Gene Expression of CXCL1 (GRO-α) and EGF by Platelets in Myeloproliferative Neoplasms

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We read with interest the recent publication by Øbro et al in HemaSphere documenting elevated cytokine levels in patients with myeloproliferative neoplasms (MPN). Specifically, their finding of a signature associated with essential thrombocythemia (ET) that included three biomarkers: GRO-α, EGF and eotaxin. GRO-α was of greatest interest as elevated levels were associated with disease transformation to myelofibrosis (MF). Further, based on intracellular flow cytometry, the source of GRO-α was found to be CD56⁺CD14⁺ pro-inflammatory monocytes. Since GRO-α is stored within and secreted from platelet granules upon activation, the associated editorial hypothesized whether the circulating GRO-α could be platelet-derived. We have explored this possibility, using a targeted next-generation sequencing approach, to assess whether the expression of genes encoding these cytokines is dysregulated in platelets from patients with MPN. We also assessed EGF and eotaxin to determine whether the changes seen in circulating levels have any platelet association in MPN subtypes.

Blood was collected from 70 MPN patients (21 PV, 33 ET, 16 MF) and 15 controls, 40 of whom have been previously described. The project had ethical approval from the Sir Charles Gairdner Hospital (#2012-094, #2016-107) and University of Western Australia Human Research Ethics Committees (#RA/4/1/6366, #RA/4/1/9100), in accordance with the Declaration of Helsinki. Platelets were isolated, RNA extracted (miRNeasy Mini Kit, Qiagen) and libraries prepared (Ion AmpliSeq Transcriptome Human Gene Expression Kit, ThermoFisher Scientific). Equimolar concentrations of barcoded libraries were pooled and underwent automated template preparation and sequenced on an Ion Proton Sequencer (ThermoFisher Scientific). Statistical analysis of normalized counts was performed using DESeq2 (R/Biocondutor; http://www.bioconductor.org/) was used to normalize counts and perform differential gene expression analysis for CXCL1 (GRO-α), EGF and CCL11 (eotaxin).

Our platelet transcript data show that platelets in MF, but not ET or PV, have statistically significantly elevated CXCL1 (GRO-α) expression. This is of interest as GRO-α is produced by megakaryocytes and platelets in a thrombopoietin-inducible manner. Platelets store GRO-α in the α-granules whereupon it is released on platelet activation and granule exocytosis. This induces platelet tethering to CD14⁺ monocytes via CD62P and P-selectin glycoprotein ligand-1 (PSGL-1) binding. The platelet-monocyte binding is associated with increased monocyte activation and production of pro-inflammatory cytokines IL-6 and TNF-α, as well as transition to an adhesive phenotype, through upregulation of Mac-1 and VLA-4. In MPN, megakaryocyte production and apoptosis, megakaryocyte thrombopoietin signal transduction and platelet function are all abnormal, with the
most significant defects being in MF. Our finding of increased CXCL1 (GRO-α) platelet gene expression in MF, together with the increased GRO-α cytokine production from CD56+CD14+ monocytes in ET by Øbro et al suggests the platelet-monocyte nexus may have an important contribution to the inflammatory cytokine profile of MPN and disease progression.

Both platelets and CD56+CD14+ monocytes may therefore be sources of GRO-α in MPN. While activated pro-inflammatory monocytes were shown to be the source of GRO-α in ET, the basis for the decreased GRO-α cytokine levels in MF still requires explanation. If platelets are a major source of GRO-α, as seen in reactive thrombocytosis, it may be due to failed production, or loss of GRO-α because of hypoactive and “exhausted” platelets. Further transcriptomic, proteomic and functional studies are required to characterize the platelet-monocyte nexus and its potential role in chronic inflammation and disease progression in MPN.

**Disclosures**

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