

# Identification of prospective factors promoting osteotropism in breast cancer: a potential role for CITED2

Wen Min Lau<sup>1</sup>, Kristy L. Weber<sup>1</sup>, Michele Doucet<sup>1</sup>, Yu-Ting Chou<sup>1</sup>, Kelly Brady<sup>1</sup>, Jeanne Kowalski<sup>2</sup>, Hua-Ling Tsai<sup>2</sup>, Justin Yang<sup>1</sup> and Scott L. Kominsky<sup>1</sup>

<sup>1</sup> Department of Orthopaedic Surgery, Johns Hopkins University School of Medicine, Baltimore, MD

<sup>2</sup> Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Breast cancer metastases develop in the bone more frequently than any other site and are a common cause of morbidity in the form of bone pain, pathological fractures, nerve compression and life-threatening hypercalcemia. Despite ongoing research efforts, the molecular and cellular mechanisms that regulate breast cancer cell homing to and colonization of the bone as well as resultant pathological bone alteration remain poorly understood. To identify key mediators promoting breast cancer metastasis to bone, we utilized an immunocompetent, syngeneic murine model of breast cancer metastasis employing the mammary tumor cell line NT2.5. Following intracardiac injection of NT2.5 cells in neu-N mice, metastases developed in the bone, liver and lung, closely mimicking the anatomical distribution of metastases in patients with breast cancer. Using an *in vivo* selection process, we established NT2.5 sublines demonstrating an enhanced ability to colonize the bone and liver. Genome-wide cDNA microarray analysis comparing gene expression between parental NT2.5 cells and established sublines revealed both known and novel mediators of bone metastasis and osteolysis, including the transcriptional co-activator CITED2. In further studies, we found that expression of CITED2 was elevated in human primary breast tumors and bone metastasis compared to normal mammary epithelium and was highest in breast cancer cell lines that cause osteolytic bone metastasis in animal models. In addition, reducing CITED2 expression in NT2.5 cells inhibited the establishment of bone metastasis and osteolysis *in vivo*, suggesting a potential role for CITED2 in promoting breast cancer bone metastasis.

Breast cancer metastasis develops in the bone more frequently than any other site, occurring in approximately 80% of patients with advanced breast cancer. Bone metastases cause considerable morbidity in the form of bone pain, pathological fractures, nerve compression and life-threatening hypercalcemia. When medical and/or radiation oncologic treatments fail, surgical procedures can be performed to provide stabilization of affected bone; however, disease progression is unaltered. These facts clearly underline the immediate clinical need for the development of new preventative and therapeutic strategies for the management of bone metastasis. Critical to this challenge is the understanding of the biological interactions between cancer cells and the bone microenvironment. Despite ongoing research efforts, the molecular and cellular mechanisms that regulate breast cancer cell homing to and colonization of the bone as well as resultant pathological bone alteration remain poorly understood. Identification of factors regulating these events would be ideal targets

for preventative and therapeutic interventions against this devastating disease.

Our ability to study metastasis is limited by the models currently available, the majority of which are the product of xenografting human cancer cells into immunocompromised mice. Importantly, evidence suggests that organotropism is species-specific, thus xenograft models may not provide the most accurate method of studying the complex interactions between tumor cells and the host, which are critical to the metastatic process. Therefore in an effort to identify key mediators promoting breast cancer metastasis to bone, we utilized an immunocompetent, syngeneic murine model of breast cancer metastasis employing the mammary tumor cell line NT2.5. NT2.5 cells are derived from a spontaneous breast tumor that developed in transgenic mice (neu-N) engineered to express the nontransforming rat *neu* cDNA under the control of a mammary-specific promoter.<sup>1</sup> The human homologue, HER-2/*neu*, is overexpressed in approximately 30% of breast cancers,<sup>2</sup> which are characteristically more aggressive and associated with shorter patient survival time.<sup>3,4</sup> Following intracardiac injection of NT2.5 cells in neu-N mice, metastases develop in the bone, liver and lung, closely mimicking the anatomical distribution of metastases in patients with breast cancer. Although the intracardiac injection used in this model does not allow study of the early stages of metastasis, it does allow the study of the latter steps in the metastatic cascade (*i.e.* homing and colonization).

**Key words:** bone metastasis, CITED2, breast cancer

**DOI:** 10.1002/ijc.24780

**History:** Received 30 Apr 2009; Accepted 16 Jul 2009; Online 29 Aug 2009

**Correspondence to:** Scott L. Kominsky, Department of Orthopaedic Surgery, 720 Rutland Ave., Ross Building, Room 209, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA, Fax: +410-502-6414, E-mail: kominsc@jhmi.edu