Inhibition of Osteosarcoma metastasis by Insulin-like Growth Factor Binding Protein 5 (IGFBP5)
+1Luther, G; 1Rames R; ... Growth Factor Binding Protein 5 Suppresses Tumor Growth and Metastasis of Human Osteosarcoma. Oncogene 2011.

INTRODUCTION

Osteosarcoma (OS) is the most common primary malignancy of bone, with a peak incidence in the second decade of life. Although approximately 80% of patients have either macro- or micro- metastatic disease at the time of diagnosis, only 10-15% of these lesions can be identified with current imaging modalities. With current treatment regimens, which include wide local resection and chemotherapy, the average five year disease-free survival is only 50-65%. Despite significant advances in surgical techniques and chemotherapeutic regimens over the past few decades, there has been minimal improvement in patient survival. Therefore, there is a critical need to identify molecular markers that provide insight into both the pathogenesis and metastasis of OS.

Previously, we have shown that Insulin Growth Factor Binding Protein-5 (IGFBP5) is a potent inhibitor of OS tumorigenesis. Overexpression of IGFBP5 inhibits cell proliferation, migration, and invasion in vitro, while inducing apoptosis. Conversely, siRNA knockdown of IGFBP5 results promoted OS tumor growth and metastasis in vivo. In this study, we examined IGFBP5 expression levels in primary OS and metastatic pulmonary OS clinical specimens. We hypothesized lower expression levels of IGFBP5 in the metastatic lesions compared to the primary tumors. IGFBP5 also contains three unique domains, and its C-terminal domain closely interacts with extracellular matrix components. Given this unique function, we hypothesized that the C-terminal domain of IGFBP5 is likely responsible for the inhibition of OS metastasis.

METHODS

We searched our pathology database for patient matched primary and metastatic pulmonary OS lesions. Tissue arrays containing primary and metastatic OS specimens were generated by The University of Chicago Pathology Core Facility. The samples were subjected to immunohistochemical (IHC) staining with an anti-IGFBP5 antibody. Staining intensity was classified as 0 (no staining), 1+ (weak), 2+ (moderate), or 3+ (strong) and blinded to the clinical data.

Next, we used adenoviral (Adv) transduction to overexpress the C-terminal domain of IGFBP5 in two highly aggressive OS cell lines (MG63.2 & 143B). RFP and full length IGFBP5 were used as negative and positive controls, respectively. The transduced OS cells were assayed for cell proliferation, migration, and invasion in vitro. Briefly, cell proliferation was measured using Trypan Blue cell counting. Cell migration and invasion were measured using previously described Wound Healing and Matrigel invasion assays, respectively. Adv-tranduced OS cells were then then injected subperiosteally into the proximal posterior tibia of athymic nude mice, and we measured the ability of OS cells to form primary tumors and pulmonary metastases. Tumor size was measured every 3-4 days and the tumor volume and doubling time were calculated as previously reported. MicroCT imaging was performed on primary tumor samples and analyzed using Amira software. Histologic evaluation looking for the presence of pulmonary metastases was conducted by an independent, blinded pathologist.

RESULTS

IGFBP5 levels were consistently decreased in metastatic lesions compared to the matched primary tumors. The mean immunohistochemical score for the primary lesions was 2.5, compared to 1.0 for the metastatic lesions, and loss of IGFBP5 correlated with metastasis in the patient samples (p<0.001).

Using adenoviral transduction, the C-terminal domain was overexpressed in two highly metastatic OS cell lines, 143B and MG63.2. Semi-quantitative RT-PCR and Western Blot confirmed expression of the C-domain. Overexpression of the C-terminal domain in vitro resulted in significantly decreased OS cell proliferation (P<0.05) and increased doubling times (P<0.03). In a wound healing assay, the C-domain inhibited the ability of OS cells to migrate and close a gap (P<0.02). Cell invasion assays showed that the C-domain inhibited the ability of OS cells to invade through a Matrigel matrix (P<0.03). In vivo, overexpression of the C-terminal domain resulted in significantly decreased primary tumor growth (P<0.001). MicroCT demonstrated decreased bony invasion and destruction by OS cells transduced with the C-domain. Finally, histologic analyses showed that the C-terminal domain significantly inhibited the ability of OS cells to form pulmonary metastases (P<0.001).

DISCUSSION

Osteosarcoma (OS) carries a poor prognosis secondary to high grade lesions and frequent pulmonary metastases. While various genetic and hereditary conditions are associated with OS, the precise molecular mechanisms leading to the development of OS are poorly understood, and may be a reflection of the complexity of the disease. Therefore, there is a critical need to identify molecular markers that provide insight into both the pathogenesis and metastasis of OS.

Previously, we have shown that Insulin Growth Factor Binding Protein-5 (IGFBP5) is a potent inhibitor of OS tumorigenesis both in vitro and in vivo. In this study, we show that downregulation of IGFBP5 is correlated with the presence of pulmonary metastases in clinical patient samples. Furthermore, IGFBP5 is a 3 domain protein, and its C-terminal region has been shown to interact with components of the extracellular matrix. We have found that the C-domain inhibits OS cell proliferation, migration and invasion in vitro, and is responsible for the anti-metastatic effect of IGFBP5 in vivo. We believe further investigation is warranted to evaluate roles of the two other domains of IGFBP5. It is also of interest to delineate the signaling pathways targeted by IGFBP5 and its C-terminal domain, as this will likely provide insight into the molecular mechanisms underlying OS pathogenesis and metastasis.

SIGNIFICANCE

We have identified a novel molecular marker that inhibits OS metastasis, and the protein’s functional C-terminal domain that exerts its anti-metastatic effect. With further investigation, we believe IGFBP5 could be used as both a treatment modality and molecular marker for OS tumorigenesis and metastasis.

REFERENCES
