Tumor heterogeneity analysis using single-cell transcriptomics in Von Hippel-Lindau related renal cancer

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Introduction
Patients with Von Hippel-Lindau (VHL) disease have risk of developing multiple primary clear cell renal carcinomas (ccRCC). Our hypothesis stated that multiple independent tumors from one VHL patient might display inter-tumoral heterogeneity characterized by differences in tumor grade, metabolic, stromal, and immune cell features that may cause different response to systemic therapy. Using tissues obtained from nephrectomy performed on a VHL patient we investigated the molecular and microenvironmental factors in five independent primary tumors.

Methods
Samples from each primary tumor and from a non-tumoral region were independently processed for single-cell RNA sequencing (scRNAseq) and histology. Diagnosis, staging and grading of ccRCC were assessed by a dedicated genitourinary pathologist. For scRNAseq, cells were isolated and captured using Chromium 10x, then sequencing data were processed with the UMI tools pipeline. Unsupervised clustering was performed using the Seurat R package, and the identified cell populations were manually annotated via marker genes.

Results
Histological analysis revealed that the biggest tumor of 5.5 cm resulted pathologic tumor stage 3a (pT3a) Grade2 (G2) ccRCC, the tumor of 3.0 cm resulted pT1a G3 ccRCC whereas the three smallest lesions of 0.5, 0.9 and 2.0 cm were pT1a G2. For scRNAseq, 10,574 cells were retained after quality filtering and integrative analysis identified 15 different cell clusters. Use of cell type markers allowed to identify cancer cells; immune cells including CD8 positive T cells, regulatory T cells, T cell natural killer like, tumor-associated macrophages (TAM), monocytes, dendritic cells, mast cells, natural killer Cells, B cells and stromal cells including myofibroblasts and endothelial cells. Each lesion had different cancer, immune and stromal composition, in particular the tumor with the highest grade tumor had less CD8+ T cells and more TAM in comparison to the other lesions. At the level of the expression on of the therapeutical target genes, HIF-2alpha target genes VEGFA, IGFBP3, TGFA, SLC2A1 and CCND1 and immune check point targets PDCD1 (PD1), HAVCR2 (TIM3), LAG3, TIGIT and CTLA4 were expressed in the cancer cells and CD8+ T cells respectively from all the lesions with some notable differences between lesions notably lower expression of most the immune check points targets in the G3 lesion.

Discussion
Inter-tumor heterogeneity within one VHL patient was revealed at the level of grade, stage and tumor microenvironment composition. Markers of potential response to systemic agents were expressed. This approach underline the importance to study intra-patient heterogeneity.