Klinikum rechts der Isar

I. Medizinische Klinik und Poliklinik

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ESC First Contact Initiative Grant Report

I would like to thank the ESC Council on Basic Cardiovascular Science for the opportunity to develop my project on platelet biology. With the support of the first contact initiative grant, I could make contact with the Cardiovascular Research Unit of Humanitas Research Hospital, Milan, Italy. During my stay at the Research Unit led by Prof. Condorelli, I could test the feasibility of deep proteomic profiling of reticulated platelets. Moreover, I developed the protocols and acquired the skills necessary to further investigate the reticulated platelet hyper-reactivity.

Reticulated platelets (RPs) are young, hyper-reactive pro-platelets newly released from the bone marrow into peripheral blood. They are larger in size, contain more RNA and, most importantly, they have a higher pro-thrombotic potential in comparison to mature, older platelets. These prothrombotic thrombocytes are known to be independent predictors of an insufficient antiplatelet response to thienopyridine and aspirin treatment in patients with coronary artery disease. In addition to these findings, elevated RPs have been shown to be an important prognostic risk factor for high on-thienopyridine platelet reactivity, as well as for major adverse cardiovascular events not only in patients with coronary artery disease but also in severe sepsis and during post-operative treatment. These findings highlight their role as a novel biomarker in different clinical scenarios as well as the translational potential of this scientific field.

However, the reason for this correlation as well as the intrinsic hyper-reactivity of RPs was, so far, unknown. The mRNA and miRNA of RPs have not been characterized so far although they are very likely to influence aggregability and biology of RPs.

As platelets contain only a very low amount of RNA, the RNA extraction and analysis of these cells is extremely challenging and it requires very sensible tools. With the support of the first initiative Grant, I spent 6 Weeks at the Cardiovascular Research Unit of

Humanitas Research Hospital, Milan, Italy. With the local know-how in biomarkers, microparticles analysis and low input RNA-sequencing, I was able to test the feasibility of a wide mRNA and miRNA analysis in immature platelets. With the support of the research unit of Humanitas Research Hospital, I was able to perform a Total- and small-RNA sequencing of RPs after sorting for the first time.

Transcriptome analysis of RPs revealed an enrichment of several pro-thrombotic pathways and transcripts of transmembrane proteins including the collagen receptor GP6, the thromboxane receptor A2, and the thrombin receptors in RPs. In addition, we detected an upregulation of calcium channels involved in platelet activation. If confirmed in pathological settings, these findings may provide a first biological explanation of an insufficient response to antiplatelet drugs in patients with high levels of RPs. Concerning downregulated transcripts in RPs, we found differentially regulated inflammatory proteins, which might indicate that platelets play different roles in inflammatory processes in dependence on their degree of maturity. However, the downregulation of both proinflammatory proteins like IL7 and anti-inflammatory genes such as ANXA1 does not allow to define a clear phenotype polarization. Small-RNA sequencing detected a total of 155 miRNAs, the most abundant of those correlating with recent characterizations of platelet transcriptome. Despite the various content of miRNAs in platelets, we found only 9 miRNAs differentially regulated in RPs. Interestingly, all 9 miRNAs were consistently downregulated in RPs. These miRNAs have not been correlated to platelet reactivity so far, and this is the first time that they are described to negatively correlate with a prothrombotic phenotype.

In conclusion, thanks to the support of the first initiative grant I performed the first comparative transcriptome analysis of RPs and mature platelets and I could describe for the first time a differential enrichment of transcripts involved in platelet activation[1]. The clear upregulation of prothrombotic signaling in RPs may provide, at least in part, the first biological explanation of RPs hyper-reactivity and it may offer a new therapeutic target in patients with high on-treatment platelet reactivity.

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1. Bongiovanni D, Santamaria G, Klug M, Santovito D, Felicetta A, Hristov M, et al. Transcriptome Analysis of Reticulated Platelets Reveals a Prothrombotic Profile. Thromb Haemost. 2019 Sep 1;.