RAT MODEL OF CHONDROSARCOMA: LUNG COLONIZATION-ASSOCIATED DIFFERENCES

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INTRODUCTION:
Despite advances in the management of chondrosarcoma, pulmonary metastasis remains the most significant cause of death in these patients. To improve survival rates in patients a better understanding of its biology is necessary. To characterize the molecular basis of tumor lung colonization, two rat Swarm chondrosarcoma (SRC) tumor lines showing difference in growth properties was utilized. The TGO tumor line, is more close in growth properties to the original spontaneous tumor that developed in a female Sprague Dawley rat in the 1960’s (1), than the JWS tumor line that grows much faster. With a subcutaneous injection of a tissue tumor sample (~1x10^6 cells), in 35 days, a 11 gram tumor will develop for the TGO and a 35 gram tumor for the JWS tissue line. The hypothesis that the JWS, faster growing SRC tumor, would colonize to the lung more efficiently than the TGO tumor when injected in to the tibia medullary cavity, an orthotopic site, was tested in this study.

METHODS:
SRC tissue-lines: Two tissue-lines were used in this study that have been maintained by serial subcutaneous passages by Dr. Oegema (Rush University) of the TGO line since 1975 and the JWS line, originating from Drs. Kimura and Hascall (NIDR, NIH), passed by Dr. Stevens since 1981.

Tumor induction and tumor analyses: An animal care protocol was approved from the University of Iowa for these studies. Tumor induction was performed by injection of Sprague Dawley, male rats, three weeks old (TGO, n=19; JWS, n=20) orthotopically in the tibia medullary cavity of the right leg with subcutaneously grown tumor. The animals were euthanized at 35 days and tumor lung colonization was determined for each animal, tissue samples of tumors from the lung and tibia were then taken for histological (safranin O/fast green staining) and ultrastructural (transmission electron microscopy) analyses. Additionally, tissue samples were taken for cell isolation (2) and used for determining degree of invasive capabilities utilizing an invasion assays developed in Dr. Hendrix’s laboratory (penetrating membrane composed of laminin and collagen type IV) in a gelatin base in a 24h culture. Additionally, at time of injection of the tibia with tumor, subcutaneous tumors were initiated for each SRC line (TGO and JWS) and were analyzed following 35 days post injections, as performed for the tibia and lung tumors.

RESULTS:
Tibia transplantation of the JWS line resulted in pulmonary tumors in 52% (11/20) of the animals, and while for the TGO line only 11% (2/19) of the animals developed tumors in their lungs. Histological analysis of the tibia showed extensive expansion of the tumors in the medullary cavity with more aggressive penetration of the JWS-line into the bone than the TGO line. Ultrastructurally cells of the tibia appeared less health with fragmentation of endoplasmic reticulum and less mitochondria and Golgi than seen of tumor growing subcutaneously and in the lung. Of the lungs, no histological and ultrastructural difference was apparent between the two tumor tissue lines. However, with comparison of the lung tumors to tumors grown subcutaneously, lung tumors were encapsulated with a thinner layer of connective tissues than tumors grown subcutaneously, and the lung tumors appeared less vascularization than those grown subcutaneously. In all three tissue sources, the JWS line demonstrated a more aggressive invasion capability than that of the TGO line. See Table I for values and comparison between tumor lines and tissue source for cell invasion assay.

| Table I. Invasion assay |
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| **Tissue** | **JWS** | **TGO** |
| SQ | 12.0±0.7% | 3.0±0.1% |
| Tibia | 12.0±4.0% | 5.5±0.5% |
| Lung | 9.00% | 2.0±0.4% |

DISCUSSION:
This study demonstrates that two rat chondrosarcoma tumor lines, of which the JWS line drifts with phenotype growing faster than the original tumor, and that of the TGO line, have different lung colonization properties. Characterizing the molecular base for this difference would lead to a better understanding of chondrosarcoma lung metastasis. For example degree of metastasis is often correlated with ability of cells to penetrate through basement membrane in order move through tissues. By examining for difference in expression levels of metalloproteinase between these two tumor lines used in this study would give an incite to the mechanism whereby an increase in lung colonization is occurring. The rat model of chondrosarcoma lung colonization permits easily manipulative techniques to be explored in deciphering the complexity of chondrosarcoma metastasis.

REFERENCES: