# Characterisation of the Oncogenic Signaling Cascade in Fumarate Hydratase Deficient Cancer Cells Using Subcellular-resolved Systems Biology Approaches

## Investigators


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## Project

Altered cellular metabolism is an emerging hallmark of cancer[1]. In the past decade it has been demonstrated that in order to support unrestrained chronic proliferation, cancer cells undergo a complex metabolic rearrangement aimed to convert nutrients into building blocks such as nucleotides, protein and lipids[2]. This metabolic transformation is orchestrated by several oncogenes and tumor suppressor genes and is shaped by environmental cues such as hypoxia and nutrient deprivation[3,4]. Interestingly, it has been recently demonstrated that mutations in key metabolic enzymes, by inherently deregulating metabolism, could promote tumorigenesis[5]. This is the case of few mitochondrial enzymes such as fumarate hydratase (FH) and succinate dehydrogenase (SDH). In particular, the loss of FH leads to the Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), a cancer syndrome characterized by a severe form of papillary renal cancer. A striking characteristic of FH-deficient tumors is that they accumulate significant amounts of the Krebs cycle metabolite, fumarate. It was demonstrated that the accumulation of fumarate in these cells inhibits the aKG-dependent prolyl hydroxylases (PHDs), a class of enzyme involved in the hydroxylation of the hypoxia inducible factors HIFs (see[5] for a review). When PHDs are inhibited, HIFs are no longer hydroxylated and, as a consequence, their ubiquitin-dependent degradation is halted. While some of the oncogenic signals in FH-deficient cancer were related to HIFs stabilization, the situation appears nowadays more complex and

encompasses some unexpected role of these metabolites, from post translational modification to epigenetics[6]. Of note, it has been recently shown that fumarate can trigger a post translational modification called succination, whereby it binds to active thiol residues of proteins. In particular, it was shown that it binds and inactivates the negative regulator of Nrf2, an antioxidant response gene[7], initiating a genetic reprogramming that promotes cancer progression. These results suggest that deregulated cellular metabolism might contribute to tumorigenesis acting at multiple levels, affecting both the genome and the proteome of the cell. However, how metabolism communicates to these layers is only partially known.

This project will investigate the multi-scale changes caused by the loss of FH and how these changes lead to cancer predisposition by combining subcellular proteomics and metabolomics data with computational analysis and mathematical modeling.

**Aim 1. Identification of signaling pathways affected by FH deficiency using phospho-proteomics and subcellular proteomics.**

Initially, the successful candidate will characterize the activation of signaling pathways in different cellular models of FH deficiency (both human and mouse cell lines) by performing quantitative proteomics and phospho-proteomics in the Lilley laboratory. This will involve both unsupervised and targeted analyses on specific pathways expected to be deregulated in response to fumarate accumulation. The experiments will be designed within the Frezza laboratory, which has significant experience and knowledge of the system[8], and the data analysed within the Saez-Rodriguez group, specialized in computational analysis of phospho-proteomics data[9]. The student will then apply a new method, developed in the Lilley laboratory, which is based on LOPIT (localisation of organelles proteins by isotope tagging)[10,11]. We will use this approach to map the location of thousands of proteins simultaneously within a cell, including mapping phosphoprotein isoforms to determine the effect of phosphorylation on cellular location of proteins in response to FH deficiency.

**Aim 2. Metabolic characterization of FH-deficient cells.**

The project will then involve the metabolic characterization of these cells by means of state of the art metabolomics carried out in Frezza's laboratory. While some aspects of the metabolic changes in these cells have already been studied, the candidate will perform unsupervised analyses to define the metabolic footprint and fingerprint of these cells. Of note, we will use the fractions generated in Aim 1 to build a subcellular-specific map of the metabolome of these cells.

**Aim 3. Build a predictive and mechanistic mathematical model of the interplay of signal transduction and metabolism.**

Finally, the data gathered both from proteomics and metabolomics will be integrated in a mathematical model developed in the Saez-Rodriguez laboratory by using their platform CellNOpt (www.cellnopt.org) to model signaling networks. The Saez-Rodriguez group is extending the platform to integrate metabolic networks thus this will be an ideal application of this method[12]. A starting network including the relevant pathways will be built based on existing

knowledge available in databases. This network will be converted into a predictive mathematical model, using CellNOpt by training it to the data generated in Aims 1 and 2.

**References**


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## Further information

Although previous experience in the all three areas involved in the project, i.e. metabolomics, proteomics and systems biology is not a prerequisite, the student will have the opportunity to develop proteomic, metabolomics and computational skills in an interdisciplinary environment. The student will be based at the Lilley lab but with full access to all resources and extensive interactions with the Frezza and Saez-Rodriguez groups.