Abstract: P752

Title: TRANSCRIPTOMIC ANALYSIS OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS) IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA (CIN)

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Background:

CIN is a disorder characterized by prolonged, unexplained reduction of peripheral blood (PB) neutrophil counts. Immune mechanisms implicating lymphoid and myeloid populations have been involved in the pathophysiology of CIN by affecting the survival of the bone marrow (BM) granulocytic progenitor cells (Papadaki et al, 2003). We have previously shown that CIN patients display lower percentages of PB and BM MDSCs compared to age/sex matched healthy individuals (Bizymi et al, Blood 2022,suppl1). Given that MDSCs normally suppress T-cell responses, we hypothesize that the lower number of MDSCs in CIN may contribute to the maintenance of T-cell activation associated with the disease.

Aims:

We have previously studied the quantitative characteristics of PB and BM MDSCs in CIN and their suppressive effect on T-cell proliferation. In the current study we evaluate the transcriptome profile of MDSCs in CIN to better characterize their functionality.

Methods:

From our cohort of CIN patients and healthy controls previously evaluated for MDSC quantification, we studied the transcriptomic profile of PB MDSCs from 6 patients and 5 controls. MDSC subsets (polymorphonuclear, PMN and monocytic, M) were sorted by flow-cytometry (BD FACSAria III) from the mononuclear cell fraction using the combination of CD33PC7/CD15PC5/HLADRECD/CD14PE/CD11bFITC monoclonal antibodies. RNA was extracted, libraries were produced via 3′ quant-seq FWD strand specific protocol (Lexogen) and were sequenced at 75bp single end length (Nextseq500). Bioinformatic analysis, to map reads to human genome, count/normalize number of reads per gene, and assess statistically differential expressed genes, was performed using the R software, Bioconductor packages and the metascape.org webpage.

Results:

We found that patient and control MDSCs express genes typically mediating immunomodulatory properties, such as S100A8 and NFκB. However, when comparing patients and controls by protein-protein interaction enrichment analysis, several differences were detected. Specifically, in patient PMN-MDSCs, 645 genes (related to positive regulation of lymphocyte proliferation, programmed cell death and signaling by interleukins) were upregulated, whereas 796 genes (related to cellular response to DNA damage, stress and death, chromatin organization, neutrophil degranulation, protein phosphorylation, inflammatory response) were downregulated. In patient M-MDSCs, 205 genes (related to T-cell activation, regulation of cell-cell adhesion, adaptive immune system, leukocyte mediated immunity) were upregulated, whereas 107 genes (related to amino-sugar metabolic process, DNA synthesis for DNA repair, response to toxic substance, interferon signaling, innate immune responses) were downregulated. The upregulated genes in both cell types were indicative for $CD33^{+}$ myeloid cells according to PaGenBase and were found in diverse inflammatory and auto-immune conditions according to DisGeNET.

Summary/Conclusion:

The transcriptome profile of MDSCs in CIN indicates typical immunosuppressive properties. Patient MDSCs, however, show differential expression of a number of genes compared to healthy individuals. Patient MDSCs highly express genes related to T-cell activation/responses, while they express at a lower-level genes related to

innate immunity functions. Genes important for cell viability, such as repair of DNA damage and response to toxic substances, are also expressed at a lower level. These data show altered properties of MDSCs in CIN that may contribute to the pathophysiology of the disease.

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Keywords: Neutropenia, Gene transcription, Immunology