Platelet proteomic progress and restraining mechanisms in glycoprotein VI-mediated thrombus formation

Impact

Cardiovascular diseases (CVD) provide the great threaten to man, of which thrombosis in the arteries or veins are still leading causes of death[1-3]. Platelets are the smallest anucleate blood cells that are released by megakaryocytes in the bone marrow[4]. Platelets fulfill a key role in the formation of a thrombus or clot and also support the blood coagulation system. Hence, the formation of a platelet plug prevents excessive blood loss when a blood vessel is damaged or ruptured[5]. However, dysfunctional platelet activation in thrombus formation at a ruptured site of an atherosclerotic plaque can cause pathological occlusive thrombus formation, which predisposes for stroke or heart infarction. Currently, antiplatelet drugs are widely used in the clinical treatment after arterial thrombosis, including aspirin, clopidogrel, prasugrel and ticagrelor[6]. However, all these antithrombotic drugs are associated with a risk of bleeding, and their efficacy can vary greatly between individuals.

Glycoprotein VI (GPVI) is the major signaling collagen receptor on the surface of platelets, which induces thrombus formation by binding to collagen directly, or by binding to fibrin in second instance. In GPVI-deficient mouse, only a moderate increase of the bleeding time was observed, while arterial thrombosis was substantially impaired. This points to a crucial role of GPVI in arterial thrombosis with limited contribution to hemostasis; and this makes GPVI to a novel potential antithrombotic target[7]. As indicated below, this thesis on human platelets aims to contribute to the support for a selective antithrombotic action mechanism through GPVI signaling inhibition or through GPVI blockage.

Because of their anucleate structure, platelet signaling activities are regulated by post-translational modifications (PTMs). To unravel these, mass spectrometry-based proteomics analyses are becoming one of the powerful tools for defining the protein alterations and receptor-mediated signaling cascades in platelets[8,9]. Here, we revealed the full composition of platelet proteome by a global comparison of platelet proteomes and transcriptomes. The generated large datasets – also to compare human and mouse platelets

– can now be used to elucidate the mechanisms of GPVI-induced thrombus formation, and ultimately for determining protein targets downstream of GPVI for use in the clinic.

With the development of state-of-the-art mass spectrometers, proteomics techniques are increasingly applied in the research of platelet protein composition, platelet signaling cascades and platelet-related diseases. The overview on these aspects in **Chapter 2** provides in-depth insight into earlier and current proteomics methods for detecting protein changes in patients after antiplatelet treatment or presenting with a platelet-based disorder. However, in spite of this wide use of proteomics methods, still only part of the platelet proteins has been identified in comparison to the detected mRNAs, likely to limitations in the proteomic techniques. To get a comprehensive idea on the theoretical platelet protein composition, we conducted a quantitative global comparison of the human and mouse platelet proteomes and the corresponding platelet (and megakaryocyte) transcriptomes, which assisted in our understanding the variety of functions carried out by platelets (**Chapters 3 and 4**). For the latter purpose, we developed an integrative platelet protein function classification scheme.

Throughout this work, three restraining factors for the hitherto limited protein identification by mass spectrometry-based proteomics were seen. These were: (i) (peri)nuclear localization; (ii) low transcription and (iii) low translation of the expected (megakaryocytic) proteins. Based on these restraining factors, we could establish and validate a prediction model for the full platelet proteome, in which around 10,000 platelet proteins are expected out of the more than 20,000 predicted ones with any corresponding transcript level. In addition, we confirmed a high correlation between the human platelet and megakaryocyte transcriptomes, and even higher correlations between several included datasets of human platelet transcriptomes. However, the composed mouse platelet transcriptome was less well correlated with the median human platelet transcriptome; and the same was true for the two platelet proteomes. On the other hand, the

overlap of orthologous transcripts between the two species was very high, especially for the highest abundance transcripts. The same also held for the highest abundant human and mouse platelet proteins. Together, these studies thus provided a better understanding of the (complete) platelet proteome with the help of platelet transcriptome information.

Platelet proteomics can also be used for monitoring proteins changes in platelet related diseases. In **Chapter 5**, using stable isotope labeling we detected a panel of 50 differently iloprost-regulated protein phosphorylation sites in platelets from patients with Albright hereditary osteodystrophy syndrome (AHO syndrome), and we confirmed that the upregulated proteins were protein kinase A-dependent. This indicated that indeed platelet phosphoproteome analysis can help to understand alterations in platelet function in such patients.

In **Chapters 6-8**, we investigated signaling pathways that may enhance or restrain the GPVI-induced processes of platelet activation and thrombus formation. In **Chapter 6**, we observed that the activating role of GPVI was only moderately activated by binding to fibrin(ogen), by only triggering microthrombus formation on surfaces of fibrin or fibrinogen upon whole-blood flow. The blockage effects of GPVI fiber or Syk let us conclude for a nonredundant role of GPVI and the integrin αIIbβ3 in fibrin-induced thrombus formation. Stated otherwise, our data suggest a partial overlap of the effects of clinical-relevant antagonisms of GPVI and αIIbβ3.

In **Chapter 7**, we established that the pharmacological inhibition of the Src homology 2 domain containing protein tyrosine phosphatases, Shp1 and Shp2, under certain conditions, enhanced GPVI-induced platelet aggregation. This was the case in response to a low dose of CRP-XL, and upon blockage of the phosphoinositide 3-kinase (PI3K) pathway which is required for integrin activation. However, the blockage of Shp1/2 did not affect the signaling immediately below GPVI, while the combined inhibition of Shp1/2 and PI3K reduced rather than increased the phosphorylation of Src Tyr^{419} and phospholipase Cγ2 (PLCγ2) Tyr^{759}. These findings suggested that both

isoforms Shp1/2 compensate for the absence of PI3K activity in mediating integrin activation and PLCγ2 phosphorylation. Likely, this underscores a specific negative role of Shp2 (in integrin activation) and a positive role of Shp1 (on the PLC pathway).

In **Chapter 8**, we established that a peptide mimicking activation of the shear-dependent collagen receptor GPR56 (pGRP) showed suppressive rather than enhancing effects on collagen-mediated thrombus formation, in a way confined to a high wall-shear rate, as is relevant for arterial thrombosis. Simultaneous blockage of the focal adhesion kinase PTK2 caused a synergistical suppression of the thrombus formation. In the same chapter, we found that interfering peptides with the calcium and integrin-binding protein 1 (CIB1), pCIB and pCIB^{m} were able to suppress collagen-induced thrombus formation only at high shear rate. These findings pointed to a shear-dependent role of PTK2, CIB1 and integrin αIIbβ3 in collagen- and GPVI-mediated platelet activation and thrombus formation.

Overall, this thesis provides a powerful tool for further investigating the platelet protein composition and to reveal the relations of the platelet proteome and transcriptome across human and mouse. In addition, this thesis provides novel insights into the precise regulation and mechanism of the GPVI-mediated signaling and thrombus formation. Therefore, I am confident that my findings will give a better background for understanding the possibilities and limitations for GPVI-related antithrombotic drugs in CVD.

References

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