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MOLECULAR IMAGING

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Our lab develops new ways to visualise cancer – we create novel molecular tracers that image metabolic reprogramming, a hallmark of cancer, and use state-of-the-art methods such as PET/MRI to non-invasively detect and characterise tumour development. This year, we have been developing technologies to image metabolic responses to cancer treatment. Our goal is to develop a better understanding of how cancer drugs work, identifying when those drugs succeed or fail, and supporting the use of more effective therapies.

The primary focus of our work is to develop new methods to non-invasively image cancer metabolism and then apply these techniques to investigate the causes and consequences of metabolic heterogeneity in high-fidelity mouse models of cancer. Our research has two main themes, first we develop and validate novel technologies such as new metabolic radiotracers and new quantitative methods. Second, we exploit PET as a biological imaging modality and investigate the molecular mechanisms and vulnerabilities underlying regional tumour metabolism. The goal of our work is to validate imaging biomarkers for visualising *in vivo* metabolic phenotypes and, by investigating the liabilities of these phenotypes, determine if we can use metabolic imaging to identify susceptibilities that we can use to guide therapy in individual patients.

Visualising metabolic heterogeneity and plasticity in lung cancer

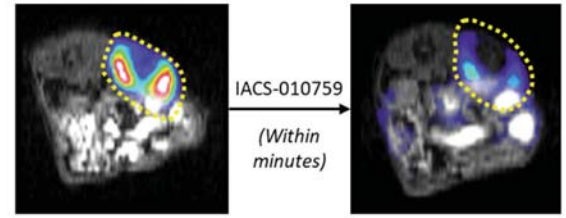
Metabolic heterogeneity presents both a challenge and an opportunity to imaging. Due to heterogeneity, it is unlikely that a single imaging test will detect cancer in all cases. However, if we could develop a complementary panel of PET tracers and develop a better understanding of how PET imaging signatures relate to underlying metabolic and molecular features of cancer, we could potentially identify metabolic differences between or within patients and use this information to stratify treatment.

Lung cancer has large regional variations in glucose uptake, hypoxia and blood flow; regions of high and low perfusion within the same lung tumour have striking differences in metabolism. To understand the significance of these imaging signatures we need to relate them to the underlying genetics and metabolism of tumour sub-regions.

To address these challenges, we have developed a dual tracer approach – combining [^{11}C]acetate as a tool for imaging fatty acid synthesis and [^{18}F]FDG, a surrogate of glucose uptake – to visualise and deconvolve regional tumour metabolism. Using dual-isotope positron emission tomography, we imaged the LSL-Kras$^{G12D/+}$ p53$^{fl/fl}$ mouse model of lung adenocarcinoma and found that tumours arising from the same genetic lesions and in the same tissue-of-origin produced two spatially heterogeneous metabolic subtypes. One subtype was characterised by high uptake of the radiolabelled tracer [^{18}F]FDG and the other by high [^{11}C]acetate uptake. Evident on dual-isotope autoradiographs, these tumour sub-regions appeared to demonstrate reciprocal metabolic phenotypes within the same mouse.

To investigate the molecular mechanisms underlying these imaging subtypes we developed a dual-isotope tracking method, DIOPTRA, and traced [^{11}C]acetate and [^{18}F]FDG within the same lesions *ex vivo*. Unbiased molecular profiling of these regions showed distinct transcriptional, proteomic and metabolic signatures. Regions with higher glucose consumption were more proliferative with activation of cell cycle genes, Myc targets and the unfolded protein response. While regions of high acetate uptake showed signatures of fatty acid metabolism, reactive oxygen species, tricarboxylic acid (TCA) cycle and oxidative phosphorylation.

To establish metabolic pathway activity in each subtype we compared PET imaging to metabolic pathway flux measurements using stable isotope tracing with [U-^{13}C]glucose and [U-^{13}C]acetate. FDG-avid tumours utilised glucose for synthesis of serine and glycine and used acetate to replenish the TCA cycle intermediates. In contrast, acetate-avid



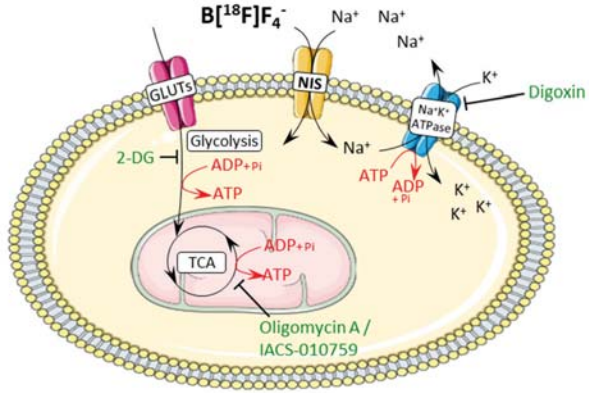


Figure 1 Metabolic sensing of energy charge *in vivo*
Rapid decrease in [^{18}F] tetrafluoroborate PET uptake within minutes of administration of drugs (2-DG, oligomycin and IACS-010759) targeting ATP production.

tumours used glucose for TCA anaplerosis and glutamine biosynthesis while using acetate for synthesis of palmitate, suggesting marked differences in metabolic pathway activation in the two subtypes.

This is the first example of using non-invasive radionuclide imaging to identify cancer subtypes within lung adenocarcinoma. As this imaging is eminently applicable to the clinic, we aim to develop these imaging signatures to identify subtype-specific cancer vulnerabilities.

Imaging energy stress in real-time by in vivo PET imaging of the sodium iodide symporter

Despite recent advances in our understanding of tumour metabolism over the last several years, relatively few metabolic cancer treatments have been successfully translated. Predicting how well drugs targeting metabolism will work in the clinic is a real problem. Testing drugs solely in cell culture models, although relatively straightforward, does not provide a good indication of how well that drug will work in animal models or patients. Recent efforts to make more physiologically relevant cell culture media are an important step, but there is still not a substitute for *in vivo* testing. However, *in vivo* experiments are long and complex which limits throughput. Here we set out to make an *in vivo* system allowing a rapid and non-invasive readout of drug efficacy for metabolic treatments. We used a positron emission tomography (PET) imaging reporter gene, sodium iodide symporter, previously used for cell tracking, and exploited the fact that NIS-mediated PET uptake is coupled to the sodium gradient maintained in an ATP-dependent fashion by Na^+/K^+ ATPase activity. We showed that targeting metabolic pathways that lead to energy stress also led to decreases in NIS-mediated radiotracer uptake (Figure 1). Importantly, this happened very quickly, and using PET imaging, we could sensitively detect drug effects within minutes of their administration, suggesting NIS could act as a rapid *in vivo* sensor of energy stress.

This tool can be used by any laboratory with access to PET imaging and it could be easily adapted to SPECT imaging using sodium pertechnetate, which would eliminate the need for radiochemistry facilities. Radionuclide imaging is very sensitive and quantitative, meaning that NIS can be used in any *in vivo* system without issues like penetration depth or tissue pigmentation that affect optical reporters. In parallel, we are developing a number of *in vivo* tools, including a palette of lentiviral vectors carrying NIS and a germline reporter mouse carrying a Cre-inducible NIS, so that the approach presented here can be used more widely, facilitating its further dissemination. This tool can be a useful means to obtain a rapid indication of the efficacy of drugs that target energy pathways *in vivo*.

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