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1. Biology and preclinical

FUNCTIONAL DISSECTION OF THE SINGLE CELL TRANSCRIPTOMIC LANDSCAPE OF GENOTYPICALLY-IDENTIFIED POLYCLONAL PLASMACELLS IN MULTIPLE MYELOMA MICROENVIRONMENT

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Multiple Myeloma (MM) is a plasma cell (PC) dyscrasia usually preceded by asymptomatic stages defined as Monoclonal Gammopathy of Undetermined Significance (MGUS) or Smoldering MM (SMM). Progression is driven by cell-intrinsic factors in the clonal PCs population as well as by changes in the tumorigenic microenvironment (TME). Even if the role of immune and non-immune cells has been extensively studied, the disruption of polyclonal PCs and their role in contributing to TME dysregulation has never been deeply studied. To address this shortcoming, we aimed to dissect the transcriptional landscape of polyclonal PCs in asymptomatic/symptomatic PC dyscrasia , through integrated analysis of single-cell B-cell receptor (BCR) genotyping and single cell RNAsequencing (scRNAseq) in the same single cells. To investigate the CD138pos polyclonal PCs, scRNAseq and scBCR analysis were applied in a cohort of n=46 patients (n=7 MGUS, n=16 SMM and n=23 newly diagnosed NDMM patients). Cells derived from healthy donors (HDs) sourced locally (n=1) and from publicly available repositories (n=14) were used as controls, as well as cells gently shared from collaborators (n=3). Here, we collect a total of n= 213,146 CD138pos PCs, revealing the presence n= 170,428 clonal and n= 42,718 polyclonal cells in patients with PC dyscrasias. The polyclonal nature of the latter group was confirmed by lack of identity with the clonal BCR sequence at nucleotide level and by the lack of CNAs. By the supervised marker genes expression analysis, we have tracked polyclonal PCs across disease stages, revealing the upregulation of genes of TME and inflammation (i.e. ICAM1, VIM, ITGB7), autophagy (i.e. SQSTM1) and interferon pathway (i.e. IFI6, IFITM1) in polyclonal PCs derived from patients, as well as HLA-DPA1 and HLA-DOB, that are fundamental modulators of immune response. The Gene Set Enrichment Analysis showed that inflammatory pathways as HALLMARK ALLOGRAFT REJECTION, HALLMARK INFLAMMATORY RESPONSE, IFN related pathways, resulted overexpressed in MGUS, SMM and NDMM, highlighting a climax of increasing inflammatory status from HD to NDMM. Furthermore, inflammatory and TME PCs marker genes as CD19, GPRC5D and ICAM1 were expressed by SMM patients progressing to MM, while downstream targets of FOS linked with cellular senescence via NF-

kappa-B activation (i.e. HRF3B, CDKN1A, PEL1) were specifically upregulated in asymptomatic patients characterized by immunoparesis, where hallmarks of TNFa, UV RESPONSE, APOPTOSIS, HYPOXIA and P53 signaling were overexpressed. Finally, we performed a network analysis between polyclonal PCs and TME in each patient, highlighting an increased suppressive interaction between polyclonal PCs and effector CD8 T cells, mainly in NDMM. Collectively, our findings provide a comprehensive analysis of the transcriptomic landscape of corrupted polyclonal PCs in patients with asymptomatic and symptomatic PCs dyscrasias, as the first time, showing functional disruptions that may contribute to disease pathogenesis through promoting an inflammatory milieu, as well as explain the typical immunoparesis seen upon progression.

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