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

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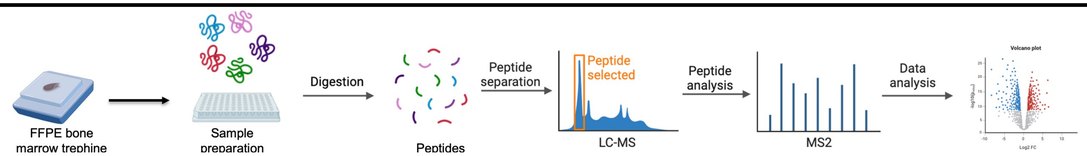
INTRODUCTION

Proteomic changes in bone marrow trephines of patients with myeloproliferative neoplasms (MPNs) is largely unexplored. In this study, we have taken an unbiased approach to investigating changes underlying the increased thrombotic risk in MPNs through mass spectrometry based proteomic analyses.

METHODS

	PV	ET	PMF	Control
Number	20	20	20	20
Median Age	61.5	57	69	63
Male	50%	30%	65%	60%
JAK2V617F	100%	50%	50%	N/A

Table 1 *Patient demographics*



- 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 who were age- and sex-matched patients with limited-stage B-cell lymphoma and uninvolved bone marrow, from the Royal Melbourne Hospital.
- We performed gene set enrichment analysis (GSEA) to identify differentially expressed gene pathways between the MPN subgroups and controls.

RESULTS

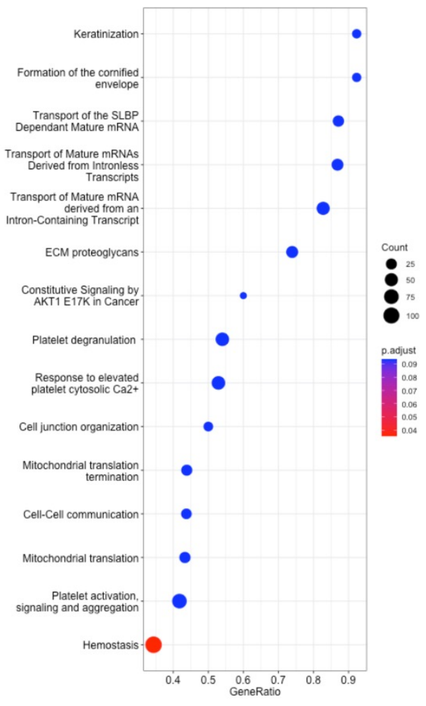


Figure 1 *GSEA analysis in ET vs controls*

Figure 2 *GSEA analysis in PMF vs controls*

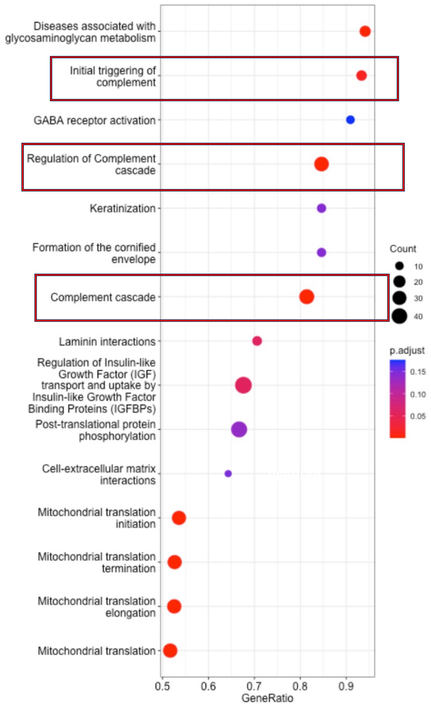


Figure 3 *GSEA analysis in PV vs controls*

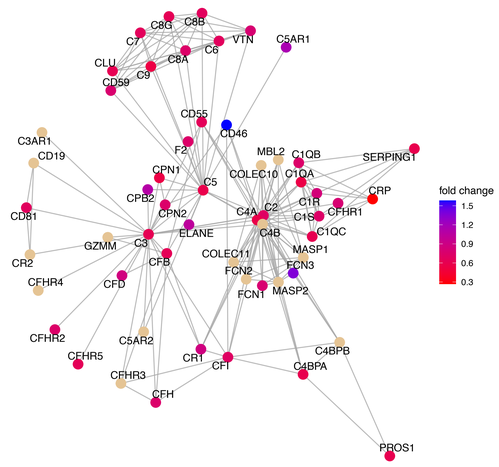


Figure 4 *Interactive plot of proteins in Complement Cascade pathway in PV compared to control. Mean PV protein expression expressed as fold change vs control, blue = high, red = low, brown = not detected.*

<u>On GSEA analysis there was</u>

- **Upregulation of haemostasis pathway in ET when compared to control samples**
- **Upregulation of haemostasis, extracellular matrix (ECM) organization, integrin cell surface interactions, response to elevated platelet cytosolic calcium, ECM proteoglycans, elastic fibre formation, non integrin cell surface interactions, molecules associated with elastic fibres and downregulation of lagging strand synthesis pathways in PMF when compared to control samples.**
- **Upregulation of mitochondrial translation initiation, elongation and termination pathways, and downregulation of complement cascade, initial triggering of complement, and regulation of complement cascade pathways, and diseases associated with glycosaminoglycan metabolism pathway in PV when compared to control samples.**

<u>Complement cascade pathway</u>

- **Soluble complement proteins, including Factor B, Factor D, C1S, C1R, C2, C3,C4A, C5, C6, C7, C9, and components of C1Q, C8, and C4B, and 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 compared to controls suggesting increased consumption and activation.**
- **Membrane-bound complement regulatory proteins, including CD55, CD59, and CD35 were reduced in PV samples despite pan-myelosis**

DISCUSSION

- MPNs are associated with an increased risk of thrombosis, and unsurprisingly there was an upregulation of related pathways such as haemostasis pathway in ET and PMF, and integrin cell surface interactions and response to elevated platelet cytosolic calcium pathways in PMF.
- There was decreased expression of soluble complement proteins and membrane bound complement regulatory proteins suggesting increased activation of the complement pathway, and consumption of soluble complement proteins in the bone marrow of PV patients, relative to controls.
- 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).
- Dysregulated complement activation is most pronounced in PV patients and this could play a crucial role in thrombosis associated with PV

REFERENCES

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2. de Bont, Cynthia M., Wilbert C. Boelens, and Ger JM Pruijn. "NETosis, complement, and coagulation: a triangular relationship." *Cellular & molecular immunology* 16.1 (2019): 19-27.

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