Next Generation Sequencing reveals profound transcriptomic differences between reticulated and non-reticulated platelets from healthy donors, CCS- and STEMI patients

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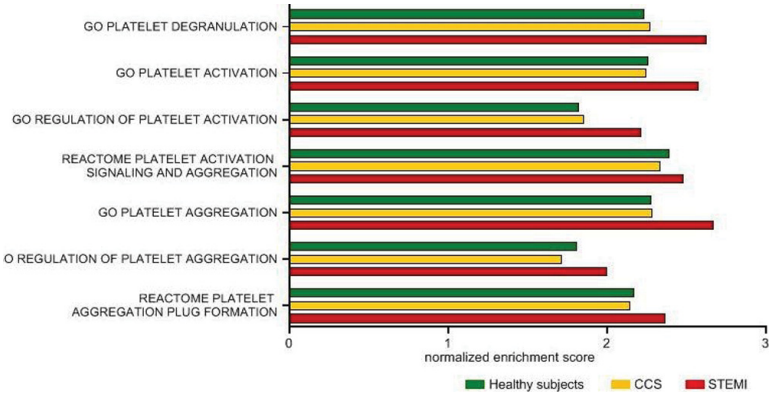
Background: The youngest circulating platelets – so called reticulated platelets (RP) – represent a highly prothrombotic platelet subpopulation. Previous studies showed that patients with chronic coronary syndrome (CCS) as well as patients with ST-elevation myocardial infarction (STEMI) have higher amounts of RP compared to healthy subjects. It has been suggested that intrinsic properties of RP impact on cardiovascular risk. However, it is unknown if transcriptomic alterations contribute to the prothrombotic properties of RP.

Purpose: This study sought to investigate differences in the transcriptomic landscape of sorted RP versus non-RP, i.e. young and old platelets, in healthy subjects, CCS- and STEMI-patients.

Methods: Blood samples were obtained from healthy subjects as well as from patients with CCS/STEMI (n=8 each) the day after PCI. After staining with SYTO 13, platelets from each donor were sorted into a RP and a non-RP fraction based on their RNA-content. Next Generation Sequencing (NGS) was applied to generate sequencing reads for sorted RP and non-RP from the 3 cohorts. Data was analyzed by use of the Freiburg bioinformatics platform "Galaxy".

Results: Investigation of transcriptomic alterations in non-RP versus RP by differential gene expression analysis revealed a total number of 2,476 transcripts that were differentially expressed in platelets from healthy donors, 2,075 in CCS-patients and 1,852 in STEMI patients, respectively (adj. $p<0.05$ in all analyses). Comparison of these transcripts revealed a large overlap of 500 mRNAs which were downregulated and 660 mRNAs which were upregulated in RP in all 3 cohorts. However, there are also distinct groups of transcripts that are differentially expressed in only one of the 3 cohorts. Gene ontology (GO)-analysis of the 500 uniformly enriched transcripts in RP yielded 38 overrepresented GO-terms. A large group was related to cytoskeleton and shape change. Furthermore, GO-terms associated to the platelet activation cascade were overrepresented. Upregulated transcripts included well-known examples like GP6 and GP9, P-selectin, integrin β3, integrin a-IIb, and tubulin α4a. GO-analysis of enriched transcripts in non-RP showed a large group associated to mitosis and cell nucleus/DNA which is surprising since platelets neither contain DNA nor a nucleus. Gene set enrichment analysis (GSEA) determined higher normalized enrichment scores for several gene sets associated to platelet degranulation, aggregation and activation in the STEMI-cohort. Gene sets affecting cell adhesion and platelet calcium homeostasis were overexpressed in particular in CCS-patients.

Conclusion: NGS-results indicate a highly prothrombotic transcriptome of RP from each cohort with high amounts of differentially expressed transcripts overlapping. However, GSEA identified gene sets that are particularly overexpressed in CCS- or STEMI-patients which might contribute to platelet hyperreactivity in these cohorts.



Gene set enrichment analysis