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POSTER ABSTRACTS

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

Proteomic Screening Identifies Megakaryocyte Derived PF4/Cxcl4 As a Critical Driver of Myelofibrosis

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Background: Despite advances in understanding the genomic landscape of Myeloproliferative Neoplasms (MPNs), the precise pathological mechanisms driving fibrotic progression in MPNs remain incompletely understood. Previous research has shown that Megakaryocytes (Mks) play a role in MPN pathology by promoting inflammation and extracellular matrix (ECM) deposition by activated stromal cells. However, the specific factors derived from Mks that contribute to the inflammatory milieu and myelofibrosis progression are not well defined.
Aims: To investigate the events that drive bone marrow (BM) fibrosis due to abnormal megakaryocyte-stromal crosstalk, we employed mass spectrometry-based proteomics in a myelofibrosis-like mouse model. Our goal was to identify differentially expressed proteins involving Mks, platelets and BM cells in fibrotic versus non-fibrotic conditions.
Methods: We induced myelofibrosis in mice through repeated injections of supra-pharmacological doses of the thrombopoietin-mimetic Romiplostim (TPO ^{high}). Subsequently, we isolated Mks, BM cells and platelets from TPO ^{high} and control mice (injected with saline) and analyzed protein extracts using LC-MS/MS assay. Cytokine levels in plasma and BM cell-free fluids of treated mice were assessed using cytokine arrays. To evaluate fibrosis-related markers, we analyzed the expression of myofibroblasts markers (a-SMA, Vimentin) and ECM synthesis (Fibronectin, Type III Collagen) in mouse embryonic fibroblasts (mEFs) and human BM mesenchymal stromal cells after stimulation with recombinant PF4 using western blot. mEFs were co-cultured with BM-derived Mks in direct and indirect co-culture systems in the presence of increasing TPO concentrations. In some experiments, Mks were transfected with siRNA specifically targeting CXCL4 gene or control siRNA before co-culture with mEFs. Additionally, we quantified plasma PF4/Cxcl4 levels using ELISA assay in a large cohort of MPN patients and normal controls. We also sorted CD41/CD61 Mks from BM aspirates of MPN patients and evaluated CXCL4 mRNA levels through qPCR.
Results: We observed that signaling pathways related to cytoskeletal reorganization, cell adhesion and inflammation, were commonly activated in Mks, platelets and BM cells of TPO ^{high} mice compared to control mice. The MSP-RON signaling pathway and the Unfolded Protein Response were specifically activated in Mks and platelets, respectively. Among the differentially expressed proteins,the chemokine PF4/Cxcl4 was up regulated exclusively in the proteasomes of the TPO ^{high} mice. We confirmed Increased levels of PF4/Cxcl4 in plasma and BM cell-free fluids of fibrotic mice using cytokine arrays. *In vitro* stimulation of BM-derived Mks with high TPO concentrations resulted in increased synthesis and secretion of PF4/Cxcl4. Recombinant PF4/Cxcl4 was rapidly internalized through surface glycosaminoglycans by mEFs and human BM mesenchymal stromal cells, leading to myofibroblast differentiation. Importantly, we demonstrated that these mechanisms were interconnected during Mk-mEF cross-talk in co-culture systems with high TPO concentrations. Genetic down-regulation of CXCL4 in Mks, prior to co-culture in TPO-saturated conditions, mitigated the profibrotic phenotype of mEFs in terms of ECM synthesis and myofibroblast markers expression. Finally, we found higher plasma levels of circulating PF4/Cxcl4 and increased expression in BM-sorted Mks in patients with MPNs.
Conclusions: Our findings identify a crucial role of Mk derived PF4 in the fibrosis progression of MPNs, providing further support for the potential therapeutic strategy of neutralizing PF4.

Disclosures Cattaneo: *Novartis, Pfizer, Incyte, BMS, GSK:* Honoraria. **Iurlo:** *Novartis, Pfizer, Incyte, BMS, GSK, AOP Health:* Honoraria.

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