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

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Abstract

Background—Magnetic Resonance Imaging (MRI) has the potential to non-invasively inform about tumor micro-environment. A correlation between arterial spin labelled (ASL) MRI and tumor vasculature has been previously demonstrated, however, its correlation with tumor cellularity is unknown.

Purpose—To assess intratumor heterogeneity of perfusion and diffusion *in vivo* in clear cell renal cell carcinoma (ccRCC) using MRI, and correlate these findings with tumor vascularity and cellularity at histopathology.

Patients and Methods—23 ccRCC patients underwent ASL and diffusion-weighted MRI prior to surgery after signing an informed consent in this prospective IRB-approved, HIPAA-compliant study. Quantitative ASL perfusion and diffusion were measured in two areas within the same tumor with high and low perfusion. Microvessel density (MVD) on CD31 and CD34

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DISCLOSURE

The remaining authors have stated that they have no conflicts of interest.

immunostains and tumor cellularity in anatomically co-registered tissue samples were correlated to MRI measurements (Spearman; $P < 0.05$ statistically significant).

Results—ASL perfusion ($p < 0.0001$), CD31 MVD ($p = 0.02$), CD34 MVD ($p = 0.04$) and cellularity ($p = 0.002$) from high and low perfusion areas were significantly different across all tumors. There were positive correlations between (1) tumor cellularity and CD31 MVD ($\rho = 0.350, P = 0.021$); (2) CD31 and CD34 MVD ($\rho = 0.838, P < 0.0001$); (3) ASL perfusion and cellularity ($\rho = 0.406, P = 0.011$); (4) ASL perfusion and CD31 MVD ($\rho = 0.468, P = 0.003$); and a negative correlation between tissue diffusion coefficient and cellularity ($\rho = -0.316, P = 0.039$).

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.

MICROABSTRACT

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.

Keywords

Kidney cancers; Intratumor heterogeneity; Arterial spin labeling; Diffusion weighted imaging; Tumor vascularity; Tumor cellularity

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignancy arising in the kidney and accounts for 2–3% of cancers in adults. RCC is a heterogeneous disease with three major histopathological subtypes: clear cell RCC (ccRCC, 70–80%), papillary RCC (pRCC, 10–15%), and chromophobe RCC (chRCC, 3–5%)¹. Compared to papillary and chromophobe tumors, clear cell RCC 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 ccRCC⁷ 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 non-invasive 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 between quantitative imaging biomarkers, cellularity and tumor aggressiveness in a variety of tumors^{8,9} with higher cellularity being associated with worse overall survival¹⁰. Thus, a non-invasive 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 on active surveillance.

Arterial spin labeled (ASL) perfusion magnetic resonance imaging (MRI) allows direct quantification of blood flow without exogenous contrast agents¹¹. Using this technique, the magnetization of the incoming arterial blood to the tissue of interest is inverted by radiofrequency (RF) 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 perfusion¹². ASL perfusion allows differentiation of renal masses with different histopathology based on their perfusion levels¹³. Compared to other subtypes, clear cell RCC demonstrates heterogeneous moderate-to-high perfusion levels¹³.

Diffusion-weighted MR imaging (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 al¹⁶. However, the monoexponential diffusion model is prone to variability in ADC values, especially when different b values are used during the DWI acquisition¹⁷. 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 flow¹⁸.

In addition to the ability to differentiate different histologic subtypes and tumor grade, these quantitative MRI techniques can be applied non-invasively 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 therapy¹⁹. 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 (IRB)-approved, Health Insurance Portability and Accountability Act (HIPAA)-compliant study. Fifty consecutive patients were 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 neoplasm, confirmation of non-ccRCC diagnosis after

nephrectomy, and renal failure (i.e. contrast-enhanced MRI was performed although not included in this analysis).

MRI Acquisition

Prior to surgery, patients were examined on a 3T 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 = $1.2\text{--}1.3 \times 1.5\text{--}1.6$ mm, 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 operator²⁰. A single 2D coronal slice through the center of the tumor was imaged with a single-shot turbo spin-echo readout (FOV = 360×408 mm², in-plane resolution = 3×3 mm², 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 RF 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 mm², in-plane resolution = 2.7×2.7 mm², slice thickness/gap = 5/0.5 mm, TE = 60 ms, TR = 1 breathing cycle, b-values ($\times$ number of measurements) of 0 ($\times 1$), 50 ($\times 1$), 100 ($\times 1$), 200 ($\times 2$), 450 ($\times 2$), 600 ($\times 2$), and 1000 ($\times 3$) s/mm²). The diffusion-weighted gradient was applied in all three 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, Inc., Natick, MA)²¹, and then imported to the advanced open-source PACS workstation DICOM viewer (OsiriX 64-bit version). Within each tumor, two regions of interest (ROIs), each about 1 cm², were manually defined on the perfusion map by a radiologist (IP 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 (i.e. signal similar to background) were avoided. A third ROI including the whole tumor was also drawn along the tumor boundary. The mean values from these three ROIs represent the absolute blood flow in milliliters per minute per 100 gram of tissue (mL/min/100g). The ROIs and the values were extracted prior to surgery.

Similarly, three ROIs were manually drawn on diffusion images to match the location of the ROIs on the ASL perfusion map. Manual correction of the ROIs was performed if tumor location changed due to respiration, so that signal intensity of the same tumor region was measured on all diffusion images with different b-values. ROI-based diffusion parameters were quantified from both monoexponential and IVIM models using custom-written programs in MATLAB. ADC was calculated using a monoexponential model:

$$S_i = S_0 \cdot e^{-ADC \cdot b_i} \quad [1]$$

where S_i is the signal intensity at different b-values, b_i and S_0 is the fitted signal intensity at $b = 0$. An IVIM model was applied to compute pure tissue diffusion coefficient (D_t), pseudodiffusion coefficient due to blood and tubular flow (D_p), and perfusion fraction (f_p):

$$S_i = S_0 \cdot (f_p \cdot e^{-D_p \cdot b_i} + (1 - f_p) \cdot e^{-D_t \cdot b_i}) \quad [2]$$

The definitions of S_0 , S_i , and b_i are the same as those in the monoexponential model.

Histopathology

Histopathologic analysis after partial or radical nephrectomy served as the reference standard for all tumors. The final diagnosis of ccRCC was provided by a uropathologist (PK) with more than 10 years of experience. After surgery, the surgical specimen was positioned to match its anatomic orientation *in vivo* using fiducial markers placed during the surgery as previously described²¹. The pre-selected high and low perfusion areas during MRI ROI analysis were matched to locations in the specimen and color-marked for subsequent histopathologic review. A thin slice of half of the tumor specimen was collected and divided in sections that were submitted entirely for processing into formalin fixed paraffin embedded (FFPE) blocks of tissues. The edges of this slice were inked in different colors. Every step of this process was documented with photographs of the tumor specimen to facilitate anatomic co-registration of the histopathologic slides with the MRI data. The tissue blocks were fixed in 10% buffered formaldehyde solution, sliced at 3 mm intervals, and embedded in paraffin. Lastly, 4 μ m thick sections obtained from tissue sections were stained using hematoxylin and eosin (H&E), and CD 31 and CD34 immunostains.

Tumor microvessel density (MVD) was measured on CD31 and CD34 immunostaining slides which were digitally scanned and reviewed using the Aperio systems (Leica Biosystems, Melbourne, Australia). Areas in the specimen selected for tumor cellularity and corresponding to areas of high and low perfusion *in vivo* on ASL MRI, respectively, were mapped from the gross photographs onto the H&E, and CD31 and CD34 digital slides. MVD measures were obtained in these regions on the CD31 and CD34 slides using the Genie (Laica, Melbourne, Australia) pattern-recognition software package and reported as number of vessels per micrometer square (μm^{-2}). Tumor cellularity (reported in number of tumor cells per μm^2) was measured by manually counting the number of tumor cells (i.e. using the hematoxyl dye of tumor cells) on the CD34 immunostains within an area of 0.05 cm^2 of the tissue samples. During the study period, Fuhrman grade was reported in the pathology report for all patients. ISUP grading was not available.

Statistical Analysis

To evaluate intratumor heterogeneity, general linear mixed models were used to test the difference in means of quantitative MRI parameters and histopathological measurements

between high and low perfusion areas adjusted for within patient correlation. In addition, the mean and standard deviation of the MRI measurements from the whole tumor, high perfusion and low perfusion ROIs were calculated for high grade (Fuhrman grade III–IV) and low grade (Fuhrman grade I–II) tumors, respectively. Wilcoxon rank sum tests were performed to compare the difference in the distribution of the MRI measurements between high grade and low grade ccRCCs in the whole tumor, high perfusion, and low perfusion areas, respectively. Correlations between tumor cellularity and MVD measurements, *in vivo* MRI and *ex vivo* histopathological measurements, and MRI perfusion and diffusion measurements were also investigated by calculating Spearman correlation coefficients for all high and low perfusion areas combined. All statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC). P values less than 0.05 were considered as statistical significant.

RESULTS

Fifty patients with 50 renal masses underwent MRI and surgical resection and clear cell histology was confirmed in 34 of the patients. Histopathological analysis was not performed in 11 ccRCCs: poor labeling of ASL MRI (n=1); cystic tumors (n=2); no target tissue samples obtained (n=8). The final study cohort in this analysis consisted of 23 ccRCC patients, who had both MRI exam and histopathologic analysis available (Table 1). Average time from MRI to surgery was 5 ± 3 days (median 5 days, range 1–11 days). After partial (n=14) or radical (n=9) nephrectomy, histopathologic measurements were obtained in all tissue samples. In masses that had homogeneous perfusion measurements, only one representative ROI was selected for MRI and tissue measurements. Quantitative MRI measurements of tissue perfusion revealed three ccRCCs had blood flow higher than 400 mL/min/100g, which likely indicated signal contamination from large blood vessels; therefore high flow ROIs from these tumors were excluded from statistical analysis.

Representative anatomic T2-weighted image, diffusion-weighted image, and ASL perfusion map from patient with ccRCC are demonstrated in Figure 1. Gross photograph of the tumor specimen, and photomicrographs of CD34 immunohistochemistry slides correlated to the high and low perfusion areas circled on ASL perfusion maps, are also displayed, respectively. Table 2 summarizes intratumor heterogeneity of *in vivo* MRI and *ex vivo* tissue measurements across all ccRCCs. Significant different intratumor blood flow levels from ASL perfusion maps were observed (Figure 2a). The high perfusion areas on *in vivo* MRI had significant higher tumor cellularity and MVD at histopathology (Figure 2b–d). However, the mean of diffusion measurements from all tumors didn't show significant difference between high and low perfusion regions of tumors. Table 3 includes quantitative ASL perfusion and diffusion results for high grade and low grade ccRCCs for the whole tumor, and for the high perfusion and low perfusion areas within the same tumor, respectively. ASL perfusion measures from the whole tumor and low perfusion area had higher mean values of blood flow in high grade ccRCCs compared to low grade ccRCCs, although these differences did not reach statistical significance. Moreover, lower mean diffusion coefficients (ADC and D_t) across all tumors were shown in high grade compared to low grade ccRCCs. However these differences were not statistically significant.

Figure 3 demonstrated representative Spearman correlation results. Tumor cellularity had a significant positive correlation with MVD on CD31 ($\rho = 0.350$, $P = 0.021$) (Figure 3a), however the positive correlation with CD 34 did not reach statistical significance ($\rho = 0.266$, $P = 0.085$). A significant positive correlation existed between CD31 and CD34 MVD measurements ($\rho = 0.838$, $P < 0.0001$) (Supplemental Figure). Comparing MRI and tissue measurements, ASL perfusion exhibited moderate statistical correlation with tumor cellularity ($\rho = 0.406$, $P = 0.011$) (Figure 3b) and MVD from CD31 ($\rho = 0.468$, $P = 0.003$), but the correlation with CD34 was not statistically significant ($\rho = 0.303$, $P = 0.064$). Tissue diffusion coefficient without contribution of tumor perfusion, D_t , showed a significant negative correlation with tumor cellularity ($\rho = -0.316$, $P = 0.039$) (Figure 3c), whereas ADC showed weaker correlation with tumor cellularity without statistical significance ($\rho = -0.214$, $P = 0.169$). Comparing quantitative MRI measurements, diffusion coefficients from monoexponential and IVIM models, ADC and D_t , were significantly correlated to each other ($\rho = 0.679$, $P < 0.0001$). ASL perfusion showed negative relations with diffusion coefficients, ADC and D_t , respectively. However the Spearman correlations were not statistically significant ($\rho = -0.302$, $P = 0.066$ for ASL vs. ADC; $\rho = -0.280$, $P = 0.088$ for ASL vs. D_t).

Discussion

Tumor angiogenesis leads to increase in the number of microvessels and blood flow and it is an essential process for tumor growth and the development of metastasis in clear cell renal cell carcinoma^{5,6}. This process is however highly heterogeneous in ccRCC, with tumor vasculature varying drastically in different areas of the same tumor²¹, and likely linked to the underlying genetic heterogeneity that characterizes this disease⁷. Noninvasive quantitative magnetic resonance imaging (MRI) methods provide an opportunity to study tumor microvasculature and microenvironment beyond the morphological characteristics of the tumor. In this study, we applied arterial spin labeled (ASL) perfusion and diffusion-weighted imaging (DWI) techniques to investigate intratumor heterogeneity of blood flow and cellularity in ccRCC *in vivo*. We confirmed a correlation between blood flow measures *in vivo* and tumor cellularity.

In our study, ASL and DWI MRI provided a non-invasive quantitative assessment of tumor vascularity and cellularity, both of which may be useful as surrogates of tumor biology in longitudinal follow up studies of patients undergoing active surveillance. Changes in these quantitative measures may indeed precede tumor growth and predict tumor progression and metastatic potential. Moreover, changes in tumor cellularity may correlate with response to therapy in the metastatic setting²² and targeted tissue procurement (e.g. percutaneous biopsy, post-surgery) of tumor areas with presumably higher cellularity may lead to better quality genetic data based on prior studies²³. The use of ASL and DWI in these clinical settings deserves further investigation.

Previous studies have shown that quantitative ASL perfusion measures correlate with different histopathologic diagnosis in renal masses¹³, and could potentially predict antiangiogenic treatment response in metastatic renal cell carcinoma²⁴. ASL perfusion quantification has also been correlated with quantitative DCE pharmacokinetic parameters

and tumor microvessel density²¹. In our study, after accounting for intratumor heterogeneity on the quantitative ASL perfusion map, we confirmed a positive correlation between ASL perfusion and MVD. Our results also suggested higher mean blood flow levels in high grade ccRCCs compared to low grade ccRCCs in our patient cohort, although this difference was not statistically significant. Importantly, we demonstrated higher cellularity in the high perfusion regions compared to the cellularity in the low perfusion region within the same tumor in all but three homogeneous tumors included in our study. This finding is consistent with prior observations pinpointing tumor angiogenesis as a key step to promote tumor growth and suggests the usefulness of ASL as a marker of cell proliferation. Moreover, our results support the use of *in vivo* MRI as a platform to facilitate targeted tissue procurement for correlating histological and/or molecular alterations with pathophysiologic alterations in the microenvironment within the same tumor.

Using DWI acquisitions, an apparent diffusion coefficient (ADC) can be calculated utilizing a monoexponential diffusion decay model, which has shown promise in distinguishing high-grade from low-grade ccRCC^{14, 15}. An inverse relationship between ADC and tumor cell density was found in pediatric tumors²⁵, prostate cancer²⁶, uterine cervical cancer²⁷, breast cancer²⁸, and pancreatic endocrine tumors²⁹. The significant inverse linear correlation between ADC and cellularity reported by Manenti et al¹⁶ in renal malignancies has not been reproduced in other studies³⁰. Furthermore, previous studies focused in whole-tumor analysis and did not take into consideration the presence of intratumor heterogeneity. Moreover, the contribution of high tissue perfusion levels in the kidney, and potentially in ccRCC, likely plays a role in the reported high variability of ADC measures³¹.

The biexponential IVIM model applied in our study to fit DWI data allows for separation of pure tissue diffusion from pseudodiffusion due to capillary perfusion¹⁸. Such model has been used to determine the histological subtype of renal masses^{32, 33}. In our study, DWI was acquired using multiple b-values to allow the quantification of tumor diffusion using both monoexponential and IVIM models. Diffusion coefficients from both models, ADC and D_t , were significantly correlated, with monoexponential model overestimating tissue diffusion compared to IVIM model. However, the mean of diffusion parameters from the high and low perfusion ROIs across all tumors was not statistically different ($p>0.05$). Moreover, lower diffusion coefficients were noted in high grade tumors, suggesting higher tumor cellularity, compared to low grade tumors although this difference did not reach statistical significance. A negative relationship was found between diffusion coefficient and tumor cellularity when combining measurements from high and low perfusion ROIs in the same tumor. However, the IVIM-derived D_t parameter (i.e. pure tissue diffusion coefficient) correlated statistically with tumor cellularity, whereas a correlation with ADC was not found. This finding further illustrates the value of the IVIM model in excluding the perfusion contribution to DWI signal for better estimate of tissue diffusivity and characterization of tumor cellularity.

Our study has several limitations. First, intratumor heterogeneity was investigated using only two regions within the same tumor, identified as high and low blood flow areas from ASL perfusion imaging. By doing this, we were able to compare the *in vivo* quantitative MRI measures with the *ex vivo* histopathological findings of anatomically co-registered tissue specimens from the same region of the tumor. It would be virtually impossible to compare

whole tumor MRI assessments with quantitative tissue analysis given the inherent heterogeneity of ccRCCs. Other image analysis methods, however, such as histogram and texture analysis, may provide information about heterogeneity in the whole tumor. Second, despite efforts to match imaging data to post-surgical tissue samples, misregistration between the two data sets continues to be a challenge. Further improvements including 3D printing to create MRI-based molds of the tumor are being considered to facilitate targeted tissue procurement. Third, a mismatch between perfusion and diffusion images due to different imaging acquisition methods (e.g. slice thickness) is possible. Fourth, the use of histology as a reference standard for MVD and cellularity is not without challenges. For example, areas of tumor necrosis within a given region of interest need to be excluded during this analysis potentially leading to an over-estimation of these measures. Fifth, only twenty-three patients with ccRCC (16 ccRCCs of Fuhrman grade II and 7 ccRCCs of Fuhrman grade III) were included in this study. This small sample size limited the assessment of the tumor grade based ASL and DWI measurements, as well as the correlation of radiological or histopathological findings to Fuhrman grade.

CONCLUSION

Clear cell renal cell carcinoma demonstrated intratumor heterogeneity on quantitative ASL and DWI MRI, and some of the perfusion and diffusion measures correlated to tissue-matched histopathologic features. Specifically, histopathologic analysis of tumor areas corresponding to high perfusion regions on ASL had higher MVD and demonstrated higher cellularity compared to tumor areas with low perfusion on ASL. A negative correlation between *in vivo* MRI diffusion measures and tissue cellularity was also confirmed supporting the use of these MRI techniques for assessment *in vivo* of the heterogeneity in tumor angiogenesis and changes in tumor microenvironment. These findings provide the rational for using quantitative ASL and DWI MRI to monitor tumor changes in active surveillance RCC patients. Moreover, the noninvasive *in vivo* MRI may offer an opportunity to direct percutaneous biopsies to a specific region of a renal mass during pre-surgical histological assessment. To our knowledge, this is the first demonstration of a correlation between tumor vascularity and cellularity in ccRCC. We confirm the previous observation of a negative correlation between tumor diffusion coefficient and cellularity. Our MRI-directed tissue procurement provides a basis for exploring molecular alterations that drive tumor angiogenesis and growth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CLINICAL PRACTICE POINTS

- Quantitative ASL MRI at 3T can be used to non-invasively assess tumor vascularity, and provide evidence of tumor angiogenesis and tumor cell proliferation in clear cell renal cell carcinoma.
- Intravoxel incoherent motion diffusion-weighted imaging at 3T can improve tissue diffusion quantification *in vivo* and correlation with tumor cellularity at histopathology.
- ASL perfusion and diffusion MRI can provide *in vivo* assessment of heterogeneity in tumor vascularity and tumor microenvironment in primary ccRCC.
- ASL and DWI offer information that may be helpful to monitor interval changes in tumor microenvironment in patients with ccRCC undergoing active surveillance.

Figure 1. Representative T2W (A), DWI (B), and ASL perfusion map (C) of ccRCC (white line) and gross specimen photo (D) of the same tumor. Selected areas in the tumor with high perfusion and low perfusion areas on MRI are marked in black and yellow, respectively, and corresponding areas were examined at histopathology. Photomicrographs (10× magnification) of CD34 immunohistochemistry in the high and low perfusion areas (E and F, respectively) confirm differences in microvessel density (i.e. number of blood vessels stained in brown color) in the same tumor. Similarly, tumor cellularity indicated by the number of nuclei (arrows in red) per field was markedly increased in the area with high flow (E) compared to the area of low flow (F).

Figure 2.

Boxplots of the MRI and tissue measurements categorized by high flow (HF, red) and low flow (LF, yellow) areas within the tumors. Significant intratumor differences of *in vivo* ASL perfusion and *ex vivo* tumor cellularity and MVD measurements are demonstrated. The lower, middle, and upper bars of the box are the 25th, 50th (median), and 75th percentile of the measurements. The circle inside the box indicates the mean value. The intra-quartile range (IQR) is defined as the range between the 25th and the 75th percentile. Outliers (shown as asterisk) are defined as more than 1.5*IQR from the box. The whiskers extend to the range of the measurements that are not outliers.

Figure 3.

Comparisons between tissue and MRI measurements are presented by scatter plots, in which measurements of high and low perfusion areas from the same tumor are connected with a dash line. A regression line (in solid black) is drawn to demonstrate the overall linear relationship. The corresponding Spearman correlations both within and across patients were also derived: (A) a mild positive correlation between cellularity and MVD CD31 ($\rho=0.350$, $P=0.021$); (B) a moderate positive correlations between ASL perfusion and cellularity ($\rho=0.406$, $P=0.011$); (C) a mild negative correlation between tissue diffusion coefficient, D_t , and cellularity ($\rho=-0.316$, $P=0.039$).

Table 1

Clinical and histological characteristics of patients with ccRCC. Tumor size is in the form of mean $\pm$ standard deviation.

Characteristic	No. of Patients	%	
Age (years)	60 $\pm$ 10		
Gender			
Male	16	69.6	
Female	7	30.4	
T Stage			Tumor Size (cm)
T1a	10	43.5	3.4 $\pm$ 0.3
T1b	8	34.8	5.5 $\pm$ 1.1
T3	5	21.7	7.8 $\pm$ 3.4
Histology			
Fuhrman Grade			Tumor Size (cm)
II	16	69.6	4.9 $\pm$ 1.5
III	7	30.4	6.9 $\pm$ 3.2
Sarcomatoid	0	0	
Cystic Component	2	8.7	
Necrosis	5	21.7	

Table 2

Intratumor heterogeneity of *ex vivo* tissue histopathology and *in vivo* MRI measurements for the assessment of intratumor heterogeneity (all data are in the form of mean $\pm$ standard deviation).

	High perfusion area	Low perfusion area	P value
ASL Perfusion (mL/min/100g)	233.89 $\pm$ 66.00	92.93 $\pm$ 61.79	< 0.0001 *
Cellularity (μm^{-2})	0.00424 $\pm$ 0.00093	0.00332 $\pm$ 0.00113	0.002 *
MVD CD31(μm^{-2})	0.00055 $\pm$ 0.00020	0.00041 $\pm$ 0.00021	0.016 *
MVD CD34 (μm^{-2})	0.00045 $\pm$ 0.00020	0.00034 $\pm$ 0.00015	0.044 *
ADC ($\times 10^{-3}$ mm ² /s)	1.92 $\pm$ 0.36	1.99 $\pm$ 0.50	0.565
D _t ($\times 10^{-3}$ mm ² /s)	1.29 $\pm$ 0.49	1.33 $\pm$ 0.52	0.942
f _p (%)	37.18 $\pm$ 8.89	34.38 $\pm$ 12.34	0.553
D _p ($\times 10^{-3}$ mm ² /s)	14.79 $\pm$ 11.01	15.22 $\pm$ 8.16	0.653

* denotes statistical significance.

Table 3

Quantitative ASL perfusion and diffusion MRI measurements from the whole tumor, high perfusion and low perfusion areas within the tumor for high grade and low grade ccRCCs.

		High Grade	Low Grade	P value
ASL Perfusion (mL/min/100g)	Whole tumor	153.15 ± 58.83	136.61 ± 57.56	0.628
	High perfusion area	225.65 ± 76.59	238.38 ± 63.04	0.802
	Low perfusion area	107.79 ± 72.67	85.50 ± 57.09	0.478
ADC ($\times 10^{-3}$ mm ² /s)	Whole tumor	1.99 ± 0.40	2.18 ± 0.34	0.367
	High perfusion area	1.78 ± 0.35	1.99 ± 0.36	0.351
	Low perfusion area	1.94 ± 0.40	2.02 ± 0.56	0.725
D _t ($\times 10^{-3}$ mm ² /s)	Whole tumor	1.24 ± 0.52	1.55 ± 0.39	0.242
	High perfusion area	1.08 ± 0.33	1.39 ± 0.54	0.192
	Low perfusion area	1.14 ± 0.39	1.42 ± 0.57	0.359
f _p (%)	Whole tumor	38.98 ± 11.67	33.21 ± 7.74	0.066
	High perfusion area	39.17 ± 9.90	36.19 ± 8.56	0.351
	Low perfusion area	40.09 ± 5.08	31.71 ± 13.91	0.231
D _p ($\times 10^{-3}$ mm ² /s)	Whole tumor	13.85 ± 7.29	12.59 ± 8.11	0.740
	High perfusion area	16.58 ± 11.48	13.90 ± 11.10	0.433
	Low perfusion area	13.37 ± 4.19	16.14 ± 9.57	0.794

No statistically significant difference was found in tumor grades.