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論 文 審 査 の 結 果 の 要 旨

博士の専攻分野の名称	博士（　歯学　）	氏名	Nushrat Sarmin
学 位 授 与 の 条 件	学位規則第 4 条第 ①・2 項該当		

論　文　題　目

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）

論文審査担当者

主　　査　　教授　藤井　万紀子　　印

審査委員　　教授　宮内　睦美

審査委員　　教授　寺山　隆司

〔論文審査の結果の要旨〕

MicroRNAs (miRNAs) are small non-coding RNA molecules that act on RNA silencing in post-transcriptional regulation of gene expression. Some of miRNAs are enclosed in micro vesicles that are secreted into extracellular fluids, and thereby play a role in micro vesicle-based cell-cell communication in a wide variety of biological processes. Recently, the research group including this applicant (Minamizaki et al, 2020) found that matrix vesicles (MVs), in a broad sense EVs, budding from osteoblasts contain a number of miRNAs, besides playing a well-known role in bone mineralization. These miRNAs accumulate in bone matrix, of which miR-125b is released into the bone marrow microenvironment during bone resorption and inhibits osteoclast formation by downregulating Prdm1, a transcription repressor of osteoclastogenesis, in osteoclast precursors. Transgenic (Tg) mice overexpressing miR-125b under the control of the human osteocalcin promoter exhibits high bone mass with a decreased number of osteoclasts without affecting osteoblasts and bone formation.

Cancer bone metastasis frequently causes osteolytic lesions with increased activity of osteoclasts. In silico studies showed that miR-125b might target multiple genes involved in the survival and function of breast and prostate cancer cells that prone to metastasize to bone. Taken together with the results that miR-125b was selectively enclosed in MVs, and the levels of miR-125b in MVs are several folds higher than those in cancer cell lined tested (the human prostate cancer PC-3 and breast cancer MCF-7 and the murine mammary

carcinoma Py8119). Concomitant with these, not only MVs isolated from mouse osteoblast cultures but also miR-125b mimic suppressed cancer cell proliferation, migration and/or invasion in vitro. Py8119 cells tagged with luciferase (Py-Luc cells) were injected into wild-type (WT) and Tg mice via the caudal artery. Luc activity in femurs was detected as early as 17 days post-injection in WT mice but not in Tg mice. These signals at day 21 post-injection were significantly lower in Tg than WT mice, in proportion to Py8119 cell mass in WT vs. Tg hind limb bones. 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. Candidate genes were enlisted through database searches 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.

In conclusion, this study showed that miR-125b in bone matrix suppressed bone metastasis possibly via its dual actions in inhibiting osteoclastogenesis and blocking cancer cell activities. These findings may provide a novel therapeutic target for osteolytic bone metastasis. Therefore, all the committee members admitted that this dissertation is of sufficient value to confer the Doctor of Philosophy in Dental Science to NUSHRAT SARMIN.