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Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome

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Introduction

Platelet integrity and function critically depend on lipid composition. However, the lipid inventory in platelets was hitherto not quantified. Here, we examined the lipidome of murine platelets using lipid-category tailored protocols on a quantitative lipidomics platform. We could show that the platelet lipidome comprises almost 400 lipid species and covers a concentration range of seven orders of magnitude. A systematic comparison of the lipidomics network in resting and activated murine platelets, validated in human platelets, revealed that less than 20% of the platelet lipidome is changed upon activation, involving mainly lipids containing arachidonic acid. Sphingomyelin phosphodiesterase-1 (Smpd1) deficiency resulted in a very specific modulation of the platelet lipidome with an order of magnitude up-regulation of lyso-sphingomyelin (SPC), and subsequent modification of platelet activation and thrombus formation. In conclusion, this first comprehensive quantitative lipidomic analysis of platelets sheds light on novel mechanisms important for platelet function, and has therefore the potential to open novel diagnostic and therapeutic opportunities.

Workflow

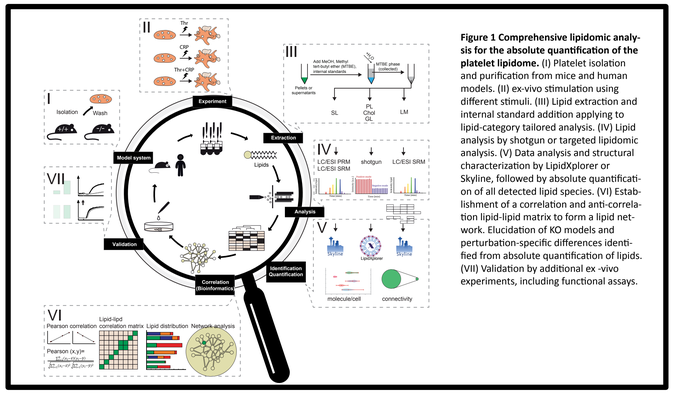

Figure 1 Comprehensive lipidomic analysis for the absolute quantification of the platelet lipidome. (I) Platelet isolation and purification from mice and human models. (II) ex-vivo stimulation using different stimuli. (III) Lipid extraction and internal standard addition applying to lipid-category tailored analysis. (IV) Lipid analysis by shotgun or targeted lipidomic analysis. (V) Data analysis and structural characterization by LipidXplorer or Skyline, followed by absolute quantification of all detected lipid species. (VI) Establishment of a correlation and anti-correlation lipid-lipid matrix to form a lipid network. Elucidation of KO models and perturbation-specific differences identified from absolute quantification of lipids. (VII) Validation by additional ex -vivo experiments, including functional assays.

Results

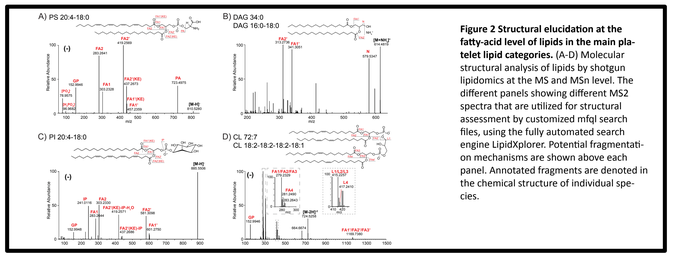

Figure 2 Structural elucidation at the fatty-acid level of lipids in the main platelet lipid categories. (A-D) Molecular structural analysis of lipids by shotgun lipidomics at the MS and MSn level. The different panels showing different MS2 spectra that are utilized for structural assessment by customized mfql search files, using the fully automated search engine LipidXplorer. Potential fragmentation mechanisms are shown above each panel. Annotated fragments are denoted in the chemical structure of individual species.

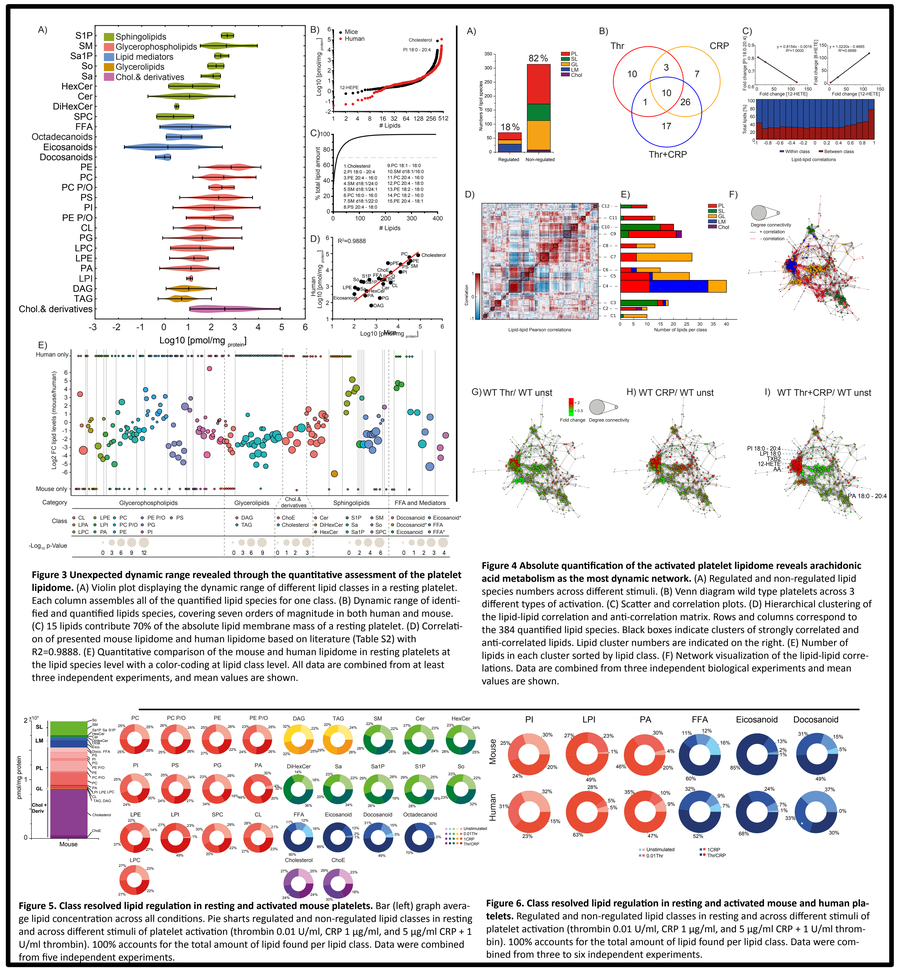

Figure 3 Unexpected dynamic range revealed through the quantitative assessment of the platelet lipidome. (A) Violin plot displaying the dynamic range of different lipid classes in a resting platelet. Each column assembles all of the quantified lipid species for one class. (B) Dynamic range of identified and quantified lipids species, covering seven orders of magnitude in both human and mouse. (C) 15 lipids contribute 70% of the absolute lipid membrane mass of a resting platelet. (D) Correlation of presented mouse lipidome and human lipidome based on literature (Table S2) with R2=0.9888. (E) Quantitative comparison of the mouse and human lipidome in resting platelets at the lipid species level with a color-coding at lipid class level. All data are combined from at least three independent experiments, and mean values are shown.

Figure 4 Absolute quantification of the activated platelet lipidome reveals arachidonic acid metabolism as the most dynamic network. (A) Regulated and non-regulated lipid species numbers across different stimuli. (B) Venn diagram wild type platelets across 3 different types of activation. (C) Scatter and correlation plots. (D) Hierarchical clustering of the lipid-lipid correlation and anti-correlation matrix. Rows and columns correspond to the 384 quantified lipid species. Black boxes indicate clusters of strongly correlated and anti-correlated lipids. Lipid cluster numbers are indicated on the right. (E) Number of lipids in each cluster sorted by lipid class. (F) Network visualization of the lipid-lipid correlations. Data are combined from three independent biological experiments and mean values are shown.

Figure 5. Class resolved lipid regulation in resting and activated mouse platelets. Bar (left) graph average lipid concentration across all conditions. Pie sharts regulated and non-regulated lipid classes in resting and across different stimuli of platelet activation (thrombin 0.01 U/ml, CRP 1 µg/ml, and 5 µg/ml CRP + 1 U/ml thrombin). 100% accounts for the total amount of lipid found per lipid class. Data were combined from five independent experiments.

Figure 6. Class resolved lipid regulation in resting and activated mouse and human platelets. Regulated and non-regulated lipid classes in resting and across different stimuli of platelet activation (thrombin 0.01 U/ml, CRP 1 µg/ml, and 5 µg/ml CRP + 1 U/ml thrombin). 100% accounts for the total amount of lipid found per lipid class. Data were combined from three to six independent experiments.

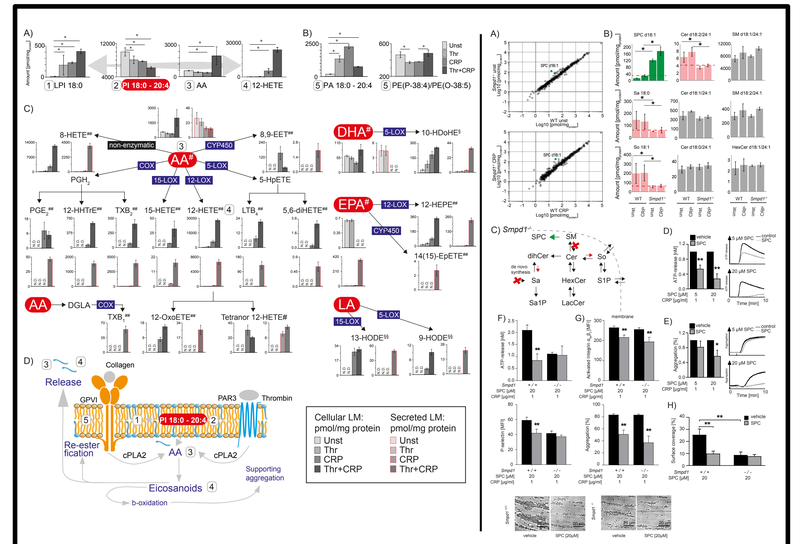

Figure 7 Mediator production and release in platelets is stimulus dependent and centered. (A) Concentration (pmol/mgprotein) of 12-HETE, arachidonic acid and PI 18:0-20:4. (B) Bar graphs of various mediator precursors during different stimuli. (C) Analyzed mediators in platelets and their corresponding secretome level. Bar graphs with black framed colors display mediators in platelets while bars with red framed indicate mediators in the corresponding secretome. (D) Measured lipid regulation during platelet activation. The absolute quantities are reported in pmol/mgprotein. #: the lipid class of free fatty acids (FFA); ##: Eicosanoids; §: Docosanoids; §§: Octadecanoids. N.D.:not detectable. Data were combined from three independent experiments, displayed as mean values, and the error is represented as standard deviation of the mean.

Figure 8 Quantitative lipidomics reveals a SPC dysregulation at the trans-Golgi network in the Smpd1-/- mouse strain. (A) Correlation of murine lipid concentrations between wild type (WT) and acidic sphingomyelinase knockout (Smpd1-/-) mutant mice in resting platelets (upper panel) and after stimulation with CRP (1 µg/ml; lower panel). (B) Regulated and non-regulated sphingolipids in resting and activated platelets. Data from three independent experiments were combined. (C) Sphingolipid metabolism in WT and Smpd1-/- mice indicates a shift towards lyso-sphingomyelin. (D-I) Functional platelet assays. ATP-release, P-Selectin, Aggregation, Surface coverage.

Summary and Outlook

The present study is the first to quantify the platelet lipidome, which comprises almost 400 lipid species and covers a concentration range of seven orders of magnitude. The platform presented here permits uniquely a systematic assessment of the lipidomics network in resting and activated murine platelets, and demonstrates the feasibility of performing absolute and comprehensive quantitative platelet-lipidome analysis. The operation of our platform yielded in identification of sphingomyelin phosphodiesterase 1 as a specific modulator of the platelet lipidome, involved in the regulation of lyso-sphingomyelin levels being critical to platelet dense granule release and thrombus formation.
This approach opens new doors to study the functional consequence of the (genetic) deficiency of particular proteins and other molecules involved in platelet lipid metabolism regulation. Thus, the application of this workflow might be helpful to identify lipids affected in (patho-) physiological platelet activation and modified in thrombo-inflammatory diseases such as atherosclerosis, coronary artery disease or acute myocardial infarction with the potential to identify novel biomarkers or targtes for antihrombotic treatment strategies.

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