PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

@IMAGINE INSTITUTE

PROJECT

- **FILE #07**
- **ACRONYM:** DarHaiomics
- **TITLE:** Integrated Gene and Proteomic Signatures in Darier and Hailey-Hailey Diseases

LABORATORY

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LABORATORY PRESENTATION AND RESEARCH TOPICS

- **SUPERVISOR HDR:** Pr. Alain HOVNANIAN, alain.hovnanian@inserm.fr

- **SPECIFY THE TEAM NAME:** Genetic skin diseases: from disease mechanisms to treatments, IMAGINE Institute, Paris

- **CO-SUPERVISOR:** Dr Sonia GAUCHER, sonia.gaucher@aphp.fr

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DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** genetic skin disease, Darier disease, Hailey-Hailey disease, mRNA sequencing, proteomics, epidermal differentiation, acantholysis, calcium, SERCA2, SPCA1

- **ABSTRACT**

This project aims at identifying the gene and proteomic signatures of two rare and severe orphan genetic skin diseases, namely Darier disease and Hailey-Hailey diseases, whose genes were identified by the host laboratory. Although both diseases are caused by haploinsufficiency of Ca^{2+} pumps of the endoplasmic reticulum (Darier disease) or the Golgi apparatus (Hailey-Hailey disease), the precise cellular and molecular mechanisms underlying the pathogenesis of both diseases remains unclear. By using unbiased and combinatory mRNA transcriptomic and proteomic studies from affected and non-affected skin biopsies, we aim to identify biological pathways amenable to specific targeting using existing biotherapies. Year 1 will involve mRNA sequencing and proteomic studies of affected and non-affected skin biopsies from 10 DD and 10 HHD patients and 10 healthy controls. *In situ* single cell RNA-Seq from patient skin biopsies will also explore spatial gene expression to gain a complete view of disease complexity. During year 2, transcriptomic and proteomic data will be validated experimentally and will be compared with transcriptomics and proteomics of keratinocyte cultures from Darier and Hailey-Hailey patients to identify intrinsic epithelial abnormalities. Year 3 will aim at using biologics or siRNA strategies to

target specific biological pathways upregulated in Darier and Hailey-Hailey disease using a 3D organotypic skin model. A combined and integrated analysis of deregulated biological pathways identified by proteomic and transcriptomic analyses will provide a comprehensive picture of cellular and molecular disturbances in both diseases. We expect to gain a comprehensive view of disease complexity and to identify biological pathways which could be targeted by new specific treatments. This project should the foundation for the development of precision medicine relying on the demonstration of proof of principles in 3D organotypic skin models.

- **DESCRIPTION OF THE PROJECT**

Introduction

Darier disease (DD) and Hailey-Hailey disease (HHD) are rare and severe dominant genetic skin diseases characterized by acantholysis (cell-to-cell separation) of the epidermis. Additionally, DD is associated with abnormal keratinization and the presence of apoptotic cells while HHD is not. Both diseases can manifest as extensive inflammatory skin lesions, are worsened by triggering factors, and cause a considerable burden. DD and HHD are caused by haploinsufficiency of Ca^{2+} pumps of the endoplasmic reticulum (*ATP2A2* encoding SERCA2) and the Golgi apparatus (*ATP2C1* encoding SPCA1), respectively (Sakuntabhai *et al.,* 1999, Hu *et al.* 2000, Sudbrak *et al*. 2000). In addition, SPCA1 also transports Mn^{2+} and maintains Mn^{2+} concentrations in the Golgi. There is currently no specific treatment and both diseases are orphan diseases which cause considerable distress with major unmet medical need.

Objectives

We propose to use an unbiased and combinatory approach to capture the gene and proteomic signatures of DD and HHD *in vivo* and *in vitro* in comparison with healthy controls, using a unique cohort of patients with DD and HHD. The project includes:

- *In vivo* comparative studies using mRNA sequencing and proteomic studies from affected and non-affected DD and HHD skin
- *In vitro* comparative studies using gene and proteomic approaches applied to DD and HHD patient keratinocytes in culture
- Functional studies aiming at testing biotherapy approaches on a 3D organotypic skin model of DD and HHD

Work Plan

WP1. The first year comprises mRNA sequencing and proteomic studies of DD and HHD from affected and non-affected patient skin biopsies. These data will correspond to gene and proteomic signature of a mixture of cell types including keratinocytes in majority, but also neutrophils, mast cells, dendritic

cells, T and B cells. We will also perform *in situ* single cell RNA-Seq from new patient skin biopsies to explore spatial gene expression and gain a complete view of disease complexity.

WP2. During the second year, transcriptomic and proteomic data will be validated experimentally using qRT-PCR and immuno-staining of patient skin sections. For *in vitro* studies, we will cultivate patient keratinocytes to identify intrinsic gene and proteomic epidermal signatures. We will check for possible N-glycosylation abnormalities in extracted proteins for HHD and DD. The generation of transcriptomic and proteomic data will allow a combinatory omics approach.

WP3. It is likely that comparison of data obtained in WP1 and WP2 will allow the identification of prominent inflammatory signaling pathways in DD and HHD. During the third year, we will specifically target keratinocyte-derived cytokines that we will have determined. We will create a 3D organotypic skin model using cultured patient keratinocytes that allow terminal differentiation. This model will be used for functional studies to test new treatments targeting specific pathways identified in WP1 and WP2.

Expected Results

We expect to find gene signatures that are different and overlapping between DD and HHD because both are acantholytic diseases caused by Ca^{2+} pumps deficiency, along with differences in epidermal differentiation for DD and N-glycosylation abnormalities in HHD. We anticipate that differentially expressed genes are involved in pathways such as cell-cell adhesion, Ca^{2+} dependent gene expression, endoplasmic reticulum stress signatures, apoptotic pathways, etc. Because our approaches are unbiased, we hope to discover novel pathways and therapeutic targets. A combined and integrated functional analysis of deregulated biological pathways identified by proteomics and transcriptomics will provide a more complete picture of cellular and molecular disturbances in both diseases. *In situ* mRNA seq has the potential to identify spatial transcriptome profile of different cell types in affected skin. We expect to gain a comprehensive view of disease complexity including inflammatory profiles of both diseases to assess whether pro-inflammatory interleukins involved in other inflammatory diseases targeted by existing biotherapies are involved. Should this be the case, they could be specifically targeted in the 3D organotypic skin models.

Ability to Succeed

This project is highly feasible because patient material (skin biopsies and keratinocytes) has already been collected and high quality mRNA data from skin biopsies have been generated. The project will benefit from the transcriptome experience in Dr. Alain Hovnanian's lab and omics facilities at Imagine (transcriptomics, bioinformatics, single cell RNA-Seq) and INEM (proteomics). Proteomic investigation will be guided by Dr. Chiara Guerrera and Dr. Mickaël Ménager will assist in single-cell RNA-Seq analysis, each of them being experts in their respective fields.

- **REFERENCES**

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EXPECTED PROFILE OF THE CANDIDATE

- **EXPERIENCE REQUIRED**
  Bioinformatics, transcriptomics, proteomics, cell culture, molecular biology.