MOLECULAR REGULATORS OF BONE SIALOPROTEIN EXPRESSION IN HUMAN METASTATIC BREAST CANCER CELLS

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Introduction

Death from cancer is usually due to the development of metastases, and the skeleton is the organ most frequently involved. Clinically, skeletal metastases are a frequently encountered event. The patient develops pain, pathologic fractures, hypercalcemia, and spinal cord compression and experiences an inexorable decline in mobility and quality of life. Recent reports have demonstrated a clinical correlation between the expression of the bone related extracellular matrix molecule, bone sialoprotein (BSP) in primary breast cancer lesions and the preferential metastasis to bone (1-3). BSP expression was found to be a poor prognostic sign when present in the primary lesion and there was a significantly increased incidence of subsequent bone metastases in patients who expressed higher levels of BSP. Additionally, it has been demonstrated that bone metastases from breast and prostate cancers express elevated levels of BSP compared to visceral metastases (4). Given the correlation between BSP expression in these malignancies and the subsequent metastasis to bone, we hypothesize that the molecular regulators of ectopic BSP expression in these cancers play important roles in the preferential metastasis to bone. In order to begin to test this hypothesis, we have used a combination of electromobility shift assays and promoter-reporter expression studies to identify the molecular regulators of BSP expression in metastatic breast cancer cells.

Methods

The human Bsp promoter constructs were cloned into PGL3 expression vector linked to the luciferase reporter (Promega). Cell transfections were performed using the Lipofectamine reagent (Gibco/Brl). In the case of stably transfected cells, the Bsp promoter construct was co-transfected with a plasmid which included neomycin resistance. Luciferase activity was determined using the Promega dual luciferase assay kit. All transient transfections were normalized to co-transfected thymidine kinase linked renilla luciferase levels. Experiments using stably transfected cells were normalized to total protein levels. All breast cell lines were obtained from the ATCC and cultured according to their specifications. The control normal human mammary epithelial cell line (HMEC) was obtained from Clonetics. Human U2OS were obtained from the ATCC and used as an additional control. Electromobility shift assays (EMSA) were carried out as previously described and supershift analyses used a Cbfa1 specific polyclonal antibody obtained from Oncogene Research (Boston, MA).

Results

Transfection studies using a series of human BSP promoter-reporter deletion constructs have identified an approximately 300 bp region which contains the cis-acting elements responsible for the positive regulation of BSP expression in metastatic breast cancer cells. Comparison of consensus binding sites in this region with known regulators of BSP expression identified multiple Cbfa1 consensus binding sites as well as two consensus homeodomain protein binding sites, an Engrailed and an Hox site. Site directed mutagenesis of the engraved binding site results in an increase in BSP promoter activity in all breast cancer cells, however, site directed mutagenesis of the Hox site preferentially enhanced expression of the Bsp promoter in metastatic cancers which preferentially metastasize to bone. EMSA analyses demonstrate that metastatic human breast cancer cells contain a novel sized Cbfa1 containing transcriptional complex. In addition, RT-PCR analysis of Cbfa1 expression identified multiple isoforms of Cbfa1 expressed in human breast cancers. Functional studies including Cbfa1 over-expression and functional inhibition through the use of competitor oligonucleotides demonstrate a functional role for Cbfa1 as a positive regulator of Bsp expression in human metastatic breast cancer cells. Cbfa1 over-expression enhanced Bsp expression in human breast cancer cells while functional perturbations of Cbfa1 transcriptional activity through the use of competitor oligonucleotides corresponding to the consensus Cbfa1 binding site demonstrate that inhibition of Cbfa1 DNA binding leads to a reduction in Bsp expression.

Figure 1. Graphic representation of the relative expression levels of the human bone sialoprotein promoter constructs. Bsp-1244, full length promoter; Bsp-435, -350, -110, -43 deletions of the promoter relative to the transcriptional start site, Enl, mutation of the engraved binding site, Hox, mutation of the Hox binding site. All values are normalized to a thymidine kinase linked renilla luciferase reporter vector.

Discussion

We have hypothesized that mimicry of an osteoblastic phenotype by metastatic breast cancers plays an important role in the preferential establishment of metastases in the skeleton. Breast cancer metastases to bone have been reported to express a variety of osteoblast related genes including BSP, however, only the expression of BSP has been clinically correlated with the preferential metastasis of cancers to bone. We have postulated that the molecular regulators of ectopic BSP expression in human breast cancers are likely to be important regulators of the process directing the preferential metastasis of cancer to the skeleton. As a first step in testing this hypothesis we have identified the molecular regulators of BSP expression in metastatic human breast cancers by comparing the expression and regulation of BSP between metastatic and non-metastatic cancers as well as between metastatic cancers with preference for bone vs. those which form visceral metastases. These studies have lead to the identification of two homeodomain factors, one of which appears to play a preferential role in the regulation of BSP in cancers with preferential metastasis to bone. In addition, we have been able to demonstrate that metastatic breast cancer cells ectopically express multiple isoforms of Cbfa1, a key transcriptional regulator of many osteoblast related genes including BSP, and that Cbfa1 acts to upregulate BSP expression in human breast cancer cells.

References


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