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

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Purpose

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.

Methods

Thrombocytes from peripheral blood of CCS patients were isolated, prepared for mass cytometry (CyTOF) and stained with a custom-made CyTOF-antibody 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.

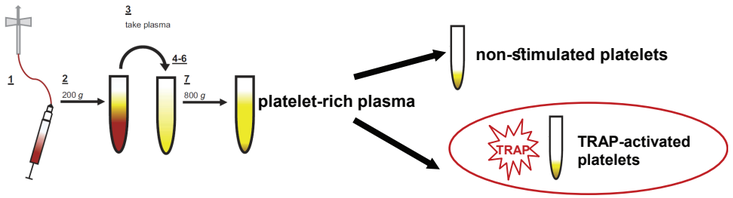

Figure 1: Experimental setup. From preparation of PRP to the two stained samples of each donor.

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). 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).

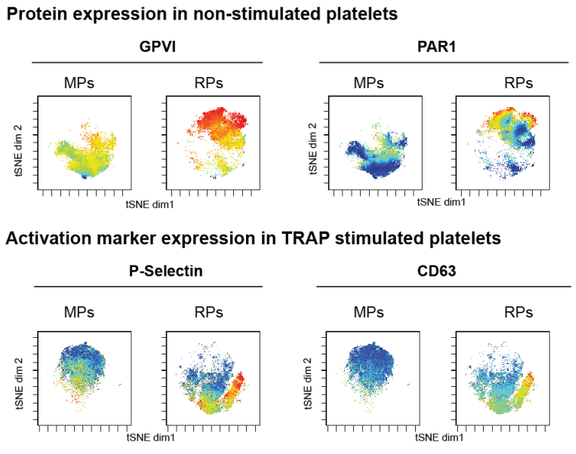

Figure 2: Exemplary t-distributed stochastic neighbor embedding plot of 1 donor colored after the expression (red=high, blue=low expression) showing the proteins A) GPVI and PAR1 in non stimulated platelets and B) P-Selectin and CD63 in platelets after stimulation with 10µM TRAP.

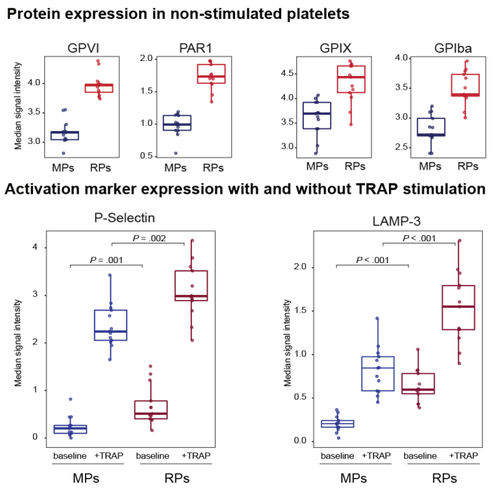

Figure 3: Median Signal Intensity of transmembrane proteins in non stimulated platelets and of activation markers in MPs and RPs population before and after stimulation with 10 µM TRAP in n=13 donors.

MPs vs. RPs at baseline, P-Selectin p=1.0*10^-3, CD63 p=6.4*10^-8. Horizontal line = median, the top and bottom=interquartile range (Q1–Q3), whisker bars indicate largest observation <= to the upper inner fence (UIF = Q3 + 1.5 × IQR) or the smallest observation >= to the lower inner fence (LIF = Q1–1.5 × IQR).

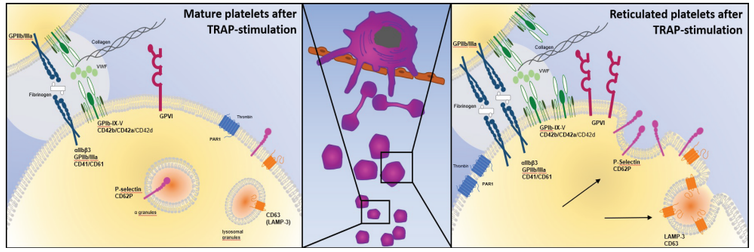

Figure 4: Illustration of protein surface expression in mature platelets and reticulated platelets after TRAP- stimulation.

Conclusion

We performed the first mass cytometric characterization of RPs in CCS.

We detected an upregulation of the activation markers P-Selectin and LAMP-3 as well as of the collagen receptor GPVI and the thrombin receptor PAR-1.

These results provides the first solid biomolecular explanation of RPs hyper-reactivity and offer unexplored therapeutic targets to tailor antiplatelet therapies.