Internship proposal for Master 2 Research

Identification of key cell-cell interactions for the biology of pluripotent embryonic stem cells

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Context

Intercellular communication is crucial for the development and maintenance of multicellular organisms. Recent research has emphasized the significance of communication between extraembryonic cells (trophectoderm and hypoblast) and pluripotent embryonic cells (epiblast) in the mammalian embryo [1,2,3]. Specifically, the extracellular matrix produced by the hypoblast plays a key role in controlling the cell proliferation of pluripotent epiblast stem cells. Our research on porcine embryonic stem cells has confirmed the importance of the extracellular matrix in influencing signaling pathways and transcription factors that regulate the pluripotency of epiblast cells [4]. One of our most original observation relies in the detection of tetraspanins CD9, CD81 and CD63 expression mostly in the trophectoderm (unpublished). These tetraspanins are known markers of extracellular vesicles (EVs), which are small lipid vesicles secreted by various cell types [5]. EVs are involved in transporting signaling proteins, cytokines, and transcription factors to recipient cells, influencing various processes including immune responses, tumor progression, and embryonic development [6,7]. Although EV-mediated cell-to-cell communication between embryonic and extraembryonic cells has been studied in the mouse embryo, the focus has mainly been on EVs secreted by mouse pluripotent stem cells [8,9]. This research aims to study the role of EVs secreted by extraembryonic trophectoderm cells and pluripotent epiblast cells in facilitating communication and influencing the biology of these cell types. This research is funded by the ANR STEM4PIGS (2025-2028) and will support a PhD student starting from September 2025.

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5. van Niel G, D'Angelo G, Raposo G. (2018) Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 19(4):213-228.
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Objectives

The main objectives of the internship will be to characterize extracellular vesicles trafficking between trophoblast and pluripotent stem cells, and to test, through *in vitro* experimentations, the transfer of EVs between cells in co-culture and their effects on the self-renewal and pluripotent phenotype of ESCs.

Methodologies

In the initial phase of the project, the master's student will collaborate with our staff to purify and characterize extracellular vesicles (EVs) from pig embryonic stem cells (ESCs) and pig trophoblast stem cells (TSCs) that are currently available in our laboratory. Utilizing optical and biochemical techniques, the student will quantify and analyze the EVs, focusing on their size, type, and cargo composition including proteome, transcriptome, and lipidome. This data will be used to compare EVs from ESCs and TSCs using various computational tools.

Moving on to the next phase of the project, the master's student will undertake the cultivation of ESCs and TSCs in culture media supplemented with purified EVs from ESCs or TSCs. Additionally, co-culture experiments between ESCs and TSCs will be conducted. The transfer of EVs from one cell type to another will be analyzed using confocal microscopy. Furthermore, the biological effects of EVs on each cell type will be characterized through gene expression studies (qPCR, RNAseq) and proteomic analysis (immunolabeling, mass spectrometry).

The student will be supervised by Hervé Acloque and Anne Burtey, who possess complementary expertise in the biology of mammalian stem cells and EVs. Our staff will also provide assistance with daily experiments. Additionally, the student will have dedicated weekly meetings with his two supervisors, alongside participating in weekly team and unit seminars.

Required skills

The candidate needs to like experimental work, in particular cell culture and molecular biology.

He/she should be comfortable with basic scientific calculation (dilution calculation, unit conversion) and common statistical analyzes. An intermediate level for the use of bioinformatics tools and of R will be a positive point. He/she will benefit from the infrastructure of a laboratory dedicated to functional genomics in a dynamic research center. Possibility of being accommodated in the research center.