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Monoclonal Gammopathies & Multiple Myeloma

IMMUNE DYSREGULATION IN MULTIPLE MYELOMA: UNCOVERING THE ROLE OF NON-MALIGNANT B CELLS

F. Viviani[1], F. Lazzaroni[1], E. Calvi[1], A. Matera[1], A. Marella[2], A. Maeda[1], M. Scopetti[2], C. De Magistris[1], L. Pettine[1], S. Fabris[1], S. Pioggia[1], A. Marchetti[2], C. Leoni[2], G. Castellano[1], M. Barbieri[1], S. Lonati[2], M. Lionetti[2], F. Passamonti[1|2], N. Bolli[1|2], M.C. Da Vià[1]

[1]IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico; [2]Department of Oncology and Hemato-Oncology, University of Milan, Italy

B cells are emerging as key contributors to the tumor microenvironment (TME), with growing evidence supporting their functional diversity and prognostic significance. However, their role in plasma cell (PC) dyscrasias' evolution, including monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM) and MM, remains poorly understood. In this scenario, our study aims to investigate the functional properties and B cell receptor (BCR) repertoire changes of B cells in the TME of PC neoplasms.

Single cell (sc) 5' RNAseq coupled with scBCRseq was performed on the CD138- cell fraction of 41 bone marrow aspirate samples collected from 35 patients at diagnosis (7 MGUS, 13 SMM, and 15 MM) and at progression to overt MM (6 paired samples). A control cohort of 12 healthy donors (HDs) (4 internally sourced, 8 from a public repository) was included. Bioinformatic analysis was conducted using the Seurat pipeline, Gene Set Enrichment Analysis with ClusterProfiler, interactome analysis with Multinichenet, and BCR repertoire profiling applying scRepertoire and Immcantation workflow.

We analyzed a total of 131,814 $CD138^-$ cells, of which 11,716 were labelled as B cells according to the BoneMarrowMap reference atlas.

In MM, compared to asymptomatic stages, there was a significant depletion of precursor stages such as pro- and pre-B cells ($p<0.05$). Differential expression analysis across disease stages revealed a transition from physiological B-cell maturation and metabolic programs (e.g., *RAG1*, *RAG2*, *TCL1A* genes, and OXPHOS, MYC, mTORC pathways) in precursor conditions –particularly in non-progressing cases– to activation- and stress-related features (e.g., *DNAJB1*, *JUN*, *FOS*, *EGR1* genes, and TNFA and IFN pathways) in MM.

To further explore B-cell functionality in MM, we performed unsupervised clustering, identifying 10 subpopulations that we subsequently used as a reference to annotate HD, MGUS, and SMM samples. Notably, small pre-/transitional and mature B cells showed both stressed and IFN-driven phenotypes. We also detected subsets of NR4A+ and atypical memory B cells, which were also identified and validated in an external dataset. In both datasets, these two populations were consistently more prevalent in non-progressing precursors and MM samples, respectively. BCR repertoire analysis of mature B cells revealed a lower rate of class-switch recombination and somatic hypermutation (SHM, $p<0.0001$) in progressing asymptomatic and MM samples. Consistently, a trend toward higher *IGHA1* usage was observed in MGUS and non-progressing cases. BCR diversity, assessed using Hill's index, was highest in MM, suggesting increased reactive polyclonality. These findings highlight the evolving phenotype of specific B-cell populations in PC dyscrasias. In asymptomatic stages, B cells retain more physiological function, whereas in MM they display increased activation, greater polyclonality, and reduced SHM, suggesting a less specific repertoire.