Whole Exome DNA Sequencing

These same tissue blocks were used to extract tumor DNA for sequencing, which determined the presence of somatic mutations in ccRCC tumors. Sequence reads were aligned to the reference human genome with the Burrows-Wheeler Aligner (BWA)[1], and insertion/deletion realignment and quality score recalibration were performed with the Genome Analysis ToolKit (GATK)[2]. Tumor specific mutations were identified with Strelka[3] and MuTect[4], and were annotated to determine genic context (ie, non-synonymous, missense, splicing) using ANNOVAR[5] and summarized using in-house Perl and R scripts. A summarized outline of the above methods is provided in the Supplementary Material, along with the entire scripts utilized for this analysis.

RNA-seq Immune Cell Expression and IMmotion150 Angiogenesis and T-effector scores

A subset of patients in the IF cohort (n = 92) had tumor samples that had previously undergone bulk RNA sequencing of macro-dissected tumor samples. The TruSeq RNA Exome kit (Illumina) for 50 million 100–base pair paired-end reads was utilized, and RNA sequence reads were aligned to the human reference genome in a splice-aware fashion using Spliced Transcripts Alignment to a Reference (STAR) [6], allowing for accurate alignments of sequences across introns. Aligned sequences were assigned to exons using the HTseq package[7] to generate initial counts by region. Normalization, expression modeling, and difference testing were performed using DESeq2 [8]. A summarized outline of the above methods is provided in the Supplementary Material, along with the entire scripts utilized for this analysis.

RNA sequencing data was analyzed for cell-type enrichment using the xCell bioinformatic pipeline[9]. xCell uses a compendium of validated gene expression signatures for 64 individual cell-types derived from thousands of expression profiles. Single-sample gene set enrichment analysis scores were adjusted for spillover compensation to generate an adjusted enrichment score for each cell type within

the specimen, which is referred to as the xCell score[9]. xCell scores were generated for each of the 64 cell-types for each tumor specimen. Notably, the tumor samples utilized for RNA sequencing were almost entirely composed of tumor tissue, by volume, and therefore are expected to most closely resemble the tumor-core zone from the IF analysis. Relevant comparisons were made between xCell cell-types and corresponding mIF marked cell types.

Angiogenesis and T-effector scores were generated from the RNA-seq data per the IMmotion150 gene signatures, in a manner according to the original publication[10]. Angiogenesis: VEGFA, KDA, ESM1, PECAM1, ANGPTL4, and CD34. T-effector: CD8A, EOMES, PRF1, IFNG, and CD274.

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