CRUK Cambridge Centre Clinical PhD project

PhD Project Title	**Hypoxia and chromatin remodelling in lung cancer.**
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Laboratory Location	Cambridge Institute for Medical Research

Project Outline	**Aims and objectives** Solid tumours, such as non-small cell lung cancers (NSCLC), are often characterised by their ability grow and metastasise in oxygen and nutrient poor environments, but how is this achieved? Our work has identified a novel pathway for regulating cell growth in cancer cells through accumulation of a rate-limiting step in the Krebs cycle (2-oxoglutarate) and an evolutionary conserved but poorly understood post-translational modification in humans, termed lipoylation (1,2). The overall objective of this research is to examine how lipoylation controls cancer cell growth and to determine if targeting enzymes involved in lipoate formation may be of benefit in NSCLC. **Key aims are:** **Aim 1)** To determine how changes in protein lipoylation affect cell growth through metabolic regulation of the Krebs cycle or through remodelling chromatin. **Aim 2)** To use unbiased CRISPR/Cas9 forward genetic screens to identify genes that confer synthetic lethality when lipoylation is impaired.
Experimental plan	**Aim 1.** The HCC15 cell line (NRAS- and STK11-mutant), represents a robust model of studying metabolic changes in NSCLC (3), and will be used to explore how lipoylation affects cell growth. CRISPR/Cas9 deletion of lipoate enzymes will inform on how lipoylation affects metabolism (through modulating activity of the 2-oxoglutarate dehydrogenase complex) and cell fates (chromatin remodelling and activation of the HIF response). Lipoylation of mitochondrial proteins will be determined by quantitative mass spectrometry. Further studies will focus on chromatin remodelling enzymes (e.g. histone demethylases) found to be regulated by lipoylation directly or indirectly through changes in 2-oxoglutarate levels. **Aim 2.** We have shown that cancer cells that have adapted to loss of lipoylation can still survive, suggesting that alternate metabolic pathways can compensate for lipoate loss. Identifying these compensatory mechanisms may provide novel therapeutic strategies. By taking cells that are deficient in lipoylation we can use forward genetic approaches that are well established in the lab, to identify genes that confer synthetic lethality. Characterising the genes and pathways involved will form a substantial part of this project.
Main Techniques	• Molecular biology • CRISPR/Cas9 genetic screens and CRISPR knock-in • Flow cytometry • Quantitative mass spectrometry • Metabolomics • Mammalian cell tissue culture • Protein Biochemistry

Key References	1. Burr SP, Costa AS, Grice GL, Timms RT, Lobb IT, Freisinger P, Dodd RB, Dougan G, Lehner PJ, Frezza C, Nathan JA. Mitochondrial Protein Lipoylation and the 2-Oxoglutarate Dehydrogenase Complex Controls HIF1α Stability in Aerobic Conditions. *Cell Metabolism* 2016 Nov 8;24(5):740-752. 2. Bailey PSJ, Nathan JA. Metabolic Regulation of Hypoxia-Inducible Transcription Factors: The Role of Small Molecule Metabolites and Iron. *Biomedicines*. 2018 May 17;6(2). 3. Faubert B, Li KY, Cai L, Hensley CT, Kim J, Zacharias LG, Yang C, Do QN, Doucette S, Burguete D, Li H, Huet G, Yuan Q, Wigal T, Butt Y, Ni M, Torrealba J, Oliver D, Lenkinski RE, Malloy CR, Wachsmann JW, Young JD, Kernstine K, DeBerardinis RJ. Lactate Metabolism in Human Lung Tumors. *Cell.* 2017 Oct 5;171(2):358-371.