687 MDSC GENE EXPRESSION ANALYSIS IN PATIENTS WITH CANCER AND THE RESPONSE TO INHIBITION OF BRUTON'S TYROSINE KINASE

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Background Myeloid-derived suppressor cells (MDSC) are an immunosuppressive immature population of myeloid cells that are elevated in cancer patients. Increased levels of MDSC has been linked to dysregulated anti-tumor responses and reduced efficacy of immune checkpoint therapies thus making them an attractive target. MDSC express Bruton's tyrosine kinase (BTK) and can be depleted using ibrutinib, an FDA-approved irreversible inhibitor of BTK. BTK inhibition leads to reduced MDSC expansion/function in murine models and significantly improved activity of anti-PD-1 antibodies. In this study, single cell RNA-seq (scRNA-seq) was used to characterize the gene expression of MDSC from different cancer types and the effect of ibrutinib on MDSC gene expression.

Methods Peripheral blood mononuclear cells were isolated from patients with melanoma (n=2), head & neck (n=1), and breast cancer (n=1). MDSC were isolated via fluorescence activated cell sorting. MDSC isolated from melanoma patients (n=2) were treated in vitro for 4h with 1 uM ibrutinib or DMSO and scRNA-seq was performed using the Chromium 10x Genomics platform. ScRNA-seq samples were analyzed using the standard integrative workflow of Seurat v3, which addresses the sample heterogeneity. Cell clusters were identified using Seurat and annotated using SingleRversion3.12. Identification of gene markers for each cell cluster and cell-cluster-specific differential expression analyses were conducted using Seurat.

Results Baseline gene expression of MDSC from patients with breast and head & neck cancer revealed similarities among the top expressed genes (S100A8, VCAN, and LYZ). In vitro ibrutinib treatment of MDSC from patients with melanoma resulted in significant changes in gene expression within the MDSC cluster compared to DMSO treatment. GBP1(-1.72 log fold change), IL 1β(-1.27 log fold change), and CXCL8(-0.63 log fold change) were among the top downregulated genes ($p<0.001$) and RGS2 (0.68 log fold change) and ABHD5(0.52 log fold change) were among the top upregulated genes ($p<0.001$). MDSC subset (PMN-MDSC, M-MDSC, early-MDSC, and CD14+/CD15+ double positive) gene expression changes mirrored total MDSC gene changes. Ingenuity pathway analysis revealed significant downregulated pathways including TREM1 ($p<0.001$), nitric oxide signaling ($p<0.003$), and IL-6 signaling ($p<0.004$). Multiple genes associated with cellular movement (CXCL8, CXCL10) and activation of macrophages (CXCL10, CCL3) were downregulated ($p<0.001$). PCR analysis on isolated melanoma MDSC (n=2) treated in vitro with ibrutinib verified downregulation of CXCL8 (0.42 fold decrease, $p<0.05$) and CXCL10 (0.40 fold decrease, $p<0.001$).

Conclusions Analysis via scRNA-seq revealed similar gene expression patterns for MDSC from different cancer patients. There was downregulation of multiple genes and pathways important to MDSC function and migration after BTK inhibition.

Ethics Approval The study obtained ethics approval.
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