**FINAL PUBLISHABLE SUMMARY REPORT**

Receptor Tyrosine Kinases (RTKs)-dependent signaling control many cellular processes. Tight regulation of signaling propagation and specificity is often lost in various diseases, such as cancer. Endocytosis, the process by which cells sort RTKs for either degradation or recycling, is a potent regulator of signaling specificity and duration, resulting in different cellular outcomes. However, the molecular bases of endocytosis-dependent signaling are not fully understood.

Here, we focused on Epidermal Growth Factor Receptor (EGFR) and Fibroblast Growth Factor Receptors 1 and 2 (FGFRs 1 and 2) as RTKs model systems to define the signaling cascades that are specifically regulated by the different endocytic pathways. Our study was based on an unbiased approach consisting of mass spectrometry (MS)-driven quantitative proteomics (in particular phosphoproteomics), followed by the use of appropriate functional assay to validate the results.

Overview of the work:

* Establishment and optimization of a workflow for the enrichment and the analysis of phosphopeptides (phosphoproteomics).
* Establishment of a new protocol for the enrichment of tyrosine-phosphorylated containing peptides.
* Generation and analysis of biological replicates of phosphoproteomes of the three mentioned RTKs taken at different time points. We characterized the dynamic activation of signaling cascades, focusing on very early (upon 1 minute stimulation), early (upon 8 minutes) and late (upon 40 minutes) events.
* Employment of a large-scale quantitative strategy based on pull-down assays using either phosphopeptides or SH2 domains as baits to further characterize RTKs-dependent signaling cascades.
* Use of siRNA and confocal microscopy to validate the results obtained in the phosphoproteomics screening.

Results and Conclusions:

* The stimulation of each RTK with ligands that induce either receptor degradation or recycling results in the activation of RTK-specific signaling cascades. We have not found a common signature associated to each endocytic route.
* For each RTK we found specific signaling events and cellular processes associated to one or the other ligand: both ligand specificity and timing are required for the activation of the right intracellular cascade resulting in the right cellular response.
* Already upon 1 minute stimulation (very early events), we observed significant differences in both RTKs phosphorylation and signaling activation.
* In particular, a very early molecular switch controls the fate of FGFR2, one of the RTKs considered in this project.
* The combination of advanced MS-based functional proteomics (phosphoproteomics, phosphopeptides pull-down assays) with more classical biochemical (i.e. siRNA screening) and cellular (i.e. microscopy) techniques allowed us to perform not only a system-wide analysis of RTKs entire cascades but also to further characterize some of their previously unknown individual components.
* We believed that such a system biology approach shed light on some previously uncharacterized molecular aspects of RTK signaling and regulation.
* Finally, since aberrant RTK signaling and trafficking are hallmarks of various diseases, including many cancers, the implications of our studies extend beyond basic research, paving the way to better define the pathogenesis of RTK-driven diseases, with the potential to identify novel therapeutic targets.