Metastasis, the spread and growth of cancer cells from primary tumor to distant organs, is the most dreadful and deadly development during cancer progression. A notable feature of this process is the variation in metastatic tissue tropism displayed by different types of cancer. In the case of breast cancer, as well as prostate, lung and skin cancers, most patients with advanced disease develop bone metastases, which are a common cause of morbidity and sometimes mortality.

The traditional models for cancer metastasis suggest that during tumor progression, cancer cells acquire multiple alterations that render them increasingly competent to establish metastatic lesions in specific organs. We investigated the genetic tools utilized by breast cancer cells to achieve tissue-specific metastasis to bone by using the MDA-MB-231 human breast cancer cell line as a model system. These cells form typical osteolytic bone metastases when inoculated into the arterial circulation of athymic mice. Their bone metastatic activity is enhanced by TGF-beta, a ubiquitous cytokine that inhibits growth of normal epithelia and early stage tumors but stimulates invasion and metastasis of aggressive tumors. By in vivo selection of MDA-MB-231 cells, we have isolated subpopulations with enhanced metastatic abilities to bone. By transcriptomic profiling of these isolates, we identified a gene set whose expression pattern is associated with, and promotes the formation of metastasis to bone but not other tissues. Cells with the bone metastasis gene profile are present in the parental population and become selected in vivo as highly metastatic entities. By over-expressed, these genes promote osteolytic bone metastasis by acting co-operatively. Two of these genes, interleukin-11 and CTGF, encode osteolytic and angiogenic factors whose expression is further increased by the bone-derived prometastatic cytokine TGF-beta. Inhibition of TGF-beta signaling by stable siRNA-mediated repression of Smad4 blocked the positive feedback of bone matrix to tumor cells and inhibited formation of bone metastasis.

Metastatic recurrences frequently happen in cancer patients many years or even decades after removal of the primary tumor. Tumor cells can be disseminated from the primary tumor early during cancer progression, and remain as dormant solitary cells or micrometastases in distant sites until certain genetic or epigenetic events convert them into overt, fast-growing macrometastasis. Despite the clinical importance of tumor dormancy, however, very little is known about the molecular mechanisms underlying aggressive conversion of tumor cells from their dormant state.

In our model system, only a very small percentage of individual cells in the parental MDA-MB-231 population possess the bone metastasis gene profile that we identified. These cells generated large osteolytic bone lesions in mice within 5 weeks of inoculation. Most cells in the parental population do not express any of the bone metastasis genes and are poorly metastatic to bone. Micrometastases generated by these cells failed to grow exponentially and eventually disappeared, as shown by highly sensitive bioluminescence imaging. In rare cases, after six months of incubation, a small percentage of mice develop large osteolytic bone metastases. Tumor cells isolated from these lesions efficiently generated bone metastasis when they were re-inoculated into nude mice. We subjected these spontaneous metastatic variants to DNA microarray and array CGH (comparative genomic hybridization) analyses and found a very high degree of similarity between these cells and their weakly metastatic progenitors, suggesting that a limited number of genetic and epigenetic changes are required for the aggressive conversion from the dormant state. Surprisingly, we did not find the expression of our previously identified bone metastasis genes, such as CXCR4 or IL-11, in the highly metastatic variants converted from dormant cells. We are currently investigating the different genetic tools that were utilized by these cells for bone metastasis. Our data illustrated the

**Keywords**: Breast Cancer, Bone Metastasis, Microarray Profiling
plasticity of late stage breast cancer cells and redundant routes for metastatic progression.

References