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POSTER ABSTRACTS

631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Mechanical Checkpoint Regulates Monocyte Differentiation in Fibrotic Matrix

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Abstract Myelofibrosis (MF) is a progressive, myeloid malignancy characterized by deposition of collagen and reticulin fibers in the bone marrow (BM). Previous studies have shown that monocytosis is associated with poor prognosis in MF, highlighting a potential pathogenic role for monocytes in MF. Although many studies have addressed the role of cell-intrinsic and soluble extracellular factors in MF development, it is currently unknown if mechanical properties of fibrotic BM contribute to aberrant differentiation of myeloid cells and of monocytes in particular.

We first defined the stiffness and viscoelastic properties of healthy and fibrotic BM. Stiffness is defined as the resistance of a matrix to deformation, while viscoelasticity is the rate of dissipation of an applied stress over time. Independent of stiffness, an applied stress relaxes rapidly in a more viscous, liquid-like matrix, whereas in a more elastic, solid-like material, stress relaxes slowly.

We next generated a cohort of fibrotic and non-fibrotic mice by transplanting retrovirally transduced JAK2V617F or empty vector (EV) control hematopoietic stem and progenitor cells (HSPCs) into lethally irradiated recipients. Femurs from these mice were harvested seven months post-transplant, as well as from age- and sex-matched healthy primary mice. Nanoindentation was performed to measure BM stiffness and viscoelasticity. Fibrotic BM showed higher stiffness, as well as trending higher elastic, solid-like properties, compared to BM of control mice.

We then aimed to study the effect of matrix stiffness and viscoelasticity on monocytes. Human BM-derived monocytes were encapsulated in stiff, viscous or stiff, elastic hydrogels and cultured in the presence of GM-CSF, IL-4, and PGE2 for 3 days, followed by nanoString and flow cytometry analyses. Cells in elastic gels upregulated gene sets associated with co-stimulatory molecules and cytokine receptor signaling, MHC class II antigen presentation, and regulation of extracellular matrix (ECM), compared to cells in viscous gels of the same stiffness. The fraction of dendritic cells (DCs) was significantly upregulated, as indicated by double-positive CD11c+CD1c+ (40.9% viscous vs 69.5% elastic of CD11b+HLA-DR+ cells) and CD80+ cells (20.9% viscous vs 62.7% elastic of CD11b+HLA-DR+ cells), and surface expression of HLA-DR (gMFI 2587 viscous vs 6334 elastic). Consistent with these findings, the fraction of pro-fibrotic SLAMF7+ cells (4.2% viscous vs 17.3% elastic) were also significantly higher in elastic gels. Together, these data suggest that stiff, elastic ECM drives pro-inflammatory polarization and differentiation of monocytes into antigen-presenting cells.

Next, we examined the role of the cytoskeleton on human monocyte differentiation. Cortical F-actin was significantly upregulated in cells in stiff, elastic gels compared to viscous gels. Cells were exposed to a highly selective small molecular inhibitor

of the γ-isoform of PI3K. Treatment with the PI3Ky inhibitor significantly reduced F-actin staining of cells in elastic gels, upregulated immature monocyte markers, reduced surface expression of HLA-DR, and downregulated the cytokines IL6, IL8, CCL4, which have previously been associated with disease progression in myelofibrosis.
In line with the above human *ex vivo* data, BM isolated from fibrotic mice (described above) showed skewing towards Ly6G-Ly6C+ monocytes (a population enriched for inflammatory monocytes) within the CD11b myeloid compartment compared to control transplanted mice or to non-fibrotic mice that were transplanted with endogenously expressing Jak2V617F cells. Additionally, the percentage of conventional DCs (cDCs) was increased in fibrotic Jak2V617F mice compared to control mice. Importantly, 16 day *in vivo* treatment with the PI3Ky inhibitor significantly reduced the fraction of Ly6G-Ly6C+ monocytes within the CD11b compartment as well as the fraction of cDCs, compared to vehicle-treated Jak2V617F mice.
In summary, fibrotic BM is stiffer and more elastic than normal BM. Our studies show that a stiff, elastic BM environment drives monocytes towards a more pro-inflammatory state which can in part be suppressed by PI3K-γ inhibition. Our results have relevance for human MF by demonstrating that a fibrotic BM niche is not just a consequence of chronic inflammation but is also inflammation-promoting.
KHV and AEM contributed equally to this work.

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