Increased Insulin mRNA Binding Protein-3 Expression Correlates with Vascular Enhancement of Renal Cell Carcinoma by Dynamic Multidetector-CT and is Associated with Bone Metastasis

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Introduction: Metastatic RCC to bone is particularly challenging because it is often resistant to chemotherapy and radiation therapy, leaving surgical intervention the only available option, which is frequently complex due to the tumor’s destructive nature within bone and its exuberant vascularity. Prior study is to: 1) develop dynamic multi-detector computed tomography (dMDCT) outcomes of renal cell carcinoma (RCC) vascularity; 2) assess dMDCT correlation with a candidate molecular marker of RCC metastasis to bone (insulin-like mRNA binding protein-3 (IMP3)); and 3) demonstrate the differential expression of IMP3 in high vs. low vascular tumors in bone.

Methods: Retrospectively obtained dMDCT from 72 patients with primary RCC were used to establish threshold values for Low, Intermediate and High tumor vascularity. Paired histopathology specimens from 33 of these patients were used for immunohistochemistry (IHC) to correlate dMDCT with IMP-3 expression. IMP-3 gene expression studies were performed on RCC and poorly vascular prostate cancer (PC) bone metastases, and on RCC 786-O and PC3 cell lines grown in vitro and in the tibiae of engrafted athymic nude mice.

Results: Real time RT-PCR demonstrated a significant 4-fold increase of imp-3 expression in RCC 786-O vs. PC3 cells in vitro (p<0.001) (data not shown), which was corroborated by IHC for IMP3 in the xenografts. (Figure 1) IMP-3 expression positively correlated with dMDCT enhancement (p<0.01) (Figure 2), and IMP3 expression by IHC was strongly positive in all RCC, but weak in PC bone metastases (Figure 3).

Discussion: Quantitation of pre-operative dMDCT is a feasible method to phenotype primary RCC vascularity, which correlates with IMP-3 expression. In situ and cell line studies demonstrate an association between high IMP-3 expression and RCC bone metastasis. Studies aimed at defining the diagnostic potential of these biomarkers of RCC bone metastasis, and functional significance of IMP-3 in RCC vascularity and tumor progression are warranted.

Significance: Patients with metastatic renal cell carcinoma (RCC) have a life expectancy of 6 months to 1 year. Currently, the mainstay of treatment for primary RCC without known metastatic disease is surgery alone. One third of patients with localized disease at diagnosis will ultimately go on to develop metastatic disease, and will only then get started on systemic therapy. Because of the high toxicity, systemic therapy is often not started until after confirmation of metastatic disease. Thus, development of molecular diagnostics to identify patients who are at high risk for metastatic disease, and for whom early treatment is indicated, may greatly improve survival in patients with aggressive forms of RCC.
Figure 1. IMP3 expression in murine xenografts. IHC for IMP3 was performed on intratibial xenograft tumors from lead-chromate (Microfil) perfused nude mice that were initially injected with: RCC (A, B), breast cancer (C), prostate cancer (D), lung cancer (E), and melanoma (F) cell lines, as previously described. Representative micrographs obtained at 400x are shown to illustrate the background staining of the secondary antibody negative control (A), and robust staining for IMP3 (brown) in the RCC (B), relative to the other xenograft tumors (C-F).
Figure 2. Use of volumetric contrast-enhance CT to correlate primary RCC vascularity with IMP3 expression in the tumor. Amira 3-D visualization software was employed to quantify primary RCC tumor lesion volume within the kidney based on the anatomic structure of the contrast-enhanced CT (CE-CT) attenuation obtained from the clinical radiology. (A) A cartoon illustration of the manual contouring of the tumor mass (pink) from the residue normal kidney (blue) tissue and non-enhanced tissue (green) within tumor is shown it illustrate the 2-D area measurements derived from the original CE-CT section (A1). These 2-D measurements were then reconstructed in 3-D to derive volumes (A2), and were also used to visualize a sagittal section (A3). Using these manipulations of the CE-CT, the kidney, tumor and vascular/nonvascular tumor volumes were determined (B). Of note is that neither tumor size nor vascular tumor volume corresponded to gross lesion enhancement attenuation, as defined by Low (black bars), Intermediate (white bars) and High (gray bars) HU described in Figure 1. However, as predicted, the vascular:tumor ratio significantly corresponded with the gross lesion enhancement attenuation (C). Direct semiquantitative assessment of tumor vascularity (MDCT enhancement) vs. IMP3 expression (IHC) as described in Figure 1 was performed on 33 tumors in this study (D), which demonstrated a significant correlation (* p<0.05 vs. Low; ** p<0.001 vs. Low; # p<0.05 vs. Intermediate; ### p<0.001 vs. +).
Figure 3. Differential IMP3 expression in renal cancer vs. prostate cancer bone metastasis. IHC was performed on retrieval tissues obtained from patients with metastatic RCC in the iliac (A), sacrum (B), femur (C) and lumbar vertebra (D), and representative micrographs obtained at 200x are shown to illustrate the robust staining (brown) versus retrieval tissue from a patient with metastatic prostate cancer in spine immunostained for IMP3 (E) and treated with the secondary antibody only (negative control, F). Arrows indicated the strong IMP3 staining in the RCC bone metastases.