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Research Paper

Increased insulin mRNA binding protein-3 expression correlates with vascular enhancement of renal cell carcinoma by intravenous contrast-CT and is associated with bone metastasis

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ABSTRACT

Purpose: To: 1) assess the correlation between CT vascularity and a candidate molecular marker of RCC metastasis (insulin-like mRNA binding protein-3 (IMP3)); and 2) demonstrate the differential expression of IMP3 in high vs. low vascular tumors.

Experimental design: Retrospectively obtained contrast CT 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 CT with IMP-3 expression. IMP-3 gene expression studies were performed on RCC and poorly vascular prostate cancer (PC) human bone metastases samples to confirm presence of IMP3 in metastatic samples from RCC. Gene expression studies were performed on RCC 786-O and PC3 cell lines to confirm the presence of high expression of IMP3 in the RCC cell line.

Results: IMP-3 expression positively correlated with CT vascular enhancement ($p < 0.01$). IMP3 expression by IHC was strongly positive in all RCC, but weak in PC bone metastases. Real time RT-PCR demonstrated a significant 4-fold increase in *imp-3* expression in RCC 786-O vs. PC3 cells in vitro ($p < 0.001$).

Conclusion: Quantitation of pre-operative CT 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 biomarkers for RCC bone metastasis, and functional significance of IMP-3 in RCC vascularity and tumor progression are warranted.

1. Introduction

There is an estimated 330,000 new cases of renal cell carcinoma (RCC) diagnosed each year worldwide, with over 100,000 deaths, and a rising incidence of 3% per year [1]. Although several advances in the treatment of metastatic RCC have occurred in the last decade, this disease remains one of the most deadly cancers with a 5-year survival rate of ~10% [2–4]. The primary treatment for localized RCC is surgical resection alone. Although systemic therapies in the form of receptor tyrosine kinase (RTK) inhibitors and targets of the mTOR protein have improved length of survival for patients found to have advanced metastatic disease, there is a

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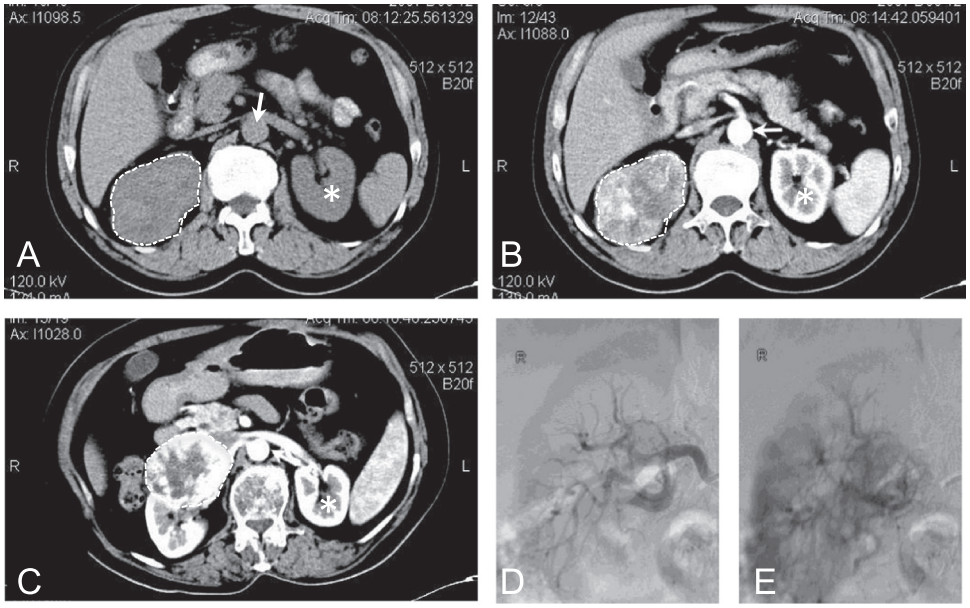

Fig. 1. *Assessment of primary tumor vascularity via CT scan in patients with malignant renal cancer.* Contrast CT scan was performed on all patients in the study to assess the vascularity of their primary renal cancer (white dashed line). In the initial analysis, patients with low vascular tumor, as illustrated in images (A) and (B); versus patients with highly vascular tumors that went on to receive digital subtraction angiography (C–E). Shown are representative: (A) pre-contrast, and (B) early arterial phase images demonstrating the initial enhancement and uptake of contrast dye (Iohexol 300 mgI/ml) at 30 s of a patient with a low vascular tumor. (C) CT scan of late phase vascular uptake showing high contrast uptake in the highly vascular tumor, which warranted the subsequent DSA. The patient's DSA confirmed the presence of a major tumor feeding vessel as seen from image obtained immediately after injection (D; 1–2 s) and late phase (E; 10 s).

reluctance to start these treatments with significant side effects in patients with only localized disease [5,6], as many patients will not go on to develop metastatic disease if treated with surgery alone. Two important trends in RCC have been noted in the past decade. The first is that the incidence of localized disease has been increasing, despite a plateau in the number of abdominal CT scans performed that would normally detect such disease [7]. Secondly, the mortality rate for patients with localized disease has also increased. This is in the setting of no improvements in incidence or mortality rates for patients with advanced disease [7]. Despite our best efforts to detect and surgically treat early stage RCC, approximately 30% of patients will go on to develop advanced metastatic disease [1]. An area in which major improvements could be made is diagnostic radiology for those patients with localized disease and a high risk of developing metastases, as early-aggressive treatments could then be justified. To this end, we aim to identify novel-clinically relevant radiologic and molecular biomarkers that can differentiate the metastatic potential of RCC in patients with local disease. This may alter surveillance and possibly the indications for starting systemic treatment.

Our research in this area has been focused on understanding the highly vascular nature of RCC, which has been largely attributed to loss of function of the von Hippel-Lindau (VHL) gene and resultant vascular endothelial growth factor (VEGF) over-expression, as it is an early event during tumorgenesis and is the most common cause for inherited RCC [8–10]. However, therapies that specifically target VEGF and its receptor have failed to demonstrate significant efficacy in clinical trials [11,12]. Moreover, our observations in a murine xenograft model of bone metastasis demonstrated that the major difference between a highly vascular RCC cell line (786-O) and a prototypical avascular prostate cancer cell line (PC3) is the presence of large smooth muscle and pericyte lined blood vessels within the tumor [13], suggests that non-VEGF signaling pathways may be more important. To test this hypothesis, we performed a microarray analysis of 786-O vs. PC3 by using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array (Chip Lot# LE23BK05) and whole data set has been documented at NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=qngrecewnbothcv&acc=GSE61942) and a summary of the notable findings is presented in Supplementary Table 1.

Based on our search criteria for candidate molecular markers of RCC vascularity, we chose to focus on insulin-like growth factor II (IGF-II) mRNA binding protein 3 (IGF2BP3 or IMP3). IMP3 is an oncofetal mRNA-binding protein and has been recently described as an independent prognostic marker for renal cell carcinoma (RCC) distant metastasis, and is associated with shorter survival [14]. It also has been noted in other cancers to be associated with cell motility and trans-endothelial migration [15]. IMP3 is a member of the highly conserved family of proteins that have been found to be associated with mRNA transport, translation and turnover. Functionally, IMPs have been shown to modulate cell proliferation, adhesion, migration and invasion [16]. IMP3 expression is almost exclusively limited to embryonic development, as its expression in most adult tissues is undetectable. However, it has recently been found to have significant expression in malignant adult tissue, including RCC [17]. Although an association between the expression of IMP3 in RCC and prognosis has recently been discovered, the predictive studies of IMP3 in clinical practice have not been evaluated [17]. More specifically, the relationship between IMP3 expression and pre-operative imaging characteristics (i.e. computed tomography (CT), magnetic resonance image (MRI) or digital subtraction angiographic (DSA)) has yet to be investigated. To address this, we evaluated the relationship between IMP3 expression in primary RCC versus tumor vascularity quantified from the pre-operative intravenous contrast CT scan. We also evaluated the expression of IMP3 via IHC from samples of bone biopsies obtained from patients with metastatic RCC to bone and compared them to patients with metastatic prostate bone disease. The goals of this study were to determine whether vascularity as assesses by contrast CT is correlated to IMP3 expression and if IMP3 expression in tumor samples could be used to stratify metastatic disease risk assessment in RCC patients. These findings