Plan Overview

A Data Management Plan created using DMP Tool

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

Title: Membrane-cytokeleton interactions in megakaryocytes and platelets

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Template: NIH-Default DMSP

Project abstract:

Abnormal platelet production and function, due to genetic factors, cancer therapy, or unknown causes, pose significant clinical risks, including bleeding and thrombotic events. Platelets are produced primarily in the bone marrow by megakaryocytes through intricate processes involving polyploidization and extensive membrane and cytoskeletal rearrangements. These include the formation of the demarcation membrane system (DMS), the surface-connected membrane reservoir necessary for proplatelet extension and release within bone marrow sinusoids. Megakaryocytes also form podosomes, which serve as mechanosensing structures to identify the most conducive sites for initiating trans-endothelial proplatelet extension. The precise molecular mechanisms responsible for these unique membrane and cytoskeletal rearrangements remain poorly understood. The membrane-shaping F-BAR protein PACSIN2 stands central in the interplay between membranes and the cytoskeleton. PACSIN2 contains an N-terminal F-BAR domain tubulating membranes and a C-terminal SH3 domain interacting with the endocytic GTPase dynamin 2 (DNM2) and actin-nucleation-promoting factor WASp. Through its F-BAR domain, PACSIN2 also interacts with the cytoskeletal and scaffolding protein filamin A (FlnA), a critical regulator of platelet production and function. Single nucleotide polymorphisms in PACSIN2 have been associated with key platelet parameters in humans. Our recent data has discovered pivotal insights into the role of PACSIN2 in megakaryocyte and platelet biology. PACSIN2 is an internal component of the initiating DMS in megakaryocytes, where its membrane tubulation activity is regulated by FlnA (PMCID: PMC4492198). Pacsin2–/– mice display a mild thrombocytopenia with slightly enlarged platelets and marked platelet-intrinsic thrombus formation defects (PMCID: PMC10841284). Pacsin2–/– megakaryocytes have a mildly defective DMS, reduced ploidy, and impaired podosome and proplatelet formation. Pacsin2–/– platelets display elevated integrin β1 activity. Deletion of integrin β1 within megakaryocytes effectively normalizes the thrombocytopenia and thrombus formation defects. We hypothesize that PACSIN2 regulates membrane-cytoskeletal interactions and integrin β1 activity to govern the formation and organization of the DMS and podosomes during megakaryocyte maturation. We propose three aims to investigate the molecular mechanisms underlying how PACSIN2 modulates platelet production and function. In Aim 1, we will characterize megakaryocyte

maturation and platelet production in the presence or absence of PACSIN2 and integrin β1. In Aim 2, we will identify the PACSIN2 effector proteins modulating actin assembly/remodeling at sites of podosome formation in megakaryocytes by proteomics and overexpression methodologies. In Aim 3, we will define the mechanisms by which PACSIN2 modulates integrin β1 activity to regulate thrombus formation and platelet hemostatic function. We anticipate that our studies will yield basic information on how PACSIN2 contributes to megakaryocyte and platelet biology.

Start date: 04-01-2025

End date: 03-31-2030

Last modified: 07-08-2024

Copyright information:

Membrane-cytokeleton interactions in megakaryocytes and platelets

Data Type

Types and amount of scientific data expected to be generated in the project: *Summarize the types and estimated amount of scientific data expected to be generated in the project.*

Describe data in general terms that address the type and amount/size of scientific data expected to be collected and used in the project (e.g., 256-channel EEG data and fMRI images from ~50 research participants). Descriptions may indicate the data modality (e.g., imaging, genomic, mobile, survey), level of aggregation (e.g., individual, aggregated, summarized), and/or the degree of data processing that has occurred (i.e., how raw or processed the data will be)

In this proposed project, data will be generated using various techniques such as flow cytometry, immunofluorescence and electron microscopy of mouse megakaryocytes, protein binding studies, and mass spectrometry. 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.

Scientific data that will be preserved and shared, and the rationale for doing so: *Describe which scientific data from the project will be preserved and shared and provide the rationale for this decision.*

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.

Metadata, other relevant data, and associated documentation: Briefly list the metadata, other relevant data, and any associated documentation (e.g., study protocols and data collection instruments) that will be made accessible to facilitate interpretation of the scientific data.

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

Related Tools, Software and/or Code

State whether specialized tools, software, and/or code are needed to access or manipulate shared scientific data, and if so, provide the name(s) of the needed tool(s) and software and specify how they can be accessed.

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

Standards

State what common data standards will be applied to the scientific data and associated metadata to enable interoperability of datasets and resources, and provide the name(s) of the data standards that will be applied and describe how these data standards will be applied to the scientific data generated by the research proposed in this project. If applicable, indicate that no consensus standards exist

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

Data Preservation, Access, and Associated Timelines

Repository where scientific data and metadata will be archived: Provide the name of the repository(ies) where scientific data and metadata arising from the project will be archived.

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.

How scientific data will be findable and identifiable: Describe how the scientific data will be findable and identifiable, i.e., via a persistent unique identifier or other standard indexing tools.

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.

When and how long the scientific data will be made available: Describe when the scientific data will be made available to other users (i.e., no later than time of an associated publication or end of the performance period, whichever comes first) and for how long data will be available.

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.

Access, Distribution, or Reuse Considerations

Factors affecting subsequent access, distribution, or reuse of scientific data: NIH expects that in drafting Plans, researchers maximize the appropriate sharing of scientific data. Describe and justify any applicable factors or data use limitations affecting subsequent access, distribution, or reuse of scientific data related to informed consent, privacy and confidentiality protections, and any other considerations that may limit the extent of data sharing.

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

Whether access to scientific data will be controlled: State whether access to the scientific data will be controlled (i.e., made available by a data repository only after approval).

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

Protections for privacy, rights, and confidentiality of human research participants: If generating scientific data derived from humans, describe how the privacy, rights, and confidentiality of human research participants will be protected (e.g., through de-identification, Certificates of Confidentiality, and other protective measures).

Not applicable.

Oversight of Data Management and Sharing

Describe how compliance with this Plan will be monitored and managed, frequency of oversight, and by whom at your institution (e.g., titles, roles).

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 - "PACSIN2 interactome"

This project will generate mass spectrometry datasets produced from PACSIN2 pulldown from 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?
PACSIN2 interactome	Dataset	Unspecified	Open	jPOSTrepo		None specified	None specified	No	No