DIFFERENTIAL EXPRESSION AND FUNCTION OF TOLL-LIKE RECEPTOR 3 WITHIN TUMOR IMMUNE MICROENVIRONMENT OF CLEAR CELL RENAL CELL CARCINOMA

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**Background** High intratumoral toll-like receptor 3 (TLR3) expression is associated with improved survival in patients with clear cell renal cell carcinoma (ccRCC). However, the function of TLR3 in the tumor immune microenvironment is poorly understood. Here, we assessed the expression of TLR3 in tumor-infiltrating immune cell subsets, and the differential gene expressions comparing TLR3+ VS TLR3- cells within each subset.

**Methods** Single-cell RNA-Seq dataset was obtained from the publication by Braun et al (PMID 33711273). Seurat version 4.0.4 was used for analysis. Uniform Manifold appreciation (UMAP) and projection reduction were performed with principal component analysis independent from previous work. Clusters were identified at a resolution of 0.8 with the Louvain algorithm using 1:20 dimensions in function. Clusters were labeled based on a combination of previous cell barcode annotation and our input. Differential gene expression was performed on normalized gene expression using FindMarkers function (only.pos = FALSE, min.pct = 0.15, logfc.threshold = 0.15) from each cluster/condition tested against all other cells. Genes were significantly elevated when Log2 fold change ≥0.20 with corrected p-value ≤0.05. Cells were segregated based on TLR3 expression if a cell has at least 1 expression unit of TLR3. Quiagen IPA was used to perform pathway analysis. IPA Z-scores of ≥2 and ≤-2 were used to determine the significance of activation and inhibition, respectfully.

**Results** A total of 164,722 cells from 13 patients with early (n=8) and advanced stage (n=5) ccRCC were included. TLR3 expression was enriched in myeloid compartment (figure 1). The top three TLR3 expressing immune cell types were: inflammatory macrophages (IM) with 636 out of 3309 cells (19.2%), CD141+ dendritic cells (DC) with 541 out of 3010 cells (18.0%), and tumor-associated macrophages (TAM) with 1209 out of 9749 cells (12.4%) (table 1). In IPA analysis, TLR3 positivity was associated with increased function of cell adhesion in DC141+DC (Z-score=2.0), response of antigen presentation in TAM (Z-score=2.2), and cell migration of IM (Z-score=2.2). Whereas TLR3 negativity was associated with inhibited cell movement of DC (Z-score= -2.1). The top differentially expressed genes between TLR3+ vs TLR3- cells of each subset was shown in figure 2.

**Conclusions** TLR3 expression is enriched in macrophages and dendritic cells within ccRCC tumor microenvironment. TLR3 signaling may promote cell adhesion, migration, and antigen presentation function of these cells.

**Ethics Approval** This research was exempt from IRB review per institutional policy. This study utilized previously published and publicly available data. Data contained in this study is not individually identifiable or potentially identifiable.
Abstract 82 Figure 3 Volcano plot showing the differential gene expression of TLR3+ vs TLR3- CD141+ dendritic cells (DC). Y-axis showing corrected p-value expressed in -Log10q, and X-axis showing the difference in gene expression measured Log2 fold change. All significant change in gene expression were colored in red.

Abstract 82 Figure 4 Volcano plot showing the differential gene expression of TLR3+ vs TLR3- inflammatory macrophages. Y-axis showing corrected p-value expressed in -Log10q, and X-axis showing the difference in gene expression measured Log2 fold change. All significant change in gene expression were colored in red.