Platelet transcriptomic changes in myocardial infarction are sex and clinical subtype-related: a step forward towards precision medicine?

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In a recently published paper Barrett *et al*. characterized platelet gene expression in females with myocardial infarction (MI), delineating a molecular signature that distinguishes patients with MI due to obstructive coronary artery disease (MI-CAD) from those with MI with non-obstructed coronary arteries (MINOCA).[1] This is the first study to assess the platelet transcriptome specifically in females with different subtypes of MI and to look at platelet transcriptome signatures in function of time after a MI, addressing important gaps in sex-specific cardiovascular research and in the time-course of these changes.

The human transcriptome is composed of a network of coding and non-coding RNAs, organized in spatial and temporal expression patterns that support multiple biological functions ensuring physiological homeostasis. Wide transcriptome variations characterize pathological states, including those of the cardiovascular system, and transcriptional portraits can identify disease-specific combinations of upregulated and downregulated genes, which in turn define precise RNA expression patterns, named "signatures".[2] A main aim of transcriptome investigations is to define reproducible RNA signatures in polygenic, multifactorial conditions with the aim of identifying clinically useful molecular biomarkers able to accurately predict disease etiology, classification and progression.[3]

For many years microarray-based methods have allowed the simultaneous screening of tens of thousands of transcripts by hybridization to pre-designed and immobilized probes. Microarray-based analysis of the human blood transcriptome[4,5] had a seminal role in the development of 'bloodomics' and has been used to investigate several cardiovascular disorders, such as coronary heart disease, atherosclerosis, dyslipidemia, hypertension and cardiac-transplant rejection. Since circulating blood is easily accessible and interacts with every organ and tissue in the body, it has been used as a surrogate to biopsy tissues for RNA profiling of several human diseases[5] based on the hypothesis that peripheral blood has the capacity to reflect various pathological changes, including atherosclerosis or tumor development.[6] However, the microarray high-throughput approach can only detect predefined transcripts, and is limited by the high amount of RNA required.

The development of next-generation sequencing technologies from the early 2000s has substantially improved the qualitative and quantitative investigation of coding RNA as well as of the increasing number of non-coding RNA species, such as microRNAs, long non-coding RNAs and circular RNAs. The advent of deep RNA sequencing (RNA-seq) technologies has permitted the large scale quantification of coding transcripts and their splicing variants in disease conditions.[7] Furthermore, RNA-seq applied to blood samples has permitted to investigate the transcriptomes of specific circulating cells, including platelets, as reported by Barret and colleagues.[1] Further methodological advances have enabled single cell RNA sequencing (scRNA-seq)[8,9] which may largely improve our understanding of the spatial and temporal transitions and interactions of healthy and diseased cells.

Differences in gene dosage compensation, deriving from X-chromosome inactivation, and in hormone levels cause dissimilarities between sexes in the expression of genes localized on sex chromosomes or involved in sex steroid pathways. A few genes display variable sex-biased expression across organs[10] that increases around sexual maturity.

Platelets contain approximately 2.2 femtograms of total RNA per cell, about 1,000-fold less than nucleated cells. Despite their small RNA content, however, platelets possess a complex transcriptome, including coding and non-coding RNAs. Transcripts from about half of the human genome are present in these terminally differentiated cells showing an unexpected complexity, and include an estimated 7800 protein-coding genes.[11] Approximately half of the transcripts in platelets encode mitochondrial genes.[12] Platelet mitochondria have been implicated in platelet activation and thrombosis by increasing reactive oxygen species (ROS) production.[13] Moreover, upon activation platelets release functional

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mitochondria and the subsequent hydrolysis of mitochondrial membranes leads to the release of inflammatory mediators, such as lysophospholipids, fatty acids, and mitochondrial DNA, that can activate leukocytes and potentially contribute to atherogenesis.[14] Of note, Barrett and colleagues found an increased expression of genes associated with mitochondrial dysfunction during the acute coronary event,[1] consistent with the high energy demand associated with platelet activation typical of coronary syndromes met through oxidative phosphorylation by mitochondria.

The platelet transcriptome also includes ribosomal RNAs (rRNAs), transfer RNA (tRNA), long non-coding RNAs (lncRNA), circular RNAs (circRNAs), microRNAs (miRNAs) and yRNA,[15] and is predominantly inherited from megakaryocytes, their bone marrow precursors, although it can also be acquired in the circulation (i.e. transferred from other cells or plasma). During megakaryocyte development RNA is transcribed, processed, and transferred into platelets. Mechanisms by which mRNAs are transferred from megakaryocytes to platelets remain largely elusive, however there is evidence that mRNAs are differentially sorted rather than randomly distributed into platelets,[16] and hints that the megakaryocyte transcriptome patrimony may change depending on the environment in which megakaryocytes mature,[17] like in inflammatory conditions such as those occurring during acute coronary syndromes.

While platelets cannot transcribe new RNA, they are competent for protein synthesis.[18] Next generation sequencing (NGS) profiling of ribosome footprints in platelets identified thousands of ribosome-bound mRNAs, which means actively translated transcripts. Interestingly, mRNA translation was found to be enhanced in platelets from septic individuals and in other acute disease conditions, with active translation of hundreds of proteins.[17,19-21]

Most coding mRNAs in platelets are mature, however platelets can also splice pre-mRNA into mature mRNA and translate it into proteins in a signal-dependent fashion upon platelet activation,[22] this contributes to the variability in the platelet transcriptome. Other factors contribute to this variability including age and genetic regulators such as expression quantitative trait loci (eQTLs). Age-related differences have been observed in platelet RNA profiles, with neonates displaying distinct transcriptomic features compared to adults, including altered expression of procoagulant and ribosomal genes.[23,24] Accordingly, a reduced platelet response to stimuli can be observed in neonates and children compared to adults.[25] Genetic variation influences platelet transcript levels, as demonstrated by studies identifying numerous cis-eQTLs - genetic variants that regulate nearby gene expression. Some of these eQTLs are linked to key platelet traits, including aggregation and platelet count, highlighting the role of genetic regulation in platelet function.[26,27] Given that mRNA and protein levels in platelets are strongly correlated[28] mRNA is often used as a surrogate to identify altered proteins or pathways in disease.

Previous studies on the platelet transcriptome in myocardial infarction (MI) showed that CD69 and MRP8/14 mRNA levels can distinguish between acute ST-elevation myocardial infarction (STEMI) and stable coronary artery disease (CAD). Additionally, protein levels of MRP8/14 showed a correlation with its mRNA expression, with elevated levels serving as an independent predictor of cardiovascular risk.[29] Subsequent studies demonstrated that MRP8/14 is linked to thromboxane-dependent platelet activation.[30] In another study comparing patients with acute STEMI versus non-STEMI (NSTEMI) showed that several transcripts display higher (FBXL4, ECHDC3, KCNE1, TAOK2, AURKB, ERG, and FKBP5) or lower (MIAT, PVRL3and PZP) expression in STEMI compared to NSTEMI platelets.[31] Another study compared the platelet transcriptome from patients with STEMI, stable coronary artery disease (SCAD) and healthy subjects (HS) and identified five differentially expressed genes in STEMI compared with the other groups: FKBP5, S100P, SAMSN1, CLEC4E and S100A12, suggesting that the expression of these five genes can be considered as an early indicator of acute myocardial infarction.[32]

These studies have consistently shown that the investigation of the platelet transcriptome may allow to identify biomarkers and to clarify pathophysiological mechanisms of acute coronary syndromes. Interestingly, differently from the above summarized studies, the recent paper from Barrett et al. assessed the transcripts enriched in platelets during acute MI also months after the acute event, showing that they remained persistently altered and that correlated with long-term cardiovascular risk.[1] These transcripts associated with important pathways for platelet function, such as the actin cytoskeleton, Rho GTPase signaling, mitochondrial dysfunction and inflammatory signaling.[1] Moreover, the biological pathways of the genes chronically decreased in platelets from MI patients were consistent with MI pathogenesis, including the decreased synthesis and metabolism of selenocysteine, an aminoacid involved in maintaining endothelial homeostasis and a non-prothrombotic platelet activation status, and confirmed the report of 35 years-ago of decreased selenium levels in patients with acute MI.[33] Impairment in selenocysteine synthesis has also been proposed as a mechanism of the coagulopathy of COVID-19 patients.[34]

In accordance with the above mentioned transcriptomic changes, key steps of protein synthesis were downregulated, such as the joining of the 60S ribosomal subunit for translation initiation, the targeting of nascent proteins to membranes and translation termination.[1] The study of protein synthesis by platelets is an evolving field of research[35] and post□transcriptional and translational mechanisms, which are now considered to be part of the platelet functional repertoire, are altered in acute disease conditions, like sepsis[17] and thrombosis.[36] On the other hand, transcripts related to inflammation, neutrophil degranulation and tumor necrosis factor-alpha (TNFα) signaling, were upregulated in women with MI-CAD.[1] These findings align with the established role of platelets in orchestrating thromboinflammatory responses. Interestingly, the study of Barret and colleagues explored not only MI due to coronary artery atherothrombosis but also MINOCA, which is significantly more prevalent in women than men, by performing RNA-seq of platelet transcripts in female patients. Interestingly, MINOCA was characterized by a distinct molecular profile associated with vesicle transport and apoptosis regulation, reflecting potentially different pathophysiological mechanisms in these two types of acute coronary syndrome.

The stability of these changes over time offers a unique opportunity for developing predictive biomarkers. In particular, the association between platelet transcriptomics and major adverse cardiovascular events (MACE) warrants further exploration in larger, longitudinal cohorts.

The application of platelet transcriptomics to the study of human disease conditions is still in its infancy but holds great potential for diagnosis, biomarker identification, patient monitoring

and personalized treatment. The different platelet transcriptome signatures of MI women with coronary artery atherosclerosis compared with those with MINOCA goes in the direction of precision medicine, providing new mechanistic insight into the platelet role and phenotype in different types of coronary artery diseases and potentially allowing to identify new biomarkers for acute coronary syndrome subtype identification. One limitation of this study, acknowledged by the authors, is that it was conducted only in women, thus future research should aim to pinpoint key differences of MI-platelet transcriptomic signatures in broader populations, including male patients and individuals with different clinical conditions.

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