Identifying Mediators of Breast Cancer Bone Metastasis Using A Novel Mouse Model

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INTRODUCTION: Bone metastasis occurs in approximately 80% of advanced breast cancer patients and despite therapeutic advances, treatment remains palliative. Despite increasing research efforts in the area of metastasis, the specific characteristics of breast cancer cells responsible for the preferential homing to and colonization of the bone are largely unknown. Our ability to study metastasis has been hindered due to our reliance on models poorly representative of clinical disease, consisting of xenografted human cancer cells in immunocompromised mice. Here, we describe the establishment of a novel immunocompetent syngeneic murine model of breast cancer metastasis that closely mimics the anatomical distribution of metastases in breast cancer patients, and its use in identifying mediators of bone metastasis.

METHODS: Intracardiac injections-Experimental protocols were approved and performed in accordance with the Johns Hopkins University Animal Care and Use Committee. Female nué-N mice were anesthetized and 10^5 cells were injected into the left ventricle. Radiographic images were obtained using a Faxitron MX-20 X-ray unit to assess bone destruction. Immunohistochemistry-Paraffin embedded sections were deparaffinized, hydrated, and antigen retrieval was performed by steaming for 20 minutes in 0.01M sodium citrate, pH 6.0. Sections were treated with 0.3% hydrogen peroxide and blocked in normal serum. Sections were then incubated with anti-Cited2 antibody for 48 h at 4°C. Proteins were visualized using 3, 3'-diaminobenzidine (DAB) and sections were counterstained in hematoxylin. qRT-PCR: Total RNA was extracted and cDNA was generated using a commercially available kit (Promega). cDNA was amplified using Sybr green on a Bio-Rad iQ5 real-time PCR detection system, and relative expression between samples was calculated by the comparative Ct method. Stable transfection-Full-length CITED2 cDNA and shRNA against Cited2 were cloned into the viral vectors pBABE and pLKO.1, respectively. Virus was generated, cells were infected, and stable clones were generated under puromycin antibiotic selection. Western blotting- Equal amounts of protein from cell lysates were resolved using 12% SDS-PAGE and protein was transferred to ECL nitrocellulose membranes, which were probed with anti-Cited2 antibody. Following incubation with horseradish peroxidase-conjugated antibody, binding was revealed using enhanced chemiluminescence.

RESULTS: Intracardiac injection of the mammary cancer cell line NT2.5, derived from a spontaneous mammary tumor occurring in the HER2/neu transgenic mouse, resulted in the development of metastases in the bone, liver, and lung, as evidenced by histological analysis. Bone metastases occurred in both the appendicular and axial skeleton, and similar to the human condition, were predominantly osteolytic as determined by digital radiography. To identify potential mediators of bone metastasis, metastatic cells were isolated from the bone, liver, and lung, using standard tissue culture techniques, and established as first generation cell lines NT2.5BO1, NT2.5LI1, and NT2.5LU1, respectively. This process was repeated several times resulting in the development of cell lines having a metastatic preference for the bone or liver, as determined by necropsy, histological analysis, and digital radiography (Figure 1). Next, we performed Affymetrix Genechip analysis to compare the gene expression profiles among early and late generation bone metastasis cell lines (NT2.5BO3 and NT2.5BO6), liver metastasis cell lines (NT2.5LI1 and NT2.5LI3), and parental NT2.5 cells. We found expression of several genes reportedly associated with bone metastasis (CTGF, ADAMTS1) to be elevated in bone metastatic cell lines relative to liver metastatic cell lines.

DISCUSSION: These data suggest that Cited2 may play a role in breast cancer bone metastasis as a master regulator of osteoclast-activating factors and signaling pathways promoting bone metastasis. Further functional characterization of Cited2 as well as other candidate genes may provide novel prognostic markers and therapeutic targets for breast cancer bone metastasis. In addition, this syngeneic, immunocompetent mouse model of breast cancer metastasis may provide a powerful preclinical tool for biological and pre-clinical prophylactic and therapeutic studies.

Figure 1: (A) Representative radiographs and (B) histomorphometry showing larger osteolytic lesions were observed in metastases from late generation bone metastatic cell line BO6 compared to liver metastatic cell line LI3 and parental NT2.5 cells. (C, D) Mice injected with late generation LI3 liver metastatic cells developed more metastatic foci on necropsy compared to those injected with BO6 bone metastatic cells.

Figure 2: Expression of Cited2 mRNA in human normal mammary epithelium (N. Mam. Org), primary breast tumor tissue (IDC), and breast cancer bone metastases (BBM).