Plan Overview

A Data Management Plan created using DMPTool

DMP ID: https://doi.org/10.48321/D1XH28

Title: Membrane-cytokeleton interactions in platelets and megakaryocytes

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Template: NIH-Default DMSP

Project abstract:

Platelet dysfunction is associated with severe clinical risks, including bleeding, such as Glanzmann's thrombasthenia, or exert thrombosis, such as myocardial infarction and stroke. Platelet function is tightly regulated by integrin activity. Platelets express two integrin β families, β3 and β1. Absence or loss-of-function mutations in β3 causes Glanzmann's thrombasthenia, a severe bleeding disorder, while gain-of-function mutations cause thrombocytopenia. Thus, differences in the activity state of integrin β3 modulates platelet homeostasis. By contrast, loss of integrin β1 in several mouse models had no effect on platelet functions, and, therefore, integrin β1 has been assumed dispensable for hemostasis. However, recent studies from our laboratory found that hyperactive integrin β1 is associated with bleeding in mice. Thus, we hypothesize that excessive integrin β1 activity causes impaired platelet function. Our hypothesis is derived from studies examining the membrane-shaping protein PACSIN2 in platelets. Several independent genomic studies found that PACSIN2 variants are associated with low platelet count and reduced platelet function. We found that PACSIN2 regulates integrin β1 function during thrombus formation based on the following evidence: 1) Pacsin2–/– mice develop profound platelet-intrinsic thrombus formation defects, where platelets form unstable thrombi that embolize abruptly, causing bleeding; 2) Pacsin2–/– platelets display integrin β1, but not β3 hyperactivity; and 3) platelet-specific deletion of integrin β1 in Pacsin2–/– mice reverses the thrombus embolization phenotype and normalizes bleeding. PACSIN2 binds the cytoskeletal and scaffolding protein filamin A (FlnA), which associates with integrin β-subunits to compete with talin and kindlins, thereby maintaining integrins in an inactive state. Our results indicate that PACSIN2 binding of FlnA mediates binding to integrin β-subunits. We propose three aims to further investigate the biochemical mechanisms underlying how PACSIN2 modulates integrin β1 activity, thereby impacting platelet hemostatic function. Aim 1 will define the biochemical mechanisms by which hyperactive integrin β1 affects thrombus formation in vivo. Aim 2 will define the biochemical mechanisms by which PACSIN2 affects integrin β1, but not β3 activity. Aim 3 will define the molecular mechanisms by which PACSIN2 mediates integrin β1 binding to FlnA. This research will provide valuable insights into the role of PACSIN2 in platelet biology and may have implications for

understanding platelet disorders associated with abnormal integrin β1 activity.

Start date: 07-01-2024

End date: 06-30-2029

Last modified: 01-19-2024

Copyright information:

Membrane-cytokeleton interactions in platelets and megakaryocytes

In this proposed project, data will be generated using various techniques such as flow cytometry and immunofluorescence of mouse platelets, protein binding studies, mass spectrometry, and X-ray diffraction. These data will be collected from a minimum of three independent experiments, comparing two or more groups (mouse genotypes; treatment conditions etc.) including but not limited to appropriate controls (genetic, solvent vehicle, etc.). The total size of the data collected is projected to be 500 GB. We expect to generate the following data file types and formats during this project: Nikon microscopic image files (.ND2), and Amersham imager 600 gel image files (.TIFF). Raw data files will be analyzed to generate CSV files of image quantitation data and to enable statistical analysis.

Data from all relevant experiments of sufficient quality to replicate research findings will be preserved and shared. This data standard includes documentation of: relevant experimental variables on a per-sample basis, raw data in a standard format, processed data on which conclusions are based in the form of standard files or tables, information about the experiment and relationships between biological samples and resulting data, and wet-lab and dry-lab protocols.

Sample collection and processing protocols and protocols for biochemical assays, including antibody and other protein concentrations, and validation information will be shared.

Data will be made available in formats that are freely available in public formats that do not require specialized tools to be accessed.

Data will be stored in common and open formats along with references to the sources of those standards when applicable.

Mass spectrometry (MS) datasets produced from this project will be processed by commercially available MS data annotation software. These datasets and generated reports will be shared with our scientific community through publicly available web-resource including PRIDE Archive, MassIVE, and/or jPOST for the proteomics data repository.

The crystallographic data will be deposited into the Protein Data Bank (PDB). Imaging data will be deposited into NCI’s Imaging Data Commons. All other data described above in the “data to be shared” section will be deposited into Zenodo or published as supplementary materials along with the manuscripts.

The crystallographic data in the PDB bank will be findable by the protein name or the author name. Each entry will be assigned an identification code by the PDB bank.

All the other published data will be discoverable online through a standard web search of the study-level metadata as well as the persistent pointer from the DOI to the dataset.

All scientific data generated from this project will be made available as soon as possible, and no later than the time of publication or the end of the funding period, whichever comes first. The duration of preservation and sharing of the data will be a minimum of 10 years after the funding period.

No anticipated factors or limitations will affect the access, distribution, or reuse of the scientific data generated by the proposal.

Controlled access will not be used. The data that is shared will be shared by unrestricted download.

In order to ensure participant consent for data sharing, IRB paperwork and informed consent documents will include language describing plans for data management and sharing of data, describing the motivation for sharing, and explaining that personal identifying information will be removed.

Lead PI Hervé Falet, PhD, ORCID: 0000-0003-0788-9204, will be responsible for the day-to-day oversight of lab/team data management activities and data sharing. Broader issues of DMS Plan compliance oversight and reporting will be handled by the PI and Co-I team as part of general Versiti Blood Research Institute stewardship, reporting, and compliance processes.

Planned Research Outputs

Dataset - "Structure of filamin A repeat 20-21 in complex with PACSIN2 and integrin β1"

This project will generate protein structure datasets produced from X-ray diffraction of human filamin A crystals in the presence of PACSIN2 and integrin β1 peptides.

Dataset - "Integrin β1 and β3 interactome"

This project will generate mass spectrometry datasets produced from integrin β1 and β3 pulldown of human and mouse platelet lysates.

Planned research output details

Title	Type	Anticipated release date	Initial access level	Intended repository(ies)	Anticipated file size	License	Metadata standard(s)	May contain sensitive data?	May contain PII?
Structure of filamin A repeat 20-21 in complex wit ...	Dataset	Unspecified	Open	RCSB Protein Data Bank		None specified	None specified	No	No
Integrin β1 and β3 interactome	Dataset	Unspecified	Open	jPOSTrepo		None specified	None specified	No	No