

**MCB Internship project 8:**  
**Role of PrP in leukocyte and endothelial cell adhesion**

**Introduction:** The prion protein (PrP) is a glycosyl-phosphatidyl-inositol (GPI)-anchored glycoprotein that resides in cholesterol-rich membrane micro-domains on the plasma membrane. PrP is mainly known for its implication in fatal neurodegenerative disorders, in which the accumulation of a misfolded isoform of PrP causes neural damage. However, the physiological function of PrP remains unclear even though a large variety of functions have been proposed. In endothelial cells, PrP accumulates at the cell-cell junctions and appears to stabilize cell-cell adhesion. In gastric cancers, however, PrP has a pro-metastatic role. PrP has also been involved in the long-term renewal of hematopoietic stem cells.

We have recently found that PrP regulates leukocyte and endothelial cell migration. Our hypothesis is that the role of PrP in migration involves the modulation of cell-cell and cell-matrix adhesion by PrP. Since adhesion is a very potent stimulus regulating cellular processes in a cell-specific manner, we hypothesize that regulation of cell adhesion by PrP lays at the basis of the multiple functions attributed to this protein.

Our current research questions are: What are the mechanisms involved in the regulation of leukocyte and endothelial cell migration by PrP? Does PrP regulate integrin- and VE-cadherin-mediated adhesion? What is the role of PrP in long-term hematopoietic stem cells?

**Project 8.1 Regulation of cell-cell and cell-matrix adhesion by PrP: identification of the PrP molecular complex**

Our current research indicates that PrP modulates cell migration. Adhesion to the substrate and to neighboring cells is essential for cells to migrate. We want to investigate if PrP is involved in cell adhesion through identifying the proteins interacting with PrP on the plasma membrane. For this purpose we have made a PrP variant that can be specifically tagged only when expressed on the cell surface. We want to isolate PrP-associating proteins by pulling-down this tagged PrP and identify the associating proteins by mass spectrometry analysis. This project will provide essential information on the molecular mechanisms of PrP-modulation of cell adhesion and migration. Also, the data obtained in this project will contribute to clarify the elusive physiological function of the prion protein.

**Techniques**

- Culture of primary human endothelial cells and of leukocyte cell lines
- Cell transfection
- Pull-down assay
- Western-blotting
- Preparation of samples for mass spectrometry analysis
- Analysis of the mass spectrometry results with the help of protein databases and literature
- Detection of selected proteins in cells by confocal laser scanning microscopy
- Cell migration and adhesion assays
- Angiogenesis assays

**Project 8.2 Regulation of hematopoietic stem cell renewal and differentiation by PrP**

PrP is required for the maintenance of the pool of long-term hematopoietic stem cells (HSCs). However, the mechanism of action of PrP is yet unknown. We want to study the role of PrP, as a modulator of cell adhesion, in the renewal and differentiation of human HSC in a series of in vitro assays to determine HSC proliferation and differentiation to the various hematopoietic lineages in cells where PrP has been silenced. The results obtained in this project may contribute to improve the conditions for the in vitro culture and expansion of hematopoietic stem cells in order to increase their number before re-infusion into patients.

**Techniques:**

- Isolation and culture of hematopoietic stem cells
- Silencing of PrP by lentiviral transduction of small hairpin RNAs
- HSC differentiation assays
- HSC proliferation assays
- Fluorescence activated cell sorting (FACS)

**Duration:** 7 - 9 months. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the project leaders, Mar Fernandez-Borja and Emilie Reinen, either by e-mail:

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