CANCER RESEARCH UK CLINICAL ACADEMIC TRAINING PROGRAMME

TRACC Programme Project Pro Forma

TRACC (to Train and Retain Academic Cancer Clinicians) is a joint Glasgow/Edinburgh programme funded by Cancer Research UK. The **MB-PhD** strand of the programme is an opportunity for medical students from either University to undertake a **3-year** PhD after their BSc year in **any discipline relevant to cancer research**. Students are provided with close mentoring and support to find the project that best fits their interests across Edinburgh and Glasgow. Your MB-PhD project proposal should be suitable for a 2nd or 3rd year medical student undertaking a 3-year intercalated PhD (having previous completed a 1-year intercalated BSc).

MB-PhD Project title: Metabolic rewiring of Cholangiocarcinoma

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Lab websites:
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https://www.liverpool.ac.uk/people/laura-randle/research
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https://www.liverpool.ac.uk/people/stephen-chapman

Research question: Cancer of the bile duct, or cholangiocarcinoma (CCA), is a tumour driven by altered metabolic processes that enable and contribute to its progression (1). These include increased glycolysis, fatty acid (FA) biosynthesis and altered amino acid that all converge to dysregulate mitochondria metabolism. Using HepG2 cells a hepatocellular cancer cell line that has retained JMJD5 expression, we have found that CRISPR knockout of the 2-oxoglutarate-dependent dioxygenase JMJD5 (aka KDM8) drives metabolic changes similar to what has been described in CCA (1) (Figure 1). Loss of JMJD5 dramatically enhances the activity of the cystine/glutamate antiporter xCT resulting in increased cellular cystine concentration driven by the export of cellular glutamate (Figure 1A). To compensate for reduced glutamate concentration the cells shut down ATP generation via FA-degradation and rely on glucose (Figure 1B) to generate ATP via glycolysis and the tricarboxylic acid cycle (TCA). Overall we observed that loss of JMJD5 in HepG2 cells increases glycolysis and fatty acid biosynthesis (Figure 1C &D) and dramatically alters the amino acid metabolism of the cells, reminiscent of what has been observed in CCA.
Interestingly, JMJD5 is highly expressed in the bile duct but lost upon CCA progression (Figure 2). Given these data, we hypothesise that loss of JMJD5 drives the metabolic reprogramming observed in CAA.

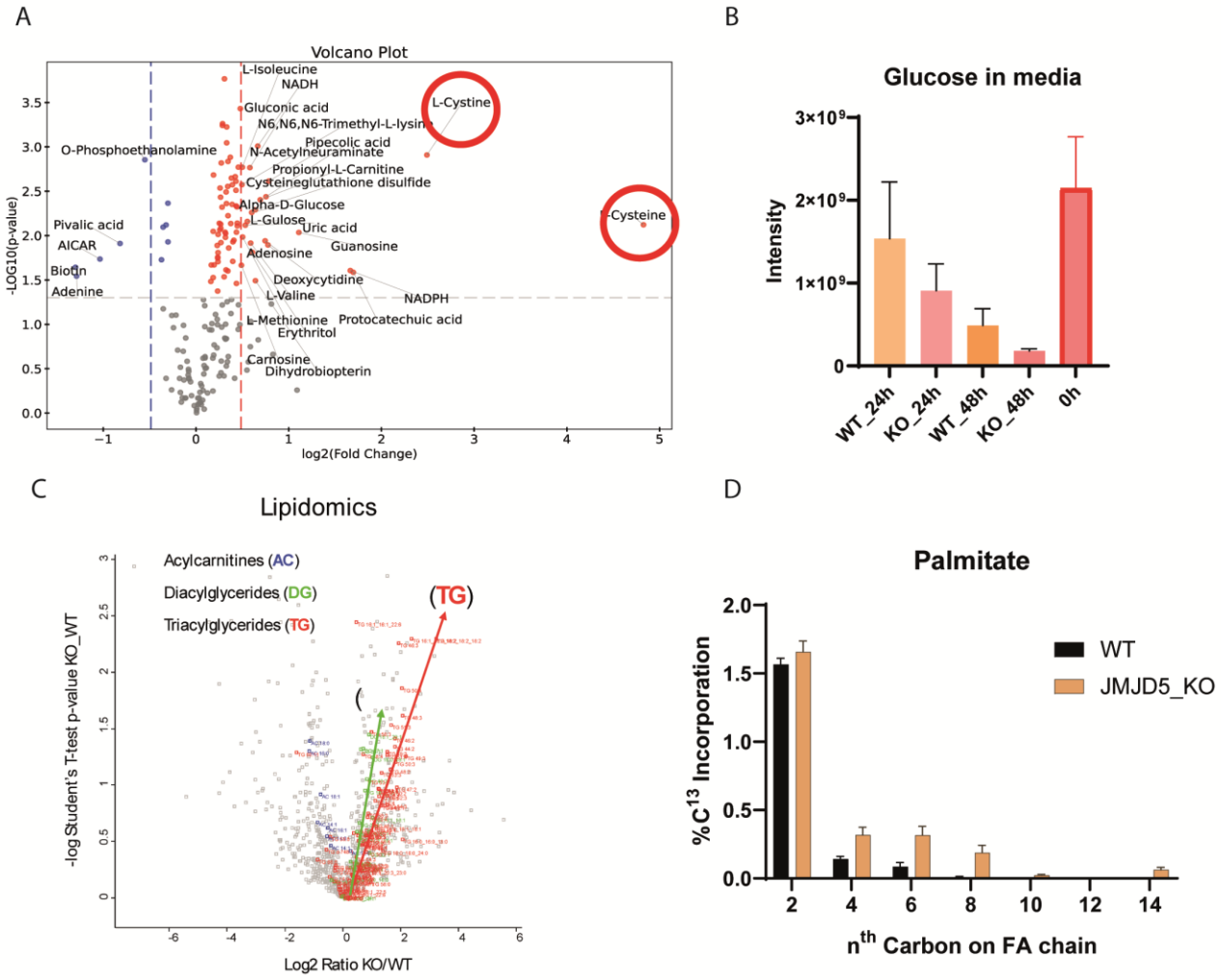

Figure 1 Loss of JMJD5 drives metabolic reprogramming in HEPG2 cells. *A) Volcano Plot of metabolomic analysis of metabolite levels comparing JMJD5 knockout(KO) over wild-type (WT) reveals that Cystine and Cysteine are the two metabolites most strongly induced by knockout of JMJD5. B) Bar-graph of glucose measured by LC-MS in media at different time points. KO and WT cells were seeded out with equal numbers and glucose concentration was determined by mass spectrometry C) Volcano Plot of Lipidomic analysis of KO over WT shows that ACs are downregulated, and TGs and DGs are upregulated in KO cells. 13C6 Glucose tracing into Palmitate shows that KO cells funnel more carbons from glucose into fatty acid synthesis.*

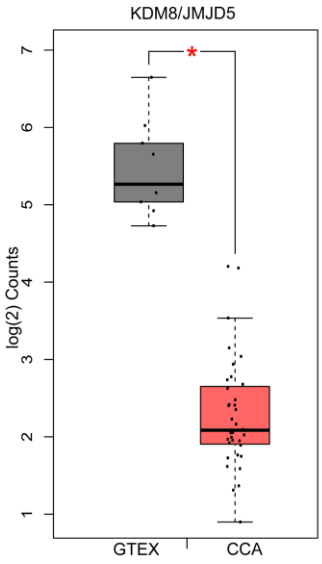

Figure 2 JMJD5 is lost in CCA. ***mRNA*** *expression levels of KDM8 mRNA in normal (GTEX) and CCA from TCGA*

Relevance to Cancer: In the healthy liver both hepatocytes and biliary epithelial exhibit metabolic plasticity allowing them to undergo significant metabolic changes, including altering their lipid metabolism, glycogen storage, and detoxification processes based on dietary intake and other environmental challenges. This plasticity is frequently lost during disease progression, would this be non-alcoholic fatty liver disease (NAFLD) or cancer. JMJD5 is frequently lost during CCA (Figure 2) and our data suggests that this loss reduces the cellular metabolic plasticity and increases the dependency of tumour cells on specific metabolic pathways. This loss of flexibility represents an actionable vulnerability that can be exploited therapeutically (2). CCA and liver cancers are types of cancer lacking targeted therapies, thus representing a disease of unmet need and increasing incidence, partially driven by the "Western" diet and alcohol consumption. We aim to elucidate metabolic networks in CCA to determine how they are altered to identify potential therapeutic networks and targets.

Techniques/model systems to be used: The Randle lab has established ex-vivo culturing of Precision Cut Tissue Slices to explore CCA disease progression (3, 4)(6). The lab is utilising the model to explore the tumour immune microenvironment and is interested in understanding CCA bioenergetics. The Kriegsheim lab is interested in how JMJD5 and loss of it regulated tumour metabolism. JMJD5 is primarily expressed in liver cells (hepatocytes and biliary epithelial cells) and is lost during hepatobiliary tumour development. The lab has used cell line models but is interested in whether these data translate in human tumour sections. Kriegsheim is the academic lead of the Institute of Genetics and Cancer Mass Spectrometry facility which has expertise in analysing the molecular landscape of cells and tissues, being at the proteomic or metabolomic level. In addition, the facility has the expertise and instrumentation to trace ^{13}C, ^{2}H and ^{15}N isotopes across metabolic networks by quantifying the isotopologue distribution of metabolic products. The data (proteomics, metabolomics, lipidomics and isotope tracing) will be integrated into a metabolic network of mammalian mitochondria (5) which can model and predict metabolism networks (Chapman lab), to generate a predictive and interrogatable computational model of CCA that will be used to first predict the metabolic switch that occurs between FA-derived ATP production towards increased glycolytic and TCA metabolism. The model will then be used to generate non-trivial testable hypotheses to reveal metabolic vulnerabilities inherent to CCA.

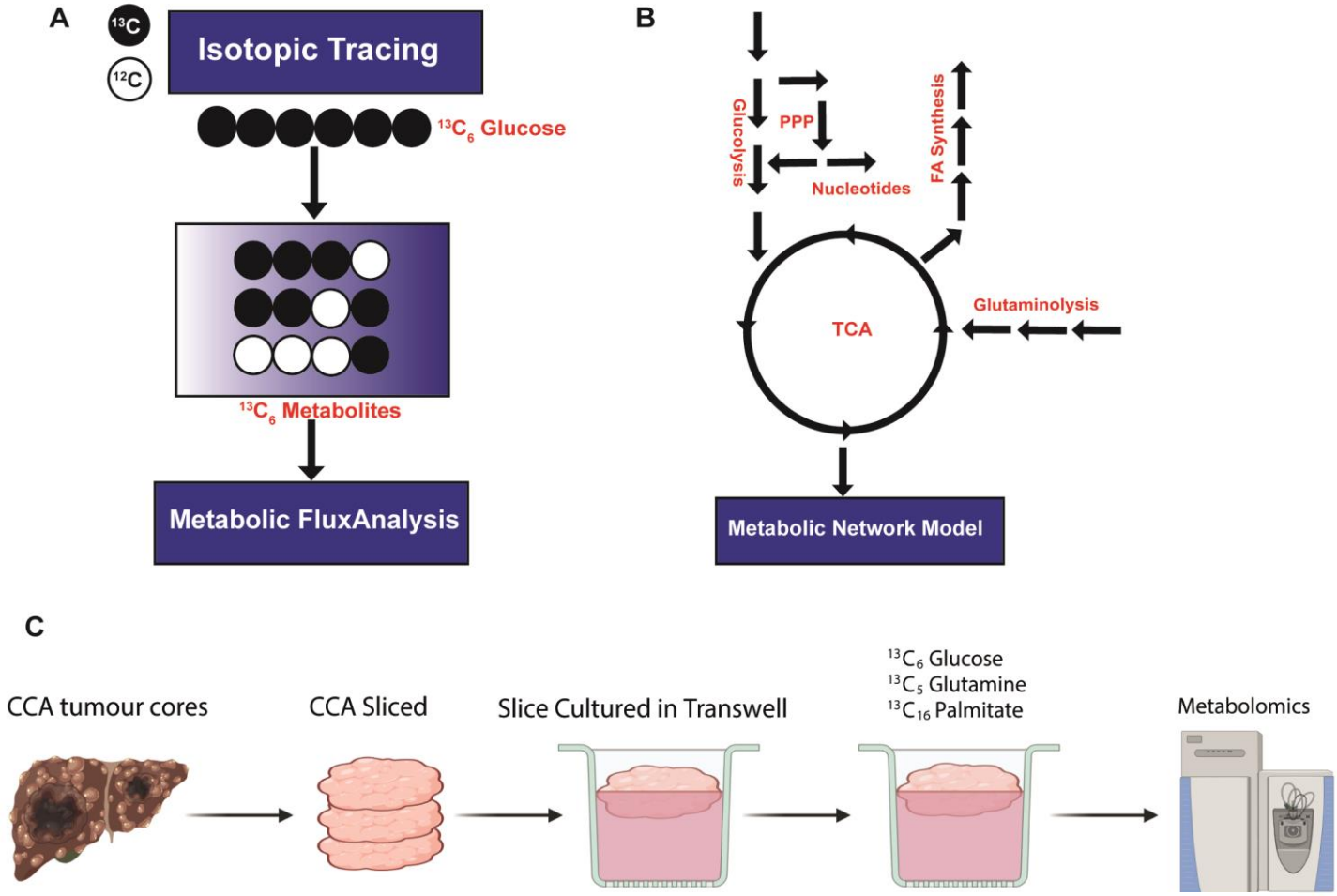

Figure 2 Metabolic tracing in ex-vivo CCA tumour slices to generate Metabolic Network Model. *A) Labelled metabolites and be added to cells and tumour slices. Metabolites are taken up and catabolised/metabolised. Heavy carbons are then traced by mass spectrometry into products, these can be used to generate B) Metabolic Network Models that are used to repeal vulnerabilities through Sensitivity analysis. We will use C) ex-vivo CCA tumour cores sliced and cultured in Transwells. Ex-plants are viable for up to 2 weeks. Labelled metabolites are added to the media and can be traced by mass spectrometry-based metabolomics*

Papers of interest:

1. Raggi C, Taddei ML, Rae C, Braconi C, Marra F. Metabolic reprogramming in cholangiocarcinoma. J Hepatol. 2022;77(3):849-64.
2. Farshidfar F, Zheng S, Gingras MC, Newton Y, Shih J, Robertson AG, et al. Integrative Genomic Analysis of Cholangiocarcinoma Identifies Distinct IDH-Mutant Molecular Profiles. Cell Rep. 2017;18(11):2780-94.
3. Chidlow SJ, Randle LE, Kelly RA. Predicting physiologically-relevant oxygen concentrations in precision-cut liver slices using mathematical modelling. PLoS One. 2022;17(11):e0275788.
4. McGreevy O, Bosakhar M, Gilbert T, Quinn M, Fenwick S, Malik H, et al. The importance of preclinical models in cholangiocarcinoma. Eur J Surg Oncol. 2025;51(2):108304.
5. Chapman S, Brunet T, Mourier A, Habermann BH. MitoMAMMAL: a genome scale model of mammalian mitochondria predicts cardiac and BAT metabolism. Bioinform Adv. 2025;5(1):vbae172.
6. Chidlow, S.J., Randle, L.E*. and Kelly, R.A., 2022. Predicting physiologically-relevant oxygen concentrations in precision-cut liver slices using mathematical modelling. PloS one, 17(11), p.e0275788.https://doi.org/10.1