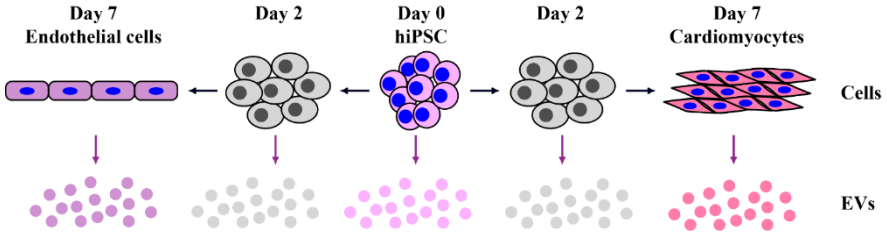
Study of lncRNA differential expression during cardio- and angiogenesis and of molecular mechanisms of their selective packaging into extracellular vesicle

Pluripotent stem cells (PSCs) can efficiently increase their number by cell division and, upon specific stimulation with signalling molecules, have the potential to become almost any specialized cell type. They are therefore of great interest in regenerative medicine where they could be applied to enhance the repair of damaged tissues. Embryonic stem cells are only found during the first stages of embryonic development and their usage poses serious ethical considerations. In 2006, scientists discovered that PSCs can also be generated by introduction of specific genes into adult somatic cells, *e.g* into easily accessible blood cells. Such cells are termed induced PSCs (iPSCs). Human iPSCs (hiPSCs) hold great potential, as platforms for human disease modelling and as source of many cell types in regenerative medicine. The regenerative potential of hiPSC can be at least partially mediated by extracellular vesicles (EVs) that can transfer hiPSC-derived biomolecules to damaged cells and tissues. EVs are small membrane-enclosed structures that are produced by all cell types and serve intercellular communication purposes. They carry proteins, lipids, DNA and RNA. The exact composition of EVs depends, among others, on the cell type and its physiological state. The RNA content of EVs is of significant interest as it is very complex and many of the contained RNAs have important regulatory functions and the capacity to influence the fate of EV-acceptor cells. It was also noticed that the RNAs are selectively loaded into EVs, but it is not yet clear how the cells achieves this selective packaging. RNA is functionally the most diverse biomolecule. RNAs transfer the genetic information from the genome to the protein-producing machinery, regulate gene expression, work as building blocks for cellular structures and catalysts of enzymatic reactions. Long-non coding RNAs (lncRNA) are recently emerging important regulators of various processes in cells. As many of them are identified in cells, but only a small number is endowed with a specific function, it is vital to focus our attention on those versatile RNAs.

In the proposed project, we would like to thoroughly investigate the lncRNA content of hiPSCs before and during differentiation to cardiomyocytes and endothelial cells. Increasing our understanding of these differentiation processes can help to enhance myocardial regeneration after cardiac injuries. RNA samples will be collected from differentiating cells and their respective EVs. The schematic representation of sample collection is presented below:



The lncRNA will be identified using genome-wide techniques: lncRNA microarrays and RNA sequencing. Both methods will allow us to detect numerous RNAs from one sample, quantify them and compare their relative abundance between samples of interest. The results will constitute the basis for selection of lncRNAs for the two subsequent parts of the project. First, we plan to find protein candidates ensuring selective RNA targeting and packaging into EVs. With this aim, we will select lncRNAs that are enriched in EVs at any of the analysed timepoints and use them as baits for the identification of proteins that bind them in cells and in EVs. The influence of identified proteins on the targeting of lncRNAs into EVs would be further tested. Second, we plan to uncover lncRNAs that are involved in differentiation regulation. We will thus select and focus on those lncRNAs which levels increase during the processes of differentiation. Candidate lncRNAs will be depleted from hiPSC by gene silencing technologies. We will then determine whether hiPSCs deprived of selected lncRNAs are able to differenciate to cardiomyocytes and endothelial cells.

The successful completion of the proposed project will increase our knowledge of the regulatory functions of human lncRNAs during stem cell differentiation. It will also provide novel information about lncRNA content in EVs derived from undifferentiated and differentiating hiPSCs. This information can be used in order to further understand the bases of the pro-regenerative effects of EVs in tissue repair. Moreover, upon identification of relevant lncRNAs, hiPSCs can be genetically modified such as to express and load into EVs higher levels of transcripts of interest which might result in increased regenerative potential of such EVs, by promoting differentiation in target cells. Furthermore, the results of this projects will help to uncover the mechanisms of selective packaging of lncRNAs into EVs. Finally, our analysis will be conducted in human cells, making the results directly applicable to human biology. Altogether, the importance of the project consists in its high potential to provide the stem cell-, EV- and RNA-interested scientific communities with interesting, novel and therapeutically-applicable data.