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International PhD PPU-IMAGINE Proposal

Call 2023/2024

PhD PROPOSAL IDENTIFICATION

PhD Project title	Integrative mRNA sequencing, proteomics and glycoproteomics to guide therapeutic repurposing in Darier and Hailey-Hailey diseases
Project Acronym	Omics in Darier and Hailey diseases
Project Keywords	Calcium pumps, desmosomes, endoplasmic reticulum stress, cell-to-cell adherence

LABORATORY PRESENTATION

Laboratory Team Name	Laboratory of Genetic skin diseases: from disease mechanisms to treatments
Laboratory Director full name	Hovnanian Alain
Department IP	Imagine Institute
Doctoral school	ED562 BioSPC
University	Paris Cité
Laboratory Telephone	(014) 275-4289
Laboratory website	https://www.institutimagine.org/fr/alain-hovnanian-182

PhD PROPOSAL

PhD Supervisor full name	Hovnanian Alain
PhD Supervisor position	Lab Director
PhD Supervisor email	alain.hovnanian@inserm.fr
Number of ongoing PhD students supervised by the Supervisor	1 PhD student in his 4th year
PhD Co-Supervisor full name	Titeux Matthias
PhD Co-Supervisor position	Theme leader
PhD Co-Supervisor email	matthias.titeux@inserm.fr
Number of ongoing PhD students supervised by the Co-Supervisor	1 PhD student in his fourth year

PhD Proposal abstract (1000 characters maximum)

Darier (DD) and Hailey-Hailey (HHD) are severe dominant genetic skin diseases characterized by acantholysis (cell-to-cell separation), with abnormal keratinization and apoptotic cells in DD. We previously shown that they are caused by haploinsufficiency of Ca2+ pumps of the endoplasmic reticulum (DD) and the Golgi apparatus (HHD). Their pathophysiology remains poorly understood although desmosome formation and glycosylation are abnormal. There is no specific treatment, and these orphan diseases cause a great distress.
We propose an unbiased approach combining bulk and single cell mRNA sequencing, global proteomics and glycoproteomic to identify abnormal biological pathways in lesional and non lesional skin from 20 DD patients, 20 HHD patients compared to 10 healthy subjects. The results will be integrated and validated by qRT-PCR, immunostaining and western-blot using patient skin biopsies and/or keratinocytes. The identification of new therapeutic targets will guide drug repurposing.

PhD Proposal (10 000 characters maximum)

I - Background and significance

Darier (DD)(or Darier-White disease) and Hailey-Hailey (HHD) (also known as benign chronic pemphigus disease), are severe dominant genetic skin diseases characterized by acantholysis (cell-to-cell separation) of the epidermis caused by desmosome rupture which is a major characteristic of lesioned areas in DD and HHD. DD is associated with abnormal keratinization (dyskeratosis) and the presence of apoptotic cells while HHD is not. Patients with DD or HHD suffer from painful keratotic papules or erosions and extensive inflammatory skin lesions. Both diseases progress in flares-ups which are worsened by triggering factors such as heat, friction, sweating, infections and stress. We previously showed that DD and HHD are caused by haploinsufficiency of calcium pumps of the endoplasmic reticulum (*ATP2A2* encoding SERCA2) and the Golgi apparatus (*ATP2C1* encoding SPCA1), respectively. SPCA1 also transports and maintains manganese homeostasis in the Golgi. The chronicity of DD and HHD, the multiple recurrence of flares and the lack of specific treatment make the management of these diseases a challenge for physicians.
The understanding of the pathogenesis of both diseases remains limited. SERCA2 and SPCA1 haploinsufficiency impairs desmosome formation and causes acantholysis. Previous studies in Darier have pointed to ER stress, impaired glycosylation of desmosomal components and abnormal trafficking of adhesion molecules including E-Cadherin and desmoplakin. Studies in HHD have reported increased oxidative stress, Notch-1 pathway activation and reduced DNA damage response.

II - Specific aims

This project aims to identify key biological pathways altered in DD and HHD to guide new therapeutic options using drug repurposing. It relies on the unbiased study of bulk and single-cell mRNA sequencing combined with proteomic and glycoproteomic analysis of lesional skin compared with non-lesional skin and healthy controls.

III - Hypothesis

Our hypothesis is that abnormal calcium concentrations in the ER and Golgi apparatus in DD and in HHD, respectively, trigger ER and Golgi stress, impair protein glycosylation and the transcription of Ca^{2+} dependent genes such as the Epidermal Differentiation Complex genes. The resulting loss of epidermal integrity triggers an inappropriate inflammatory response involving the recruitment of innate and adaptive immune cells producing pro-inflammatory cytokines and chemokines which aggravate tissue damage and acantholysis.

IV - Preliminary Studies

We have previously shown that E-cadherin and desmoplakin trafficking is impaired in DD and that reversal of ER stress improves cell-to-cell adherence in vitro.
We have collected skin biopsies from lesional and non-lesional areas from 20 patients with DD, 20 patients with HHD and 10 healthy controls (frozen OTC-embedded, RNA later, snap frozen and formalin fixed). Bulk mRNA sequencing data have been generated from the frozen biopsies stored in RNA later and will be analyzed. The other skin biopsies will be essential to perform scRNA seq, proteomic and glycoproteomic studies, and validation studies. We have also isolated primary keratinocytes from the skin biopsies of 10 DD and 10 HHD patients which will be used for validation studies.
We have successfully established protocols for bulk mRNA sequencing, scRNA seq and proteomics using patient skin biopsies with other genetic skin conditions.
All the techniques that will be used for validation, including *in situ* mRNA hybridization, immunostaining, RT-qPCR, western blotting are routinely used in the laboratory.

V - Experimental Design

Aim 1.Transcriptional profiling of lesional and non-lesional DD and HHD skin

Task 2.1 Bulk transcriptional analysis

The global specific transcriptional profile of lesional DD and HHD skin will be characterized in comparison with non-lesional DD, HHD skin and skin from healthy controls, to identify biological pathways specifically dysregulated in lesional and non-lesional DD and HHD skin. For each sample, skin sections (150µm) will be obtained for RNA extraction using the RNeasy kit (Qiagen). The Imagine genomic platform will prepare complementary DNA (cDNA) using the NuGEN Ovation RNA-Seq System to obtain the strand-specific RNA-seq libraries from RNA. Equimolar pool of the final indexed RNA-Seq libraries will be sequenced on an Illumina NovaSeq to obtain about 50 million paired-end reads per library. Differential gene expression analysis will be performed using 3 independent statistical methods (DEseq2, Voom, EdgeR). The quality of sequencing data obtained from frozen-OCT embedded samples and from RNA later samples will be compared, and biological pathways will be investigated using Ingenuity pathway and Lincs1000 softwares.

Task 2.2 Single-cell transcriptional analysis.

Part 1. Frozen OCT-embedded samples collected from 3 DD and 3 HHD patients will be analyzed first. The nuclei number obtained with this technique will be compared with the reference protocol used in the laboratory from fresh biopsies. For each sample, nuclei will be isolated from frozen skin sections (40 -100 µm) using the Chromium Nuclei Isolation kit from 10X Genomics. The Imagine single-cell platform will perform cell encapsulation, mRNA sequencing and data processing.

Part 2. Provided that the quality of the scRNA sequencing data obtained from frozen skin sections is sufficient, 2-mm skin biopsies obtained from all patients will be investigated using the newly established protocol (as described above). Alternatively, should this protocol be not satisfactory, 2x4-mm fresh skin biopsies will be proposed to 10 DD patients and 10 HHD patients for cell dissociation.

Aim 2. Proteomics (global and N-glycosylation) of lesional and non-lesional DD and HHD skin

A comparative proteomic and glycoproteomic analysis using high-resolution mass spectrometry (LC-MS/MS) will be performed in lesional DD and HHD skin compared to non-lesional DD and HHD skin and skin from healthy controls by the proteomic platform of the Structure Fédérative de Recherche (SFR) Necker. This study will initially compare 3 lesional DD and HHD skin, 3 non-lesional DD and HHD skin and 3 healthy controls for a pilot study, before investigate a total of 20 DD and 20 HHD patients and 10 healthy controls. Two types of analyses will be conducted:

Part 1. A comparative analysis of global proteomes. Proteins will be extracted and digested using the FASP metho. Peptides will be subject to prior fractionation to improve the depth of the analysis. The peptides obtained will be analyzed on a nanoRSLC-Qexactive Plus MS using a “label-free” approach.

Part 2. An analysis of N-glycosylated proteins using the glycoFASP method to map glycosylated sites of proteins.

DIANN and Spectraunaut will be used for protein and N-glycosylation analysis, and deregulated proteins in lesional and non-lesional skin will be investigated using Mass Dynamics software. Corresponding pathways will be explored using Reactome, KEGG, and GO databases. Integrated analysis of the proteome and the glycoproteome will allow to identify proteins which abundance and/or glycosylation patterns are abnormal.

Aim 3. Validation of biological cascades and identification of therapeutic targets by integrative analyses

Task 3.1 Identification of pathways and protein biomarkers by integrative analyses.

Data collected from bulk RNA sequencing, scRNA-seq, proteomic and glycoproteomic results will be integrated. Data integration of proteomics and transcriptomics data is a multifaceted process. On one hand, we will compare deregulated biological classes identified by gene expression and protein abundance analysis, enabling the recognition of key regulatory events in DD and HHD pathogenesis. On the other hand, we will explore non-canonical proteins and alternative splicing events, by searching proteomics data against transcriptomic sequences for molecular insights in the disease (using openprot.org). We will use integration methods, such as correlation analysis or network-based approaches, to uncover the relationships between genes, proteoforms, and their functional roles as previously shown.

Task 3.2 Validation of pathways by molecular and biochemical analyses.

Using patient keratinocytes and/or skin biopsies already available from 10 patients with DD and 10 patients with HHD, identified pathways will be validated using RT-qPCR, western blot, immunostaining and ELISA.

Aim 4. Drug repurposing

This multimodal and integrative profiling of DD and HHD lesional and non-lesional skin has the potential to better understand the involvement of ER and Golgi stress, abnormal Ca^{2+} homeostasis, apoptosis, impaired cell-cell adhesion, deregulated keratinisation and skin inflammation in DD and HHD and to uncover new therapeutic targets. These targets could guide drug repurposing which aims at identifying new therapeutic uses for existing drugs. Based on omics data generated by genomics, transcriptomics and proteomics, several disease targeted-based methods have emerged to explore the unknown mechanisms of existing drugs. We will conduct a systematic expression-based drug repurposing approach for lesional PC skin by integrating lesional PC skin related pathways from differentially expressed genes and drug-affected pathways from connectivity map (CMap) as previously described. The identification of candidate drugs selected from this study may lead to pre-clinical and clinical studies to validate their therapeutic potential.

Laboratory's best 5 publications

Sakuntabhai A, Ruiz-Perez V, Carter S, Jacobsen N, Burge S, Monk S, Smith M, Munro CS, O'Donovan M, Craddock N, ..., Owen M, Lathrop GM, Monaco AP, Strachan T and Hovnanian A. Mutations in ATP2A2, encoding a Ca2+ pump, cause Darier disease. Nat Genet. 1999. 21 : 271-7.

Sudbrak R, Brown J, Dobson-Stone C, Carter S, Ramser J, White J, Healy E, ... Munro CS, Strachan T, Burge S, Hovnanian A and Monaco AP. Hailey-Hailey disease is caused by mutations in ATP2C1 encoding a novel Ca(2+) pump. Hum Mol Genet. 2000. 9 : 1131-40.

Fairclough RJ, Dode L, Vanoevelen L, ... Wuytack F, Hovnanian A. Effect of Hailey-Hailey Disease mutations on the function of a new variant of human secretory pathway Ca2+/Mn2+-ATPase (hSPCA1). J Biol Chem. 2003. 278 : 24721-30.

Savignac M, Simon M, Edir A, Guibbal L, Hovnanian A. SERCA2 Dysfunction in Darier Disease Causes ER stress and Impaired Cell-to-Cell Adhesion Strength: Rescue by Miglustat. J Invest Dermatol. 2014 Jul; 134(7): 1961-70

Barbieux C, Bonnet des Claustres M, ... Schilling O, Gudjonsson JE, Hovnanian A. Netherton syndrome subtypes share IL-17/IL-36 signature with distinct IFN-α and allergic responses. J Allergy Clin Immunol. 2021 Sep 17:S0091-6749(21)01398-1. PMID: 34543653

Expected profile of the candidate

A candidate with a knowledge and if possible an experience in mRNA sequencing (bulk and/or single cell), proteomics and/or glycoproteomics will be sought. The candidate should be proficient in using R and Python analysis tools. He/she should also have a good background in molecular and cellular and some experience in cell culture, immunostaining and qRT-PCR.

Importantly, all facilities and services required for the project are available at the Imagine Institute for genetic diseases (http//www.institutimagine.org) and will be made available to the candidate in a highly collaborative spirit. Specifically, Imagine Single-cell platform (Michaël Ménager), Imagine Genomic platform (Christine Bole), SFR Necker proteomic platform (Chiara Guerrera - https://www.sfr-necker.fr/proteomics)