

Spontaneous lung metastatic osteosarcoma cells gain malignancy and both epithelial and mesenchymal markers

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Introduction: Osteosarcoma is recalcitrant, and metastasis, mostly to the lung, is a cause of poor prognosis. The 5-year survival rate is 30% for osteosarcoma patients who have metastases, while the 5-year survival rate of about 60% for osteosarcoma patients overall. Epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) are thought to be related to the metastasis process of osteosarcoma, as well as carcinomas, however, the mechanism of the EMT or MET of osteosarcoma is incompletely understood. The present study establishes a spontaneous lung-metastatic osteosarcoma mouse model, which metastasize from the tibia to the lung. With this model, the mechanism of EMT or MET of osteosarcoma was investigated, comparing characteristics of spontaneous lung metastatic osteosarcoma cells and their parental osteosarcoma cells.

Methods: Spontaneous lung metastatic 143B osteosarcoma cells (143B-SLM) were established from parental 143B osteosarcoma cells (143B-P) by as follows: 143B osteosarcoma cells with green and red fluorescent protein (GFP and RFP) were implanted into the tibia of nude mice. Four weeks after implantation, the mice were sacrificed, and their lung were harvested. Metastatic tumor tissues were imaged with GFP and RFP with a fluorescence microscope, followed by culture of the metastatic cells on plastic in normal cell culture medium. The metastatic potential of 143B-P and 143B-SLM cells was compared by testing cell invasion and migration capability in cell culture in vitro and lung metastases formation after orthotopic cell-implantation into the tibia of nude mice in vivo. All mouse studies were approved with The Institutional Animal Care and Use Committee (IACUC) protocol, based on the National Institutes of Health (NIH) Guide for the Care and Use of Animals. Epithelial-mesenchymal phenotypic expression was compared by Western immunoblotting. The Welch's t-test was performed to statistically evaluate results. Bar graphs show the mean and error bars show standard deviation of the mean. A probability value ≤ 0.05 was defined as statistically significant difference.

Results: Three different sublines of the spontaneous lung metastatic 143B osteosarcoma cells (143B-SLM-1, 143B-SLM-2, and 143B-SLM-3) were established from 3 different mice. The spontaneous lung metastatic 143B-SLM-1, 143B-SLM-2, and 143B-SLM-3 cells were more closely attached to each other than their parental 143B-P cells (Figure. 1). 143B-SLM-1, 143B-SLM-2, and 143B-SLM-3 cells showed a tendency of increased cell invasion capacity in the transwell assay in vitro ($P = 0.15, 0.1, 0.11$, respectively), and 143B-SLM-1, 143B-SLM-2, and 143B-SLM-3 cells have significantly increased migration capability in the wound healing assay in vitro ($P < 0.001$, for all), compared to 143B-P cells (Figure. 2). 143B-SLM-1 had 3 mice with lung metastases out of 7 mice which had orthotopic tumor growth in the tibia; 143B-SLM-2 had 5 metastases out of 7 mice; 143B-SLM-3 had 6 metastases out of 8 mice; in contrast, parental 143B-P had 2 metastases out of 7 mice, 4 weeks after orthotopic cell-implantation into the tibia. These results demonstrate the spontaneous lung metastatic osteosarcoma cells are more metastatic than parental cells. The spontaneous lung metastatic osteosarcoma cells showed gain of an epithelial marker, E-cadherin, as well as mesenchymal markers N-cadherin, vimentin, Snail, along with their closer cell attachment and gain of metastatic potential (Figure. 3).

Discussion: In the present study, the spontaneous lung metastatic osteosarcoma cells showed gain of both epithelial and mesenchymal markers, along with their closer cell attachment and increased metastatic potential, which can indicate that both epithelial and mesenchymal properties are required for distant metastasis in osteosarcoma, resulting in cells more attached with each other, with gain of invasion and migration capacity.

Significance: In the present study, we compared spontaneous lung metastatic osteosarcoma cells, which metastasized from the tibia to the lung of the nude mice, and their parental cells, and showed molecular differences that could be, at least in part, a new insight to the metastasis mechanism of osteosarcoma.

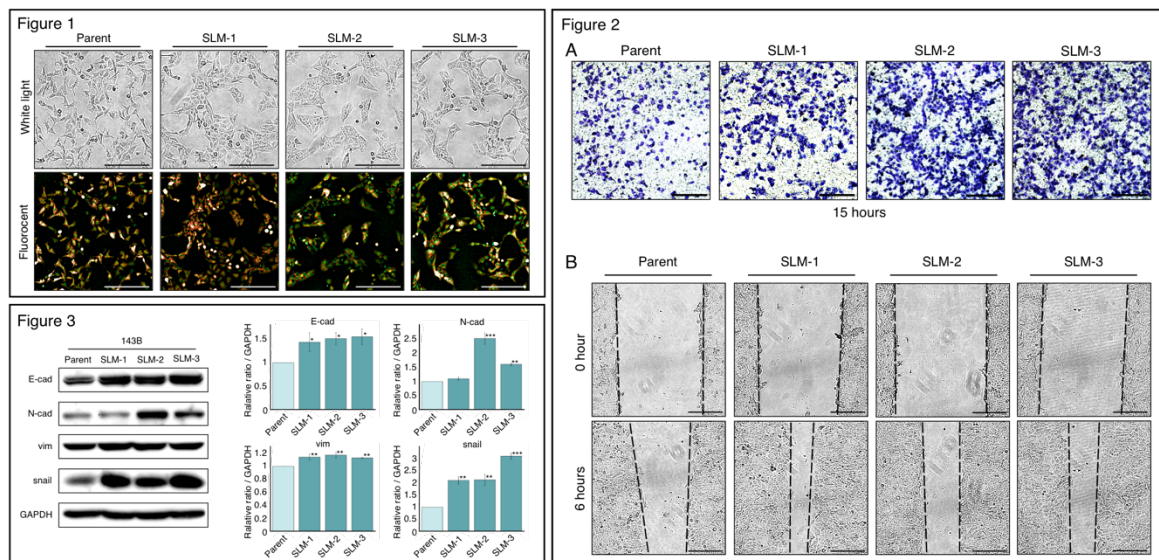


Figure 1. Cell appearance of spontaneous lung metastatic 143B-SLM osteosarcoma cells and their parental 143B osteosarcoma cells with white light and fluorescence. Parent: parental 143B osteosarcoma cells, 143B-SLM-1, 143B-SLM-2, and 143B-SLM-3: spontaneous lung metastatic 143B osteosarcoma cells.

Figure 2. Spontaneous lung metastatic 143B-SLM osteosarcoma cells have increased cell invasion and migration capacity. (A) Cell invasion capacity of 143B-P and 143B-SLM cells, with the transwell assay. Scale bar in photomicrographs: 250 μ m. Magnification: 100 \times . Parent: parental 143B osteosarcoma cells, 143B-SLM: spontaneous lung metastatic 143B osteosarcoma cells. (B) Cell-migration capacity of 143B-P and 143B-SLM cells, in the wound-healing assay. Scale bar in photomicrographs: 250 μ m. Magnification: 100 \times . Parent: parental 143B osteosarcoma cells, 143B-SLM-1, 143B-SLM-2, and 143B-SLM-3: spontaneous lung metastatic 143B osteosarcoma cells.

Figure 3. The expression of epithelial (E-cadherin) and mesenchymal markers (N-cadherin, vimentin, snail) in 143B-P and 143B-SLM cells, with Western immunoblotting. Parent: parental 143B osteosarcoma cells, 143B-SLM-1, 143B-SLM-2, and 143B-SLM-3: spontaneous lung metastatic 143B osteosarcoma cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.