From bench to bedside and back to bench: investigating the role of platelet heterogeneity in coronary artery disease

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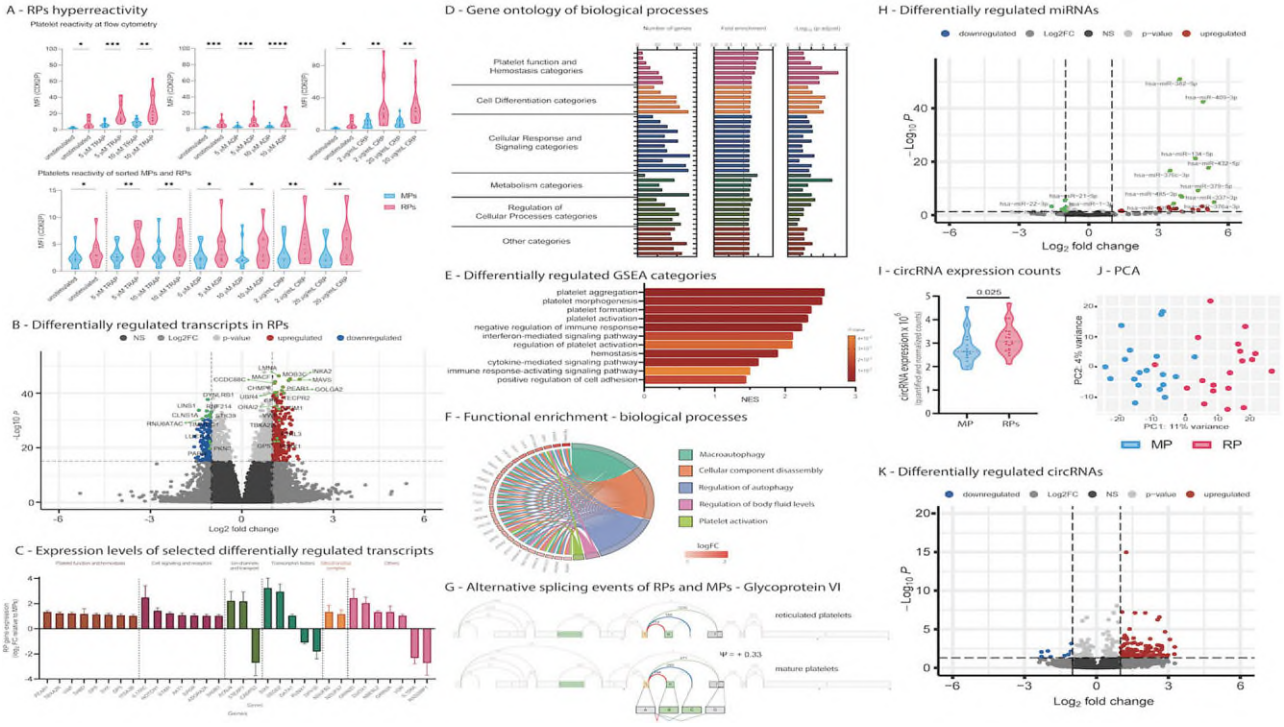
Background/Introduction: Platelets exhibit considerable heterogeneity in RNA content, size, and thrombogenicity. Our preliminary investigation of the transcriptome of the RNA-rich reticulated platelets(RPs) in healthy donors revealed an enrichment of prothrombotic transcripts compared to mature platelets(MPs). Recently, we validated the prognostic role of elevated RPs at clinical level in a modern cohort of ACS patients treated with potent P2Y12 inhibitors. However, the pathophysiology of RP hyperreactivity remains unclear, particularly its correlation with adverse events and cardiovascular death.

Purpose: To study the biology of RPs in patients with chronic coronary syndrome(CCS) and to elucidate their role in disease progression.

Methods: RPs were isolated from peripheral blood of CCS patients(N=20) and compared with MPs. Cell viability and reactivity of RPs were quantified by FACS. Deep transcriptomic profiling was performed using post-sorting bulk RNA sequencing of RPs and MPs and analyzed with ad-hoc bioinformatic pipelines. Proteomic profiling using mass cytometry(CyTOF) was used to validate the transcriptomic findings with single cell resolution.

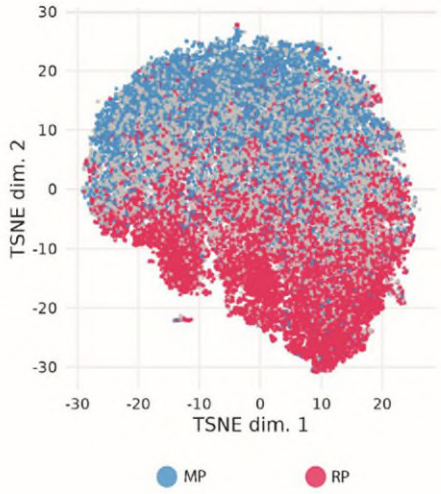
Results: FACS analysis confirmed RPs hyperreactivity showing increased P-Selectin expression upon activation with TRAP, ADP and Collagen(Fig. 1A). Total RNA-sequencing detected 2213 differentially regulated genes with an enrichment in RPs of key functional transcript such as, the von Willebrand Factor VWF(p=3.06*10-33), the thromboxane receptor TBXA2R(p=2.07*10-29) and the collagen receptor GP6(p=1.29*10-38, Fig. 1B-C). Gene ontology and gene set enrichment analyses detected an upregulation of relevant categories as "Regulation of platelet activation" and "Platelet aggregation"(Fig. 1D-F). In addition, we detected a novel alternative splicing event on the collagen receptor transcript GP6 upregulated in RPs(Fig. 1G). We detected 33 differentially regulated miRNAs, including miR-409, miR-134 and miR-432, which have been already linked to prothrombotic phenotypes(Fig. 1H). Additionally, we detected an enrichment of circular RNAs in RPs compared to MPs with several circular RNA upregulated in RPs, that have not been described before(Fig. 1I-K). Mass cytometry detected a significant upregulation in RPs of relevant proteins including the collagen receptor GPVI(p=8.98*10-12, Fig.2) and the thrombin receptor PAR1(p=5.21*10-11). Validation assays in an independent cohort detected an upregulation of the PI3K/AKT pathway in RPs which is downstream of the receptor PAR1 and GP6.

Conclusion: This study represents the first comprehensive multiomic characterization of RPs in CCS patients, providing insights into their prothrombotic phenotype and into their correlation with cardiovascular events. These findings suggest potential avenues for tailored therapeutic interventions targeting the identified upregulated pathways in patients with elevated RPs, warranting further investigation at the clinical level.

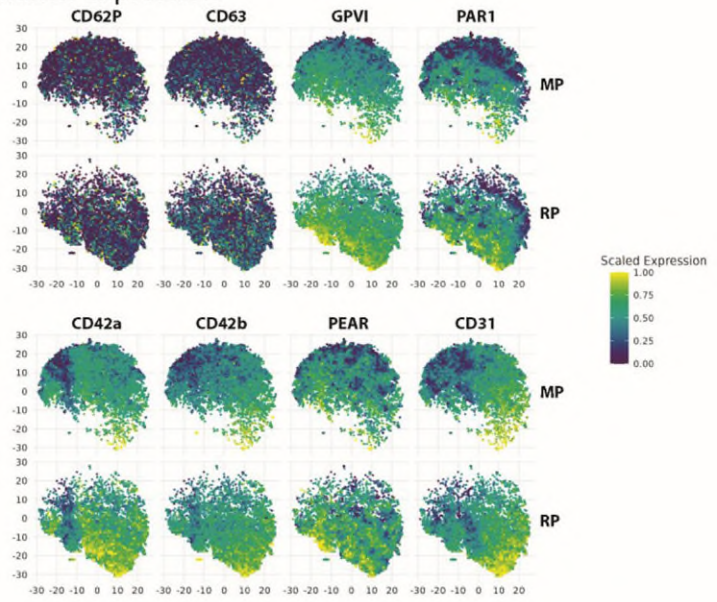

A - RPs hyperreactivity
Platelet reactivity at flow cytometry
Platelets reactivity of sorted MPs and RPs
MPs
RPs
B - Differentially regulated transcripts in RPs
NS
Log2FC
p-value
upregulated
downregulated
Log2 fold change
C - Expression levels of selected differentially regulated transcripts
D - Gene ontology of biological processes
Platelet function and Hemostasis categories
Cell Differentiation categories
Cellular Response and Signaling categories
Metabolism categories
Regulation of Cellular Processes categories
Other categories
E - Differentially regulated GSEA categories
platelet aggregation
platelet morphogenesis
platelet formation
platelet activation
negative regulation of immune response
interferon-mediated signaling pathway
regulation of platelet activation
hemostasis
cytokine-mediated signaling pathway
immune response-activating signaling pathway
positive regulation of cell adhesion
NES
F - Functional enrichment - biological processes
Macroautophagy
Cellular component disassembly
Regulation of autophagy
Regulation of body fluid levels
Platelet activation
logFC
G - Alternative splicing events of RPs and MPs - Glycoprotein VI
reticulated platelets
Ψ = + 0.33
mature platelets
H - Differentially regulated miRNAs
downregulated
Log2FC
NS
p-value
upregulated
Log2 fold change
I - circRNA expression counts
0.025
MP
RPs
J - PCA
PC2: 4% variance
PC1: 11% variance
MP
RP
K - Differentially regulated circRNAs
downregulated
Log2FC
NS
p-value
upregulated
Log2 fold change

Mass cytometry of platelet subgroups

Dimensionality reduction



Marker expression



Platelet subgroup differential protein expression