Original Study

Intratumor Heterogeneity of Perfusion and Diffusion in Clear-Cell Renal Cell Carcinoma: Correlation With Tumor Cellularity

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Abstract

Arterial spin-labeled (ASL) and diffusion-weighted magnetic resonance imaging were used to assess intratumor heterogeneity of perfusion and diffusion, respectively, in clear-cell renal cell carcinoma. Tumor areas with higher perfusion on ASL exhibited higher vascularity and cellularity at histology compared to areas with lower perfusion in the same tumor. A negative correlation between tumor diffusion coefficient and cellularity was confirmed.

Background: Magnetic resonance imaging (MRI) has the potential to noninvasively provide information about the tumor microenvironment. A correlation between arterial spin-labeled (ASL) MRI and tumor vasculature has been previously demonstrated; however, its correlation with tumor cellularity is unknown. We sought to assess intratumor heterogeneity of perfusion and diffusion in vivo in clear-cell renal cell carcinoma (ccRCC) using MRI and to correlate these findings with tumor vascularity and cellularity at histopathology. **Patients and Methods:** Twenty-three ccRCC patients underwent ASL and diffusion-weighted MRI before surgery after signing an informed consent in this prospective institutional review board–approved, HIPAA (Insurance Portability and Accountability Act)-compliant study. Quantitative ASL perfusion and diffusion were measured in 2 areas within the same tumor with high and low perfusion. Microvessel density (MVD) on CD31 and CD34 immunostains and tumor cellularity in anatomically coregistered tissue samples were correlated to MRI measurements (Spearman; $P < .05$ statistically significant). **Results:** ASL perfusion ($P < .0001$), CD31 MVD ($P = .02$), CD34 MVD ($P = .04$), and cellularity ($P = .002$) from high and low perfusion areas were significantly different across all tumors. There were positive correlations between tumor cellularity and CD31 MVD ($\rho = 0.350$, $P = .021$), CD31 and CD34 MVD ($\rho = 0.838$, $P < .0001$), ASL perfusion and cellularity ($\rho = 0.406$, $P = .011$), and ASL perfusion and CD31 MVD ($\rho = 0.468$, $P = .003$), and a negative correlation between tissue diffusion coefficient and cellularity ($\rho = -0.316$, $P = .039$). **Conclusion:** Tumor areas with high ASL perfusion exhibit higher cellularity and MVD compared to areas with low perfusion in the same tumor. A positive correlation between tumor vascularity and cellularity in ccRCC is newly reported. A negative correlation between tumor diffusion and cellularity is confirmed.

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Introduction

Renal cell carcinoma (RCC) is the most common malignancy arising in the kidney and accounts for 2% to 3% of cancers in adults. RCC is a heterogeneous disease with 3 major histopathologic subtypes: clear-cell RCC (ccRCC, 70%-80%), papillary RCC (10%-15%), and chromophobe RCC (3%-5%).[1] Compared to

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papillary and chromophobe tumors, ccRCC has worse clinical outcome with lower rates of disease-free survival and cancer-specific survival.[2,3] Molecular studies have highlighted the association between tumor angiogenesis, prognosis, and ability to metastasize.[4-6] Intratumor heterogeneity, however, is characteristically present in ccRCC[7] and likely drives the biological behavior of this disease. Moreover, assessment of intratumor heterogeneity ex vivo is challenged by the need to obtain multiple tissue samples in the same tumor. Development of a noninvasive biomarker to investigate tumor heterogeneity in vivo would be helpful to provide a preoperative assessment of RCC for optimal patient management and, potentially, selection of targeted therapies. Ideally, this biomarker would be applicable in clinical practice, provide an analysis of the entire tumor, and offer a quantitative measure that can be objectively used to characterize and monitor the natural history of RCC. Prior studies have illustrated the correlation among quantitative imaging biomarkers, cellularity, and tumor aggressiveness in a variety of tumors,[8,9] with higher cellularity being associated with worse overall survival.[10] Thus, a noninvasive biomarker of tumor cellularity could offer an objective, quantitative assessment of tumor biology that could be applied in a variety of clinical scenarios, including patients under active surveillance.

Arterial spin-labeled (ASL) perfusion magnetic resonance imaging (MRI) allows direct quantification of blood flow without exogenous contrast agents.[11] Using this technique, the magnetization of the incoming arterial blood to the tissue of interest is inverted by radiofrequency pulses and used as an endogenous contrast agent. Compared to other contrast-enhanced MRI techniques, ASL has some advantages as a quantitative biomarker, including the lack of contrast administration and the virtually negligible contribution of vascular permeability to the measurements of tissue perfusion.[12] ASL perfusion allows differentiation of renal masses with different histopathology based on their perfusion levels.[13] Compared to other subtypes, ccRCC demonstrates heterogeneous moderate to high perfusion levels.[13]

Diffusion-weighted MRI (DWI) measures the mobility of water molecules by applying diffusion-sensitized gradients. Apparent diffusion coefficient (ADC) calculated from diffusion signal decay has been applied to differentiate high-grade ccRCC, exhibiting lower ADC values, from low-grade ccRCC.[14,15] Furthermore, a significant inverse linear correlation between ADC and tumor cellularity was reported by Manenti et al.[16] However, the monoexponential diffusion model is prone to variability in ADC values, especially when different b values are used during the DWI acquisition.[17] A biexponential intravoxel incoherent motion (IVIM) model may provide more reliable ADC quantification in renal masses by separating pure tissue diffusion from pseudodiffusion due to capillary perfusion and tubular flow.[18]

In addition to the ability to differentiate different histologic subtypes and tumor grade, these quantitative MRI techniques can be applied noninvasively to study tumor heterogeneity. To date, most efforts have been directed to the assessment of heterogeneity in patients with advanced stage and its correlation with tumor response to therapy.[19] Moreover, the relationship between vascularity and cellularity in primary ccRCC has not been reported.

The purpose of this study was to investigate intratumor heterogeneity of perfusion and diffusion in vivo using quantitative ASL and DWI MRI, and to correlate MRI measurements with tumor vascularity and cellularity at histopathology in ccRCC.

Patients and Methods

Patient Population

This was a prospective, institutional review board–approved, HIPAA (Health Insurance Portability and Accountability Act)-compliant study. Fifty consecutive patients consented to participate in this study between August 2012 and August 2014. Inclusion criteria included patients with a known solid renal mass > 2.5 cm in size scheduled for a partial or radical nephrectomy at our institution, > 18 years of age, and confirmed diagnosis of ccRCC at histopathology after surgical resection. Exclusion criteria included patients with contraindication for MRI, prior local or systemic therapeutic intervention including chemotherapy for other known neoplasms, confirmation of non-ccRCC diagnosis after nephrectomy, and renal failure (ie, contrast-enhanced MRI was performed, although not included in this analysis).

MRI Acquisition

Before surgery, patients were examined on a 3 T dual-transmit MRI scanner with a 16-channel SENSE-XL-Torso coil (Achieva, Philips Healthcare, Best, The Netherlands). Coronal and axial T2-weighted images of both kidneys were acquired using single-shot turbo spin echo sequences (TE = 80 ms, TR = 1100-1300 ms, acquisition pixel size = 1.2-1.3 × 1.5-1.6 mm^2, slice thickness/gap = 5/1 mm) to localize the tumor. ASL imaging was performed using pseudo-continuous ASL (pCASL) with background suppression and timed breathing instructed by the operator.[20] A single 2-D coronal slice through the center of the tumor was imaged with a single-shot turbo spin echo readout (FOV = 360 × 408 mm^2, in-plane resolution = 3 × 3 mm^2, slice thickness = 10 mm, TE/TR = 80/6000 ms). Sixteen pairs of images were acquired from the same location with and without labeling the upper abdominal aorta. A proton density-weighted (M_0) image was also obtained using the same readout parameters without the labeling radiofrequency pulses and the background suppression. For diffusion-weighted imaging, coronal images of both kidneys were acquired using a respiratory-triggered (respiratory bellow or navigator) single-shot spin echo echo planar sequence (FOV = 180 × 408 mm^2, in-plane resolution = 2.7 × 2.7 mm^2, slice thickness/gap = 5/0.5 mm, TE = 60 ms, TR = 1 breathing cycle, b values (× number of measurements) of 0 (×1), 50 (×1), 100 (×1), 200 (×2), 450 (×2), 600 (×2), and 1000 (×3) s/mm^2). The diffusion-weighted gradient was applied in all 3 orthogonal directions to generate trace diffusion-weighted images.

Image Analysis

Quantitative ASL perfusion maps were reconstructed from the complex k-space raw data using offline reconstruction implemented in MATLAB (The MathWorks, Natick, MA),[21] and then imported to the advanced open-source PACS workstation DICOM viewer (OsiriX 64-bit version). Within each tumor, 2 regions of interest (ROIs), each about 1 cm^2, were manually defined on the perfusion map by a radiologist (I.P., with 15 years' experience in clinical body MRI studies), who was blinded to the final histopathologic diagnosis, to measure high and low perfusion areas in the same tumor. Areas without visible perfusion (ie, signal similar to background)