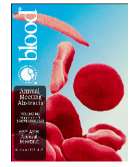
Check for updates

Blood 142 (2023) 332–333



The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Single Cell Analysis of Bone Marrow Stromal Cells Shows That a Pro-Inflammatory and Stress Response Together with Polarized Differentiation of Stromal Elements Characterize the Bone Marrow Microenvironment during Multiple Myeloma Development

Hussein Ghamlouch, PhD[1,2], Dylan Gagler, B.S.[1], Patrick Blaney, MS[1], Eileen Boyle, MBChB, PhD[1,3], Yubao Wang, PhD[4], Liming Hou, PhD[4], Ola Landgren, MD[5], Francesco Maura, MD[5], Aristotelis Tsirigos, PhD[6], Gareth J. Morgan, MD, PhD[4], Faith E. Davies, MD[7]

[1] Perlmutter Cancer Center, NYU Langone Health, New York
[2] Inserm, Villejuif, FRA
[3] Department of Hematology, UCL Cancer Institute, London, United Kingdom
[4] Perlmutter Cancer Center, Multiple Myeloma Research Program, NYU Grossman School of Medicine, NYU Langone Health, New York, NY
[5] Sylvester Comprehensive Cancer Center, Myeloma Division, University of Miami, Miami, FL
[6] Applied Bioinformatics Laboratories, New York University School of Medicine, New York
[7] NYU Langone Health, New York, NY

Current knowledge of the biological role of the non-hemopoietic components of the bone marrow (BM) niche to the pathogenesis of multiple myeloma (MM) is poorly understood. We utilized a murine model to interrogate niche forming cells early in the natural history of MM, at a precursor stage (MGUS/smoldering-like) in more detail. Studying interactions between the MM clone and the stromal components of the MM niche are challenging as they represent only approximately 0.2% of the total marrow content and are difficult to isolate. However, by isolating the stromal compartment of the BM by depleting hemopoietic cells via flow sorting combined with the use of single cell RNA sequencing (scRNA-seq) it is possible to perform an *in-vivo* analysis of the individual cellular components of the stromal microenvironment and to characterize their relative abundance, cellular differentiation state, and transcriptomic profile.

To comprehensively assess changes in the stromal cells during the early stage of MM, 5TGM1 cells were intravenously injected into aged (6 month old) KaLwRij mice. KaLwRij mice without MM were used as controls. Stromal cells were isolated following tumor engraftment in the marrow at a precursor stage and enriched as previously described (Baryawno et al, Cell 2019). scRNA-seq was performed on 5 libraries of cells using the 10X Genomics Chromium platform.

All data analysis was performed in R. After filtering out low quality cells, 45,030 cells remained for analysis. The 5 libraries were integrated with Seurat and run through a standard clustering workflow involving log-normalization, finding variable features, dimensional reduction, and clustering. Cell type annotation for each cluster was performed manually using established markers for both the stromal and immune compartments. After excluding immune cell populations, 14,219 cells remained. Select subpopulations were re-clustered by isolating the cell types of interest and running them through separate standard clustering workflows. For each population of interest, differentially expressed genes were determined using the Wilcox rank sum test in Seurat, trajectory analysis and pseudotime were computed using Monocle3, and enriched gene sets were computed using GSEAPreranked.

We identified 7 distinct populations of stromal cells including mesenchymal stromal cells (MSCs) (*Lepr*, *Adipoq*, and *Cxcl12)*, osteo-lineage cells (OLCs) (*Bglap*, *Spp1*, and *Sp7)*, fibroblasts (*S100a4*, *Fn1*, and *Dcn)*, chondrocytes (*Col2a1*, *Sox9*, and *Acan)*, pericytes (*Acta2*, *Myh11*, and *Mcam)*, and two endothelial cell (ECs) populations, arterial (AEC) and sinusoidal (SEC) (*Cdh5*, *Cd34*, and *Pecam1)*. Compared to normal BM, MM engraftment was associated with numerical differences in stromal cell populations between normal and MM. Gene set enrichment analysis showed an inflammatory and oxidative stress signal associated with the MM microenvironment. Sub-clustering analysis showed MSC differentiation was polarized away from osteocyte formation towards adipocytes with the identification of a novel population only seen in MM. Bone marrow endothelial cell populations were also substantially altered at this early disease stage, with differentiation polarized towards sinusoidal endothelial cells generating a pro-angiogenic /pro-inflammatory phenotype. An increase in cells undergoing endothelial to mesenchymal transition (EndMT) was also present.

© 2023 by The American Society of Hematology

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/332/2199220/blood-4936-main.pdf by guest on 19 May 2024

Taken together, we show, for the first time, existence of remodeling of the stromal populations induced by the MM clone characterized by a pro-inflammatory phenotype together with polarized differentiation. These changes result in the expansion of a number of key populations that increase the MM niche contributing to growth and survival signals and shaping of the content of the immune microenvironment. These changes result in a self-perpetuating signaling loop between cells, which needs to be broken therapeutically in order to stop progression, induce remission and long-term disease outcomes.
Conclusion - In early stages of MM pathogenesis, MM cells remodel the stromal microenvironment by altering the amount and function of MSCs and endothelial cells. Through favoring an adipocytic fate of MSCs, endothelial mesenchymal transition and altering the balance between arterial and sinusoidal endothelial cells, MM cells promote an inflammatory environment that contributes to MM development and progression.

Disclosures Landgren: *Merck:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees; *Amgen:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees; *Takeda:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees; *Janssen:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees; *Celgene:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees; *Adaptive:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees; *Theradex:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: Membership on independent data monitoring committees. **Davies:** *Amgen:* Membership on an entity's Board of Directors or advisory committees; *Janssen:* Membership on an entity's Board of Directors or advisory committees; *sanofi:* Membership on an entity's Board of Directors or advisory committees; *pfizer:* Membership on an entity's Board of Directors or advisory committees; *BMS / Celgene:* Membership on an entity's Board of Directors or advisory committees; *Regeneron:* Membership on an entity's Board of Directors or advisory committees; *Takeda:* Membership on an entity's Board of Directors or advisory committees.

https://doi.org/10.1182/blood-2023-182968

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/332/2199220/blood-4936-main.pdf by guest on 19 May 2024