Investigating the role of hypoxia inducible factor signalling in renal cancer driven by FH and/or SDH mutation

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The proposed PhD project within the Urological Malignancies Programme will be supervised jointly by Prof Margaret Ashcroft and Dr Christian Frezza. Through this co-supervisory partnership, the successful candidate will have the opportunity to learn state-of-the-art techniques such as proteomics and metabolomics, as well as having access to an exceptional set of experimental methods to analyse hypoxia-driven signalling pathways. As such, the successful candidate will have a unique opportunity to gain comprehensive training and expertise in cancer metabolism, which is a fundamental area of oncology.

Project Description

Background: Renal cancer is the 8th commonest cancer in the United Kingdom (CR-UK). While there has been improved clinical outcomes using targeted treatments for other cancers, the prognosis for patients with metastatic forms of renal cancer remains poor. There is an urgent need for a better understanding of the mechanisms underlying renal cancer in order to develop more effective treatments for this disease.

Fumarate hydratase (FH) and succinate dehydrogenase (SDH) are two mitochondrial enzymes of the Krebs cycle recently found mutated in hereditary and sporadic forms of renal cancer [1]. One of the proposed links between loss of these enzymes and tumorigenesis is the accumulation of the metabolite fumarate and succinate, respectively [1]. Indeed, these metabolites were shown to
inhibit the prolyl-hydroxylase-dependent degradation of the hypoxia inducible factors (HIFs), leading to the stabilisation of HIFs under normal oxygen concentrations [1]. This phenomenon, also known as ‘pseudo-hypoxia’, is considered an important driver of tumorigenesis in renal cancer. Although the accumulation of succinate and fumarate has been considered a major driver of HIF stabilisation, other mechanisms have been proposed to explain the pseudo-hypoxic signature in these cells. For instance, it was hypothesised that the prolyl hydroxylase domain (PHDs) enzymes can be inhibited by reactive oxygen species (ROS) generated in SDH and FH-deficient cells [2]. However, the presence of ROS in SDH-deficient cells has been debated suggesting that pseudo-hypoxia can be observed in SDH-deficient cells in the absence of overt oxidative stress. More recently, it was also shown that the expression of pseudo-hypoxia-related genes requires epigenetic changes orchestrated by fumarate and succinate that are mediated by ten–eleven translocation (TET) 5- methyl (SmC) dioxygenases [3] involved in maintaining DNA demethylation [4]. However, to what extent epigenetic changes contribute to the pseudo-hypoxic signature driven by HIF is still unknown.

HIFs are transcription factors, that when activated, promote a variety of cellular responses that lead to tumour cell survival and metabolic adaptation. Increased HIF activation drives tumour progression, poor prognosis and treatment resistance in most cancers [5]. In over 60% of clear cell renal carcinomas (ccRCC), the most common form of kidney cancer, HIF is constitutively upregulated due to inactivating mutations in von Hippel-Lindau (VHL). The VHL gene product pVHL, functions as an E3 ligase for HIF-α targeting it for ubiquitination and degradation by the proteasome.

While the importance of HIF activation in the context of VHL mutation in driving ccRCC is clear [6-7], the molecular mechanisms that lead to HIF stabilisation in renal cancers with mutations in FH and SDH are yet to be elucidated. Furthermore, the contribution of the HIFs to driving renal tumorigenesis in these other forms of renal cancer is not understood.

HIF plays a central role in regulating mitochondrial function and controlling metabolic adaptive responses. The possibility that mitochondrial enzymes such as FH and SDH corroborate with the HIF-hypoxia axis to drive renal tumorigenesis through novel molecular mechanisms, is of particular interest therapeutically.

**Hypothesis:** FH and/or SDH control metabolic cellular responses that interface with the HIF-hypoxia signalling pathway in renal cancer.

Overall goal and key objectives: The proposed PhD project will offer an excellent opportunity for the successful candidate to use a broad range of molecular, cellular and genetic approaches to identify molecular mechanisms underpinning renal tumorigenesis in FH- and/or SDH- deficient cancer, and to investigate the contribution of the HIF-hypoxia axis to these mechanisms.

In this PhD project, the successful candidate will:

1) Perform a comprehensive molecular characterisation of the ‘pseudo-hypoxia’ signature in FH- and SDH-deficient cells, with particular focus on exploring the role of the TETs in regulating ‘pseudo-hypoxia’ gene expression.
2) Elucidate how FH and SDH regulate the HIF-hypoxia axis, initially focusing on the role of the PHDs.
3) Determine whether FH and SDH utilize the HIF-hypoxia axis to mediate renal tumorigenesis.
4) Investigate the role of HIF-dependent and/or HIF-independent drivers in renal tumorigenesis in FH- and/or SDH- deficient cancer.
Special techniques, methodologies, and training:

1) The successful PhD candidate will have access to a unique and well-characterised toolbox of reagents, including: FH- and SDH-deficient epithelial kidney cells and a panel of patient-derived renal carcinoma cell lines.

2) The candidate will learn a broad range of molecular and cell biology techniques that are already established in the Ashcroft and Frezza laboratories. Methodologies, include: genetic modification of cells using shRNA, siRNA, and CRISPR technologies, quantitative (Q)-PCR, tissue culture, western analysis, immunostaining, 2D-migration and chemotaxis assays, 3D assays (spheroid, invasion), various mitochondrial assays (e.g. oxygen consumption rate, mtDNA/copy number, morphology, subcellular localization, mitochondrial isolation/protein analyses). The successful candidate will have access to state-of-the-art facilities, equipment and training (within the Department of Medicine, Cambridge Institute for Medical Research and MRC Cancer Unit): For example, specialised instruments, including the Seahorse Extracellular Flux Analyser (for measuring cellular respiratory endpoints), advance microscopy facilities (for confocal microscopy and real-time imaging) and flow cytometric/fluorescent cell-sorting analysers. The successful candidate will have the opportunity to attend relevant training courses for these instruments.

3) The successful candidate will have access to unique tools and state-of-the-art technologies, including: metabolomic profiling, bioinformatics and biostatistics, transcriptomics, proteomics, and methylome analysis. For example, the successful candidate will have access to the metabolomics facility located in the Frezza lab, which includes two liquid chromatography-mass spectrometry platforms. The successful candidate will be trained to perform and analyse steady-state metabolomics and metabolic flux analyses. Furthermore, the Ashcroft lab is equipped with hypoxic workstations and incubators that have capabilities for imaging and measuring respiratory endpoints under controlled oxygen environments as well as having developed unique tools to investigate hypoxia responses in cells and tissues in culture. The successful candidate will be trained to measure and analyse hypoxia responses using these unique tools and specialized equipment. In addition, our laboratories have access to facilities for RNA-sequencing and Oxidative-Bisulphate sequencing located at the CRUK Cambridge Institute for in-depth transcriptomics analyses. The candidate will learn basic and advances bioinformatics and biostatistics tools to analyse these datasets.

4) Expert co-supervisory training, support and input. The candidate will be exposed to a vibrant, multidisciplinary research environment and attend routinely scheduled one-to-one meetings with the supervisors. The successful candidate will participate in weekly lab meetings and journal clubs. In addition, the successful candidate will attend and participate in monthly Cambridge HypOxyResp Network work-in-progress meetings. The candidate will have the opportunity to present his/her results periodically at these internal joint group meetings. Moreover, the candidate will have the opportunity to attend seminars and workshops organised by the Cambridge Cancer Centre (CCC). Finally, when realising the translational aspirations of the work, as Prof Ashcroft and Dr Frezza are members of the Cambridge Renal Cancer (CamRenCan) Group and CCC Urology Theme, the proposed project has the fantastic opportunity to benefit from unique and outstanding clinical expertise in the genetics of renal cancer from our colleague Prof Eamonn Maher, and in renal cancer treatment from our colleagues Dr Grant Stewart and Prof Tim Eisen.
References


Applications

To apply for this studentship please see http://www.cambridgecancercentre.org.uk/studentships

For general enquiries please contact Tina Thorn tina.thorn@cruk.cam.ac.uk

For further information or questions relating to this project please contact:

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