**P1:108**

# Possible roles of osteosarcoma-derived exosomes in promoting pre-metastatic niche in the lung

*Tomohiro Fujiwara[1], Takeshi Katsuda[1], Nobuyoshi Kosaka[1], Akira Kawai[1], Toshifumi Ozaki[2], Takahiro Ochiya[1]*

*[1)] National Cancer Center [2)] Okayama University, Japan*

Background
Recent studies have shown the involvement of tumor-derived exosomes in tumor progression. Exosomes are nanometer-sized vesicles secreted by diverse cell types that play complex roles in intercellular communication. They comprise a ceramide- and cholesterol-rich lipid bilayer membrane, and contain both mRNA and microRNA, which can be delivered to another cells. Futhermore, neutral sphingomyelinase 2 (nSMase2), regulating the biogenesis of ceramide, were found to trigger secretion of exosomes. In this study, we investigated the role of OS-derived exosomes in lung metastasis through the regulation of ceramide signaling pathway.
Methods
We used a highly metastatic human OS cell line 143B cells expressing firefly luciferase (143B F-luc) and established a derivative cell line with shRNA knockdown of neutral sphyngomyelinase 2 (143B-F luc-KD-nSMase2). Exosomes derived from 143B F-luc were isolated by ultracentrifigation. Original 143B F-luc cells were orthotopically transplanted to the right tibia of nude mice at 1.5 × 106 cells/mouse (group 1). Mice similarly transplanted with 143B-F-luc-KD-nSMase2 cells were divided into 2 groups, and were intravenously administered 200 μL PBS (group 2) or 5 μg-exosomes/200 μL PBS (group 3) twice a week for 3 weeks. Lung metastases were monitored by IVIS system.
Results
Lung metastases were observed in 7/10 mice in group 1 at 3 weeks after orthotopic transplantation. In contrast, only 3/10 mice showed lung metastases in group 2, indicating the decreased metastases following the inhibition of exosome secretion. Remarkably, however, 7/10 mice showed metastases in group 3, indicating that systemically administered exosomes restored the metastatic ability. Histopathological examination of the lungs confirmed that the numbers of metastatic foci were dramatically reduced in group 2 compared with group 1, whereas that in group 3 was comparable to group 1.
Conclusion
We demonstrated that exosomes secreted by highly metastatic osteosarcoma cell line into the circulation promoted lung metastasis in vivo. Given the accessibility of exosomes to distant organs, we hypothesize roles of exosomes in pre-metastatic niche formation in the lung, and are now exploring the underlying molecular mechanisms.

*E-mail (main author): tofujiwa@ncc.go.jp*