions of Py8119 cells with the number of TRAP-positive multinuclear cells in Tg mice were less than those in WT mice. Concomitant with these, Tg mice had a higher survival rate than WT mice. Taken all together, these data suggest that miR-125b in bone matrix may suppress bone metastasis via its dual actions in inhibiting osteoclastogenesis and blocking cancer cell activities. These findings may provide a novel therapeutic target for osteolytic bone metastasis. We conducted database searches and attempted to do PCR array to identify possible target genes of miR-125b in cancer cells. Further investigation would be necessary to ascertain target gene of miR-125b in cancer cells.