## Single Cell Analysis of Circulating Monocytes in Prostate Cancer Patients

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Abstract: The innate immune system reacts to foreign insult in several unique ways, one of which is phagocytosis of perceived threats such as cancer, bacteria, and viruses. The goal of this study was to look for evidence of phagocytosed RNA from tumor cells in circulating monocytes. While all monocytes possess phagocytic capabilities, the non-classical CD14+/FCGR3A+ monocytes and the intermediate CD14++/FCGR3A+ monocytes most actively remove threatening 'external' cellular materials. Purified CD14-positive monocyte samples from fourteen patients recently diagnosed with clinically localized prostate cancer (PCa) were investigated by single-cell RNA sequencing using the 10X Genomics protocol followed by paired-end sequencing on Illumina's NovaSeq. Similarly, samples were processed and used as controls, i.e., one patient underwent biopsy but was found not to harbor prostate cancer (benign), three young, healthy men, and three men previously diagnosed with prostate cancer that recently underwent (curative) radical prostatectomy (post-RP). Sequencing data were mapped using 10X Genomics' CellRanger software and viable cells were subsequently identified using CellBender, removing technical artifacts such as doublets and non-cellular RNA. Next, data analysis was performed in R, using the Seurat package. Because the main goal was to identify differences between PCa patients and 'control' patients, rather than exploring differences between individual subjects, the individual Seurat objects of all 21 patients were merged into one Seurat object per Seurat's recommendation. Finally, the single-cell dataset was normalized as a whole prior to further analysis. Cell identity was assessed using the SingleR and cell dex packages. The Monaco Immune Data was selected as the reference dataset, consisting of bulk RNA-seq data of sorted human immune cells. The Monaco classification was supplemented with normalized PCa data obtained from The Cancer Genome Atlas (TCGA), which consists of bulk RNA sequencing data from 499 prostate tumor tissues (including 1 metastatic) and 52 (adjacent) normal prostate tissues. SingleR was subsequently run on the combined immune cell and PCa datasets. As expected, the vast majority of cells were labeled as having a monocytic origin (~90%), with the most noticeable difference being the larger number of intermediate monocytes in the PCa patients (13.6% versus 7.1%; p<.001). In men harboring PCa, 0.60% of all purified monocytes were classified as harboring PCa signals when the TCGA data were included. This was 3-fold, 7.5-fold, and 4-fold higher compared to post-RP, benign, and young men, respectively (all p<.001). In addition, with 7.91%, the number of unclassified cells, i.e., cells with pruned labels due to high uncertainty of the assigned label, was also highest in men with PCa, compared to 3.51%, 2.67%, and 5.51% of cells in post-RP, benign, and young men, respectively (all p<.001). It can be postulated that actively phagocytosing cells are hardest to classify due to their dual immune cell and foreign cell nature. Hence, the higher number of unclassified cells and intermediate monocytes in PCa patients might reflect higher phagocytic activity due to tumor burden. This also illustrates that small numbers (~1%) of circulating peripheral blood monocytes that have interacted with tumor cells might still possess detectable phagocytosed tumor RNA.

 ${\bf Keywords:} circulating monocytes, phagocytic cells, prostate cancer, tumor immune response$ 

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1