Internship proposal for M2R

Modeling the plasticity of mammary epithelial cells in response to cyclic activation of EMT inducers (and circadian genes): impact on animal and human health

Contact : Hervé Acloque
Laboratoire GABI, equipe GaLac UMR1313 78350 Jouy en Josas
herve.acloque@inrae.fr
tel : 0134652810

Context :

The epithelial to mesenchymal transition (EMT) is a process through which cells shift their properties, losing strong cell-cell adherence and gaining migratory capacities. This process can be reversed by a mesenchymal to epithelial transition (MET). Cells don't always undergo a full transition from one phenotype to the other, rather there exists a spectrum of cellular phenotypes ranging from fully epithelial to fully mesenchymal (Nieto et al. 2016). Cells which have undergone partial EMT are associated with increased stem/progenitor functions (Forte et al. 2017, Pastushenko et al. 2018 PMID29670281). The fate of a cell along the epithelial/mesenchymal spectrum is primarily determined by gene expression patterns. Depending on transcription factor activity, these patterns can be reversed at any time. However, in certain conditions repeated EMTs can lead to stable changes in the cells, affecting their entire lineage. One such example is the accumulation of de novo epigenetic modifications at biologically relevant sites in premalignant cells (Dumont et al 2008). In vitro, proliferating cells undergoing TGF-β induced EMT suffer mitotic abnormalities. The result of these abnormalities can be permanent changes to cell ploidy and genomic heterogeneity which can contribute to tumour progression (Comaills et al. 2016). We hypothesise that such cycles can also significantly impact the plasticity of mammary epithelial cells.

Here we enquire on the phenotypic consequences of cytokine-induced cycles of EMTs and METs on epithelial mammary cells. The object is to determine whether repeated EMT/MET cycles can have a lasting effect on these cell's plasticity, and whether mutations in circadian clock genes can modulate their response. We previously observed that experimental repetition of EMT/MET cycles seems to behave like an oscillatory system in equilibrium between an initial E state and an induced M state. Combining experimental approaches and mathematical modeling, we now want to study whether we can define a point of no return where this equilibrium is disrupted toward any E/M phenotype and whether this point of no return can poise cells toward transformation.

Objectives

The main objective of the internship will be to test through *in vitro* experimentations, the oscillatory patterns of gene expression during EMT/MET cycles, how these patterns are modified in mutated cell lines (clock genes, TP53) and whether we can define a point of no return where this equilibrium is oscillatory pattern is disrupted toward any E/M phenotype.

Methodologies

Regarding the experimental part of the project, the master student will be in charge of producing stable mutated mammary epithelial cell lines for CLOCK, BMAL1, PER2 and CRY2 genes using CRISPR Cas9 technology and to perform series of EMT/MET cycles on these mutated cell lines. In collaboration with the genomic facility @Bridge in Jouy en Josas, he will be in charge to prepare 3'Quantseq RNA-seq libraries for each of the experimental point.

Regarding the data analysis, he will be in charge to perform the primary analysis of existing RNAseq datasets of EMT/MET cycles on unmutated MCF10A and MCF12A. He will then work in collaboration with our indian and national collaborators to identify oscillating genes and specific gene signatures of E to M states. The last point will be to try to identify whether this oscillatory pattern is affected by mutations (datasets available for TP53) and whether we can identify a non return point after a defined number of cycles.

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 is also required. 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.