Supplementary Methods

A. MRI Protocol for Primary Tumors

All patients were imaged in supine position in a whole-body 3T dual-transmit MR scanner (Achieva or Ingenia, Philips Healthcare, Best, The Netherlands). Axial and coronal two-dimensional (2D) T2-weighted single shot turbo spin echo (SShTSE) images were acquired for anatomical reference: repetition time/echo time (TR/TE) = 1115/80 ms, flip angle (FA) = 90°, slice thickness = 5 mm, field of view (FOV) = 402×340 mm^2, pixel resolution = 1.4×1.3 mm^2, bandwidth = 467 Hz/pixel. Coronal three-dimensional (3D) T1-weighted fast field echo (FFE) acquisitions were then obtained with three flip angles (10°, 5°, and 2°), to generate a T1 map. Subsequently, dynamic contrast-enhanced (DCE) MRI was performed with the same FFE sequence acquired before, during, and after the intravenous (I.V.) administration of 0.1 mmol/kilogram body weight of gadobutrol (Gadavist; Bayer Healthcare, Wayne, NJ) at 2 mL/sec followed by 20 mL saline flush (1). DCE MRI acquisition parameters were: TR/TE = 3/1.53 ms, FA = 10°, slice thickness = 5 mm, FOV = variable depending on body habitus, pixel resolution = 1.5×1.4 mm^2, bandwidth = 1326 Hz/pixel, coronal orientation, 5-second temporal resolution. To minimize respiratory motion artifacts, repeated cycles of 3 consecutive dynamic phases (5 seconds each) obtained within a 15-second breath-held acquisition, followed by a 15-second free-breathing, were obtained for a total of approximately 6 minutes and 15 seconds. DCE MRI data were processed with VersaVue (iCAD Inc., Nashua, NH) to generate quantitative maps of K^{trans} and K_{ep} from the extended Tofts model (2). Since this was not an interventional study, patient cohort were not randomized into groups.

B. Image Acquisition and Analysis of Metastatic Lesions

<u>Imaging protocol</u>: MRI examinations in patients with metastatic disease were obtained as part of their standard of care. The abdominal MRI protocol included two-dimensional T1- and T2-weighted imaging, diffusion weighted imaging, and multi-phase three-dimensional (3D) fat-saturated T1-weighted images before and after I.V. administration of a bolus of gadolinium-based contrast agent (0.1 mmol/kg of gadobutrol, Bayer Healthcare). As part of the standard of care abdominal MRI, images are acquired before contrast (PRE) and during the arterial, portal, venous, and equilibrium phase. The arterial phase is somewhat equivalent to the renal corticomedullary phase and timed with a coronal MRI fluoroscopy acquisition through the heart (3). After MRI fluoroscopic visualization of the contrast in the left ventricle, two sets of breathing instructions are given followed by a breath-hold instruction at end-expiration (i.e., 'breathe-in, breathe-out, breathe-in, breathe-out and hold it'), at which point the acquisition sequence is triggered. For most patients, initiation of the arterial phase occurs at approximately 30 s after initiation

of the contrast administration. The portal, venous, and equilibrium phases are acquired 40 s, 90 s, and 120 s after the initiation of the arterial phase. Thus, the portal phase is acquired approximately 70 s after administration of contrast (i.e., center of k-space for an 18 s acquisition at approximately 79 s), closer to the frank nephrographic phase (NG). Only pre- and post-contrast 3D T1-weighted images during the portal phase (i.e., equivalent to NG phase) were used for this analysis. Image acquisition parameters varied slightly depending on the MRI scanner. The following parameters were used for acquisition with spectral fat suppression: TR= 3.2- 6.4 ms, TE=1.2- 2.4 ms, thickness 1.5-5 mm, flip angle 10 degrees, matrix 168-640 x 208-540, FOV 25-40 x 30-48 cm. For acquisitions with Dixon-based fat suppression, the following parameters were used: TR= 3.05-5.86 ms, TE=1.1/2.2 ms, thickness 3-5 mm, flip angle 10 or 15 degrees, matrix 320-560 × 320-560, FOV 30-45 × 30-45 cm.

Image analysis: First, signal intensity of the pixels within the ROI on the post-contrast image was normalized by the average of signal intensity from the same ROI on the pre-contrast image. Second, pixels with more than 15% enhancement from pre- to post- images were classified as enhancing pixels (4). Third, the percent of tumor pixels that was classified as enhancing within the ROI was defined as % area enhancement. Last, metastases were dichotomized into a High (HE) vs Low (LE) enhancement category relative to the median % area enhancement across all tumors. In a subset of data (eleven pairs of pre- vs. post-contrast datasets) originated from Philips scanners, images were rescaled using different scaling factors (slope only) by Philips' splitting tool. The scaling factors were not stored in the DICOM headers in the older version of Philips software. The subtraction images of post- and pre-contrast were available for the nine datasets with different scaling (slopes and intercepts). To ensure pre- and post-contrast images are consistently scaled, the relative scaling factor, R, was computed using pre- (S_{pre}), post- (S_{post}) and subtracted (S_{Δ}) contrast images:

$S_{\Delta} = k_1 \cdot S_{pre} + k_2 \cdot S_{post} + c.$

By using multilinear fitting, this function was fitted based on the signals from four different ROIs in liver, spleen, and muscle, drawn in homogenous regions to reduce motion artifacts. Subsequently, the relative scaling factor was computed based on: $R = -k_2/k_1$. The post-contrast images were corrected to the same scaling as the pre-contrast images using the relative scaling factor. This method was first verified in the data with known scaling factors within header and was then applied to the nine datasets. For the two datasets without the subtraction images, the signals from regions with subcutaneous fat were used to compute the relative scaling factor since the fat signals are stable in post-contrast images.

Statistical Analysis:

Associations between the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) and Memorial Sloan Kettering Cancer Center (MSKCC) models, patient groups (i.e., 1. HE AA; 2. HE IO; 3. LE AA; and 4. LE IO), and oncologic outcomes (progression-free survival [PFS], objective response rate [ORR]), were studied. Kaplan-Meier curves were used to visualize progression free survival for four tumor groups: 1. HE AA; 2. HE IO; 3. LE AA; and 4. LE IO. The cox proportional hazards model was used to test the differences in hazards rate between the four tumor groups (DCExTrt, HE AA vs LE AA vs HE IO vs LE IO). Robust sandwich covariance estimate was used to account for multiple tumors clustered within same patients. The IMDC and MSKCC score were also added as covariates respectively. Overall significance was assess by p value from Type 3 Wald tests. Pairwise hazard ratios were estimated and Tukey adjustment was use for multiple comparison. Significance level was set at 0.05. All analyses were done in SAS 9.4 (SAS institute, inc., Cary, NC). Since, this was a preliminary evaluation in the metastatic cohort, a power analysis was not performed.

C. Immunohistochemistry (IHC) Analysis

After sample hydration, antigen retrieval was performed using EnVision™ FLEX Target Retrieval Solution, Low pH in Dako PT Link followed by incubation in 3% hydrogen peroxide for 10 min. Primary antibodies for HIF1 (Novus Cat# NB100-105, RRID:AB_10001154, 1:500 dilution), and HIF2 (Santa Cruz Biotechnology Cat# sc-46691, RRID:AB_627523, 1:200 dilution). CD31 (Agilent Cat# M0823, RRID:AB_2114471, 1:50 dilution), CD34 (Agilent Cat# M7165, RRID:AB_2063006, 1:50 dilution), CD117 (Agilent Cat# A4502, RRID:AB_2335702, 1:700 dilution), CD8 (M710301-2, 1:75 dilution), and CD20 (Agilent Cat# M0755, RRID:AB_2282030, 1:300 dilution) were purchased from Agilent Technologies (Santa Clara, CA). CD163 (Thermo Fisher Scientific Cat# MA5-11458, RRID:AB_10982556, 1:100 dilution) was purchased from Thermo Fisher Scientific /Invitrogen (Carlsbad, CA). The primary antibodies were added and incubated at RT for 40 min. After washing, EnVision FLEX mouse/rabbit linker (Agilent Cat # K802121, # K80092) was applied to the tissue for 10 min followed by secondary antibody, EnVision™ FLEX/HRP (Agilent Cat # K800021), and incubated for 20 min. Sections were then processed using the EnVision™ FLEX Substrate Working Solution for 10 min followed by dehydration in a standard ethanol/xylene series, and mounting media (8310-4, Thermo Scientific). Appropriate positive and negative controls were used with each run of immunostaining. The percentage of positively stained tumor cells from the entire tumor was manually scored by a pathologist (P.K.) who was blinded to the DCE MRI results.

D. cDNA library construction, RNA sequencing, and analyses

A total of 1 μg of total RNA was purified to specifically select for polyadenylated RNA by oligo-dT beads and fragmented by divalent cations under elevated temperature. First strand synthesis was then done on the fragmented RNA using reverse transcriptase and random primers, followed by second strand synthesis using DNA polymerase I to yield cDNA fragments for library construction. The cDNA fragments were further treated with RNaseH, and carried through subsequent steps of end repair, adenylation of the 3' ends, and ligation of adapters. The cDNA library was enriched using 15 cycles of polymerase chain reaction (PCR) amplifications and then purified. The libraries were quantified using quantitative PCR (qPCR), and quality control was done using the Agilent Bioanalyzer with a DNA-specific chip. A peak at approximately 260 bp indicated the libraries were ready for pooling and cluster generation for RNA sequencing. Sequencing was performed on a HiSeq 3000/4000 platform according to the Illumina protocol, as described (5). Appropriate technical replicates were performed as part of the standard protocol. Cluster amplification of the cDNA libraries were carried out by HiSeq 3000/4000 PE Cluster Kit following the kit protocol. All samples were pooled together and sequenced on multiplex lanes to reach 120 million paired-read reads (60 M reads in each direction). Finally, high-quality RNA-sequencing reads from 180 tissue samples (144 ccRCC tumors, and 36 URPs) were aligned to the human reference genome GRCh38 (hg38) using STAR, RRID:SCR_004463 with the parameters '--runThreadN 32 --outReandsUnmapped Fastx'. FeatureCounts, RRID:SCR_012919 (Subread package, version 1.6.1) with parameters '-T 32 -p -M -O -s 2' was then used to count gene expression levels. The human genome annotation file employed by featureCounts was downloaded from UCSC table browser under the RefSeq Gene track (RefSeq, RRID:SCR_003496). All further analyses were performed under R computing environment (version 3.3.2). Gene read counts were log-scaled. Quantile normalization (preprocessCore package, version 1.45.0) was performed on the scaled gene counts to generate gene expression levels. Semi-supervised category identification and assignment were performed using (SCINA) algorithm (https://github.com/jcao89757/SCINA).

References:

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