Reticulated platelet mass cytometry reveals unexplored therapeutic targets in patients with chronic coronary syndrome

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Background: Reticulated platelets (RPs) are young, hyper-reactive thrombocytes that contain more RNA compared with mature platelets (MPs). The measurement of RPs level in peripheral blood with point-of-care systems is fast, reproducible, and inexpensive. Elevated RPs in peripheral blood predict adverse events in patients with acute and chronic coronary syndrome through unknown mechanisms. Preliminary transcriptome analyses reported an enrichment of pro-thrombotic transcripts. However, proteomic analyses are not available, and the biological features of RPs are largely unknown.

Purpose: We aimed to perform the largest proteomic characterization of RPs using mass cytometry with single-cell resolution in patients with chronic coronary syndrome (CCS) undergoing dual antiplatelet therapy (DAPT).

Methods: Thrombocytes from peripheral blood of CCS patients were isolated, prepared for mass cytometry (CyTOF) and stained with a custom-made CyTOF-panel of 20 antibodies targeting important transmembrane proteins (anti-CD9, anti-CD29, anti-CD31, anti-CD36-, anti-CD40, anti-CD41, anti-CD42a, anti-CD42b-, anti-CD47, anti-CD61, anti-CD62P-, anti-CD63, anti-CD69, anti-CD107a, anti-CD154, anti-GPVI, antiGPIIb/GPIIIa complex, anti-Par1, anti-PEAR-1 and the negative control anti-CD3 coupled with different metal isotopes). Two samples were prepared from each donor: one baseline sample (non-stimulated platelets) and one sample stimulated with 10 µM thrombin receptor-activating peptide (TRAP). According to previous experiences and common practice, we detected RPs and MPs based on their RNA content. We analyzed the results with a custom bioinformatic pipeline.

Results: 13 patients with CCS on DAPT were included in this study. Mass cytometry highlighted an expression heterogeneity of relevant transmembrane proteins in thrombocytes of CCS patients (Figure 1A-B colored according to expression level: from blue-low to red-high). CyTOF detected an upregulation of important transmembrane receptors in RPs compared to MPs in quiescent platelets: GPVI ($p<0.0001$), PAR-1 ($p<0.0001$), GPIX ($p<0.0001$), and GPIbα ($p<0.0001$, Figure 1C). After TRAP-stimulation, RPs expressed higher levels of the activation markers P-Selectin ($p=0.0016$) and LAMP-3 (CD63, $p<0.0001$) compared to MPs confirming RPs hyperactivity (Figure 1D).

Conclusion: We here describe the first biological proteomic characterization with single-cell resolution of RPs biology in CCS patients. The up-regulation of the activation markers P-Selectin and LAMP-3 as well as of specific transmembrane proteins as the collagen receptor GPVI and the thrombin receptor PAR-1 in patients treated with DAPT (schematic overview in Figure 2) provides the first solid biomolecular explanation of RPs hyper-reactivity and involvement in cardiovascular disease. Moreover, these results offer unexplored therapeutic targets to tailor antiplatelet therapy based on platelet protein expression in patients with elevated RPs

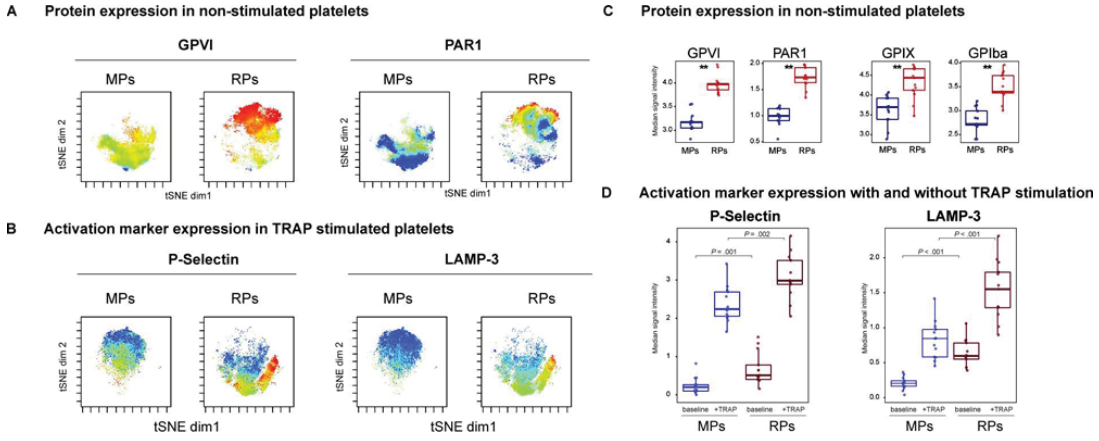

Figure 1. Platelet expression

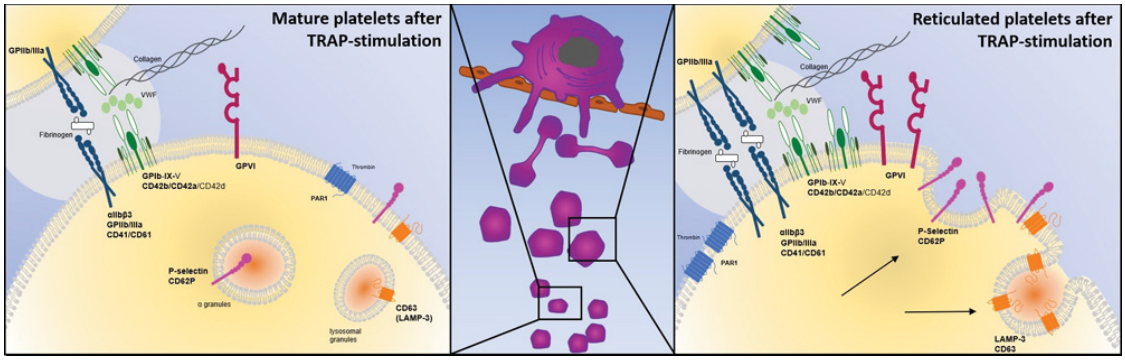

Figure 2. Schematic overview