Exploring VHL-independent regulatory mechanism(s) of HIF for therapeutic benefit.

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Principal supervisor's CRUK CC theme:

- Early Cancer Institute
- Urological Malignancies Programme

Department for student registration: Medicine
Department or institute where research will take place: Medicine (level 5 Addenbrooke's)

Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

Background: We are interested in renal cell carcinoma (RCC), the most common form of kidney cancer which is the 7th commonest cancer in the UK. Clear cell RCC (ccRCC) is the major histological subtype of RCC, and inactivation of the von Hippel-Lindau (VHL) gene occurs in the great majority of sporadic cases. VHL encodes the pVHL tumour suppressor protein, an E3 ligase for the hypoxia inducible factor (HIF) family of transcription factors. Therefore, HIF is constitutively activated in RCC, and HIF-2 drives tumorigenesis. We and others have generated small molecule inhibitors of HIF (1-9). Some of these agents are showing promise in early clinical trials, although some RCCs are resistant (6-9). Thus, identifying mechanisms that are linked to VHL loss-of-function and HIF activation in RCC is important for the development and/or stratification of new emerging treatments for advanced ccRCC. To this end, we have performed a genome-wide CRIPSR/Cas9 screen (10) in the presence and absence of a HIF inhibitor alongside global proteomic analyses. Our investigation highlights a new VHL-independent regulatory mechanism of HIF, linking metabolic signalling and tumour cell growth – that is therapeutically attractive.

Hypothesis: VHL-independent regulatory mechanism(s) of HIF can be exploited in RCC for therapeutic benefit.
Aims: The proposed MRes/PhD rotation project will offer an excellent opportunity to build on our successful CRISPR/Cas9 screen (10) and small molecule HIF targeted screening efforts (1-9). The MRes/PhD rotation project will aim to:
(1) Evaluate a new gene pathway in RCC, identified from our screening efforts.
(2) Determine the importance of a new gene pathway for HIF signalling in RCC.

MRes experimental plan:

The MRes project offers unique access to a range of well-characterized techniques and specialised reagents. The successful candidate will use gene-targeting techniques and measurement of HIF endpoints to evaluate a new gene pathway in a panel of RCC cell systems and normal cells, together with appropriate controls.

PhD project outline:

Background: We are interested in renal cell carcinoma (RCC), the most common form of kidney cancer which is the 7th commonest cancer in the UK. Clear cell RCC (ccRCC) is the major histological subtype of RCC, and inactivation of the von Hippel-Lindau (VHL) gene occurs in the great majority of sporadic cases. VHL encodes the pVHL tumour suppressor protein, an E3 ligase for the hypoxia inducible factor (HIF) family of transcription factors. Therefore, HIF is constitutively activated in RCC, and HIF-2 drives tumorigenesis. We and others have generated small molecule inhibitors of HIF (1-9). Some of these agents are showing promise in early clinical trials, although some RCCs are resistant (6-9). Thus, identifying mechanisms that are linked to VHL loss-of-function and HIF activation in RCC is important for the development and/or stratification of new emerging treatments for advanced ccRCC. To this end, we have performed a genome-wide CRIPSR/Cas9 screen (10) in the presence and absence of a HIF inhibitor alongside global proteomic analyses. Our investigation highlights a new VHL-independent regulatory mechanism of HIF, linking metabolic signalling and tumour cell growth – that is therapeutically attractive.
Hypothesis: VHL-independent regulatory mechanism(s) of HIF can be exploited in RCC for therapeutic benefit.
The PhD project with build on the MRes rotation project to evaluate a new gene pathway in RCC, and aim to:
(1) Perform profiling analyses using our unique transcriptomic and proteomic datasets. (2) Evaluate the role of a new gene pathway in HIF signalling and metabolism.
(3) Determine the contribution of VHL status using a panel of patient-derived loss-of-function VHL mutants.
(4) Perform analysis of transcriptomic datasets from patient samples.
(5) Assess patient samples using immunohistochemistry and other techniques.
(6) Extend the work to other cancer types.

PhD experimental plan:

Using a range of well-characterized techniques, specialised reagents, patient-derived cell systems, and unique transcriptomic and proteomic datasets - the successful candidate will: 1) Evaluate a new gene pathway in RCC identified from our screening efforts, using a panel of RCC cell systems and normal cells, together with appropriate controls. Alongside, perform profiling analyses using our unique transcriptomic and proteomic datasets. (2) Determine the importance of a new gene pathway for HIF signalling and metabolism in RCC, using gene-targeting techniques and measurement of HIF and metabolic endpoints. In conjunction, the contribution of VHL will be evaluated using a panel of patient-derived loss-of-function VHL mutants. Further work will extend to analyses of transcriptomic datasets from patient samples and analysis of patient samples using immunohistochemistry and other techniques.

Main techniques:

1) The successful candidate will learn a broad range of molecular and cell biology techniques that are established in the Ashcroft lab. Methodologies include genetic modification of cells using shRNA, CRISPR/Cas9, quantitative (Q)-PCR, tissue culture, western analysis, various cell analysis platforms, and bioinformatics analyses. The successful candidate will have access to state-of-the-art facilities, equipment and training within the Department of Medicine and Jeffrey Cheah Biomedical Centre.
2) Expert training, support and input will be provided. The successful candidate will be exposed to a multidisciplinary research environment, have day-to-day lab training from a senior member

of the Ashcroft group who will act as secondary supervisor, and attend routinely scheduled one-to-one meetings with Prof Ashcroft.
3) The successful candidate will have the opportunity to present their results at internal group meetings, to attend seminars and workshops organised by the Cambridge Cancer Centre (CCC) and the University, and to benefit from outstanding expertise within CamRenCan and across the broader Cambridge community.

Key references:

1 Ashcroft, M. & Jones, K. HIF Inhibitors. Patent WO 2015/079213 A1 (2015).
2 Chen, W. et al. Targeting renal cell carcinoma with a HIF-2 antagonist. Nature 539, 112-117, doi:10.1038/nature19796 (2016).
3 Cho, H. et al. On-target efficacy of a HIF-2alpha antagonist in preclinical kidney cancer models. Nature 539, 107-111, doi:10.1038/nature19795 (2016).
4 Cho, H. & Kaelin, W. G. Targeting HIF2 in Clear Cell Renal Cell Carcinoma. Cold Spring Harb Symp Quant Biol, doi:10.1101/sqb.2016.81.030833 (2016).
5 Kaelin, W. G., Jr. HIF2 Inhibitor Joins the Kidney Cancer Armamentarium. J Clin Oncol 36, 908-910, doi:10.1200/JCO.2017.77.5254 (2018).
6 Courtney, K. D. et al. Phase I Dose-Escalation Trial of PT2385, a First-in-Class Hypoxia-Inducible Factor-2alpha Antagonist in Patients With Previously Treated Advanced Clear Cell Renal Cell Carcinoma. J Clin Oncol 36, 867-874, doi:10.1200/JCO.2017.74.2627 (2018).
7 Choueiri, T. K. & Kaelin, W. G., Jr. Targeting the HIF2-VEGF axis in renal cell carcinoma. Nat Med 26, 1519-1530, doi:10.1038/s41591-020-1093-z (2020).
8 Courtney, K. D. et al. HIF-2 Complex Dissociation, Target Inhibition, and Acquired Resistance with PT2385, a First-in-Class HIF-2 Inhibitor, in Patients with Clear Cell Renal Cell Carcinoma. Clin Cancer Res 26, 793-803, doi:10.1158/1078-0432.CCR-19-1459 (2020).
9 Hasanov, E. & Jonasch, E. MK-6482 as a potential treatment for von Hippel-Lindau disease-associated clear cell renal cell carcinoma. Expert Opin Investig Drugs, 1-10, doi:10.1080/13543784.2021.1925248 (2021).
10 Thomas, L. W. et al. Genome-wide CRISPR/Cas9 deletion screen defines mitochondrial gene essentiality and identifies routes for tumour cell viability in hypoxia. Commun Biol 4, 615, doi:10.1038/s42003-021-02098-x (2021).