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POSTER ABSTRACTS

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

Dysregulated Complement Activation in Polycythemia Vera: A Novel Mechanism for Thrombosis in Myeloproliferative Neoplasms Uncovered By Proteomic Analysis

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Introduction: Myeloproliferative neoplasms (MPNs) are a group of disorders associated with an increased risk of thrombosis, with polycythemia vera (PV) considered to carry the highest risk. This study aims to investigate potential mechanisms underlying the increased thrombotic risk in MPNs through mass spectrometry-based proteomic analysis of bone marrow trephines.
Methods: We conducted data-independent acquisition (DIA) proteomic analysis on archived, formalin-fixed paraffin-embedded (FFPE) bone marrow trephine samples from 60 MPN patients (20 with PV, 20 with essential thrombocythemia [ET], and 20 with primary myelofibrosis [PMF]) and 20 controls. The control samples were from age- and sex-matched patients with limited-stage B-cell lymphoma and uninvolved, morphologically normal bone marrow biopsies, collected between 2009 and 2016 from The Royal Melbourne Hospital. Following sample preparation, purified and fractionated peptides were analyzed using DIA and a library-free search approach. Protein expression differences were determined using paired-test pair-wise comparisons and ANOVA with limma software (v.3.52.4). Gene set enrichment analysis (GSEA) using Reactome software identified differentially expressed gene pathways between the MPN subgroups and controls.
Results: A total of 4,791 proteins were quantified across all samples. Compared to the control group, 1154, 354, and 53 proteins were differentially expressed in the PMF, ET, and PV groups, respectively. GSEA analysis showed upregulation of the hemostasis pathway in ET (normalized enrichment score [NES] 1.72, adjusted p-value 0.05) and PMF (NES 1.7, adjusted p-value <0.001), as well as the platelet activation, signaling, and aggregation pathway in ET (NES 1.81, adjusted p-value 0.05) and PMF (NES 1.83, adjusted p-value <0.001). In PV, there was reduced expression of proteins involved in the regulation of the complement cascade (NES -4.45, adjusted p-value <0.001), complement cascade (-4.28, adjusted p-value <0.001), and initial triggering of the complement cascade (NES -2.69, adjusted p-value 0.01) pathways.
Soluble complement proteins, including Factor B, Factor D, C1S, C1R, C2, C3, C4A, C5, C6, C7, C9, and components of C1Q, C8, and C4B, were reduced in PV samples compared to controls. These proteins, typically produced by the liver, are often decreased following complement activation. Similarly, soluble complement regulatory proteins, such as complement factor H (CFH), complement factor H-related proteins (CFHR1, CFHR2, CFHR5), complement factor I (CFI), C1 inhibitor (SERPING1), and C4B binding protein alpha (C4BPA), were reduced in PV samples. Additionally, membrane-bound complement regulatory proteins, including CD55, CD59, and CD35, which are expressed by myeloid cells in the bone marrow, were unexpectedly reduced in PV samples despite panmyelosis.
Other upregulated pathways in MPN groups include integrin cell surface interactions, non-integrin membrane-ECM interactions, cell-cell communication, platelet degranulation, and response to elevated platelet cytosolic Ca^{2+} pathways in PMF, and mitochondrial translation pathway in PV.
Discussion: The underlying mechanisms of thrombosis in MPNs remain incompletely understood. Previous studies have highlighted the role of neutrophil extracellular traps (NETs), and upregulated adhesion molecules on platelets and neutrophils in MPN-associated thrombosis (Wolach et al., Blood 2016). Whether or not complement activation also contributes to this risk is currently unknown and could be relevant given the established interplay between NETs, coagulation factors, and the complement pathway (de Bont et al., Cell Mol Immunol 2019).
In this study, we show a decreased expression of complement regulatory proteins and increased activation and consumption of complement proteins in the bone marrow of PV patients, relative to controls, suggesting heightened thrombo-inflammation in this group. The reduction of these proteins in PV may indicate that clonal neutrophils, known to be prone to NET formation,

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are subsequently more susceptible to complement activation due to a relative deficiency of membrane bound complement regulatory proteins.

In conclusion, we show for the first time that dysregulated complement activation is most pronounced in PV patients. This may help explain the higher rates of thrombosis observed in PV compared to other MPNs.

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