

The amazing genetic complexity of anucleated platelets

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Although anucleated, platelets have a rich and complex transcriptome which includes, besides coding messenger RNAs (mRNAs) and pre-mRNAs, also non-coding RNAs (ncRNAs) such as microRNAs (miRNAs), circular RNAs (circRNAs), long noncoding RNAs (lncRNAs) and YRNAs. Actually, platelets are probably the primary source of ncRNAs in the circulation. These wide array of RNAs derives from the platelet precursors, megakaryocytes, but also exogenous RNAs present in the circulation are taken up by platelets. This diverse and dynamic transcriptome enables platelets to synthesize new proteins which modulate their functions, both constitutively and upon activation, but also to modify the phenotype of other cells with which platelets interact.

The field of platelet transcriptomics has been rapidly

growing in the last 10 years, particularly after the advent of next-generation RNA-sequencing (RNAseq) techniques. RNAseq provided new insights into the platelet transcriptome, showing that about 8,000 transcripts are present in platelets,^{1,2} and allowed to identify mutations in *NBEAL2* as the genetic cause of the Grey Platelet Syndrome, an inherited platelet disorder that had remained without a causative gene for more than 30 years.³

Several studies have shown protein synthesis by anucleated platelets, both basally and in response to activation,^{4,6} and it was shown that neo-synthesis of some selected proteins is regulated by signal-dependent pre-mRNA splicing.^{7,8}

Platelets also contain ribosomes, that are bound to translating mRNAs protecting them from degradation, and thrombin increases ribosomal occupancy on mRNAs, according with its ability to trigger protein synthesis in platelets.^{4,9}

Moreover, it was shown that mRNAs are differentially sorted from megakaryocytes to platelets rather than randomly distributed to them,¹⁰ suggesting that platelet protein synthetic capability and thus the platelet function phenotype, are regulated by the environment in which megakaryocytes mature.

miRNAs, short noncoding RNAs which regulate gene expression post-transcriptionally, represent another mechanism of protein synthesis regulation in platelets. The platelet miRNome is modified upon in vitro activation and in vivo in disease conditions, and changes of the platelet miRNome affect in turn the platelet proteome.^{11,12} The role of circRNAs, lncRNA and YRNAs in platelets is still under investigation. circRNAs enrichment in platelets can be interpreted as a signature of transcriptome degradation,¹³ but some circRNAs are selectively released in extracellular vesicles and their role is still unclear.¹⁴ Finally, lncRNAs and YRNA are abundant in platelets and might be involved in platelet reactivity.^{15,16}

Studies on platelet transcriptome have led to significant advances in the understanding of the role of platelets in health and disease. Indeed, in response to inflammatory signals, invading pathogens, cancer, or other stressors, the platelet transcriptome changes dynamically. For instance, the platelet transcriptome is altered in inflammatory disorders associated with increased cardiovascular risk, such as sickle cell disease and systemic lupus erythematosus.^{17,18} The expression of several transcripts involved in

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oxidation, immunoregulation and stress response was reported to be higher in platelets from ST segment elevation myocardial infarction (STEMI) compared to non-ST segment elevation myocardial infarction (NSTEMI) patients,¹⁹ providing new insight into the pathogenic role of platelets in acute coronary syndromes.

Aging, a condition characterized by an increased cardiovascular risk and chronic inflammation, is associated with alterations of the platelet transcriptome. Using single-cell RNA-seq of megakaryocytes, Davizon-Castillo and colleagues showed a reprogramming of the inflammatory, metabolic, and mitochondrial gene pathways in aged mice, detected also in circulating platelets, and associated with increased TNF- α .²⁰ Another recent study elegantly showed numerous differentially expressed transcripts in older healthy adults in respect to younger subjects and observed that variations of the platelet transcriptome influence platelet-leukocyte interactions and downstream cytokine synthesis contributing to chronic inflammation.²¹

Moreover, RNAseq of human and murine platelets showed that the platelet transcriptional and translational landscapes are significantly altered in sepsis, and that upregulation of *ITGA2B* is associated with higher mortality²² providing novel evidence that upregulation of protein synthetic events in platelets may play a pathogenic role in sepsis. A recent study²³ confirmed the differential expression of several genes in platelets from sepsis patients compared to controls and showed that upregulated genes were mostly related to catabolic processes and protein translation. The Authors also showed that patients with sepsis have increased levels of immature platelets and a less degraded platelet transcriptome, as indicated by decreased levels of circRNAs. The biological implications of the increased platelet turnover remain unclear, however this study first suggested that increased protein translation is a characteristic mechanism of systemic inflammation.²³

Intriguingly, there is a large overlap of the platelet transcriptome changes observed either in sepsis or in COVID-19.²²⁻²⁴ Distinctive changes in the gene-expression profile of circulating platelets of COVID-19 patients have been shown involving the pathways associated with protein ubiquitination, antigen presentation, and mitochondrial dysfunction,²⁴ suggesting a drastic change in the redox balance and in platelet metabolism leading to platelet activation and to the high thrombotic risk of COVID-19 patients. Another study similarly found in platelets from COVID-19 patients differentially expressed lncRNAs involved in the regulation of hemostasis, platelet activation and the immune response,²⁵ further confirming that platelet hyperactivation and immune-thrombosis are major players in COVID-19 complications.

Differential expression of platelet genes involved in leukocyte activation has also been shown in other viral in-

fections, for example HIV, that is associated with an increased cardiovascular risk and hyperreactive platelets.²⁶ On the other hand, anti-viral immune genes are upregulated in platelets and megakaryocytes in response to dengue and influenza infection, possibly limiting viral infection.²⁷ Platelet involvement in viral infections has led investigators to search for viruses in platelets. The entry of SARS-CoV-2 in platelets is still debated: some groups described the presence of the viral RNA in platelets,^{28,29} while others did not detect it,³⁰ or detected it in very few samples.²⁴ Other viruses, namely HIV,³¹ influenza³² and Dengue,³³ enter platelets with the latter also stealing the platelet translational machinery to replicate and produce new virions.³³

The platelet transcriptome is also altered in patients with cancer, and this has been exploited to show that the platelet transcriptome can serve as a diagnostic and prognostic platform. The uptake by platelets of tumor-associated biomolecules, mainly circulating tumor-derived mRNA, has led to the concept of “tumor educated platelets”, i.e. platelets with a highly dynamic mRNA repertoire with potential importance for cancer diagnostics. Indeed, the analysis of the platelet transcriptome can discriminate patients with cancer from healthy controls, predict the localization of the primary tumor and provide information on the molecular tumor subtypes, thus guiding clinical diagnostics and therapy selection providing from a simple peripheral blood sample what has been defined as a “liquid biopsy” of the tumor.³⁴ Similarly, it was recently shown that the platelet transcriptome may help to predict the fibrotic progression in myeloproliferative neoplasms.³⁵

Post transcriptional events in platelets also regulate key proteins related to neurodegenerative disorders. For instance, the amyloid precursor protein (APP) is present in platelets and is encoded by platelet mRNA.³⁶ APP is the metabolic precursor of amyloid A β peptides whose accumulation in brain parenchyma and cerebral vessel walls is correlated with the onset of Alzheimer disease but also plays a role in platelet function.³⁷ Recently differential spliced platelet mRNA was reported to enable blood-based diagnosis of multiple sclerosis.³⁸ Finally, elevated expression of the platelet-related miRNAs miR-22-3p, miR-146a and miR-155 were found to be a possible Parkinson’s Disease-specific miRNA signature.³⁹

New discoveries on the genetic complexity of human platelets are continuously made. A recent innovative report showed that platelets possess endogenous retro-transcriptase (eRT) activity that regulates protein synthesis by generating RNA–DNA hybrids which block translation. Inhibition of platelet eRT activity increased integrin activation in vitro and thrombosis in vivo suggesting that inhibition of platelet eRT activity may contribute to the increased risk of thrombosis observed in people with HIV treated with RT inhibitors.³⁶

It is only a matter of time, and the amazing genetic landscape of platelets will probably reveal unexpected new perspectives.

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