Assessment in the human platelet transcriptome of a predictive signature of the coronary artery disease type.

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Background. Platelets retain a small but significant amount of functionally active megakaryocyte-derived mRNA as well as protein and molecular machinery necessary for translation. The platelet transcriptome may change in coronary artery disease (CAD). We previously showed that differentially expressed transcripts do exist in platelets from patients with non-ST elevation acute coronary syndrome (NSTE-ACS) compared to those with stable angina (SA), suggesting that ACS platelets are potentially preconditioned at the transcriptional level to a higher degree of reactivity, which eventually lead to the thrombotic event.

Aims. (1) To assess in the platelet transcriptome a predictive signature of the CAD type. (2) To identify transcriptional variations related to the CAD type as compared to healthy subjects.

Methods. Total RNA was isolated from leukocyte-depleted platelets from 41 NSTE-ACS and 37 SA patients, age- and sex-matched and with no comorbidities (diabetes and kidney disease). Gene expression profiling was performed using the HumanHT v.12 BeadChips (Illumina). Data variance stabilizing transformation and robust spline normalization were conducted with the lumi R package. Genes were filtered out if less than 10% of expression data have at least a 1.35-fold change in either direction from gene's median value, and statistical differences and prediction classifiers were assessed using the BRB-ArrayTools v.4.3.0-beta2 package. Functional annotation clustering was done using the DAVID v.6.7 Bioinformatics Resources. Platelet activation state was assessed by flow cytometry, using anti-CD41, CD62, PAC1, and Tissue factor antibodies.

Results. Microarray analysis identified 4000±670 transcripts expressed in patients' platelets. Interestingly, the number of transcripts that passed the quality control criteria was lower in NSTE-ACS than in SA patients (3770±598 and 4141±733, respectively, $p<0.02$). Differential analysis was performed on the 2687 transcripts expressed in all samples: 138 were found significant with a permutation p-value ≤0.001 and a FDR<2% (121 decreased and 17 overexpressed in NSTE-ACS versus SA platelet). Notably, 58 of these transcripts are unannotated genes. Functional annotation analysis of the remaining 73 unique genes revealed that the Gene Ontology classes "Translation and Elongation" and the KEGG pathway "Ribosome" were significantly enriched in NSTE-ACS downregulated transcripts (17 and 10 genes, respectively, adjusted p-value 2.36E-10 and 1.14E-08). Prediction analysis of microarray (PAM) identified a classifier composed of 17 unique genes: cross-validation, used to assess the performance of the PAM classifier, showed that it had a rate of correct classification of 70%.

Comparison with 10 age- and sex-matched healthy subjects and functional analysis showed that several biological processes and cell compartments are altered in CAD platelets (adjusted p-value<0.05), e.g. "Wound healing", "Glycolysis", "Regulation of metabolic processes", "Membrane-bound vesicle", and "Platelet alpha-granule" genes. Of interest, CD41 mRNA is overexpressed in NSTE-ACS compared to both SA and healthy platelets, and this is paralleled by the higher antigen level assessed by flow cytometry.

Conclusion. The observed decrease of translational elongation genes in NSTE-ACS might point out an exhausted capability of *de novo* protein synthesis, which has been already exploited to sustain the platelet hyperreactive state found in unstable patients. The partial predictive capacity of the PAM classifier suggests that other variables may act as confounders.