MOLECULAR IMAGING

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Our lab develops new ways to visualise cancer – we use state-of-the-art imaging methods such as PET/MRI to non-invasively detect and characterise tumour development. We create novel molecular imaging agents targeting metabolic reprogramming, a hallmark of cancer growth. These tools have a role in clinical imaging for diagnosing cancer and directing treatment. This year we developed novel vectors for tomographic imaging of tumour initiation and treatment response in cell lines and transgenic mouse models. By identifying heterogeneity in drug response, we aim to design better combination therapies.

Rapid assessment of tumour metabolic response to treatment using sodium iodide symporter (NIS): a radionuclide imaging reporter gene

The key advantage of using radionuclide reporter genes is their high sensitivity, penetration depth and ability to provide tomographic images. The application of radionuclide imaging reporters in cancer research has been principally in targeted radiotherapy, cell tracking and assessment of tumour growth. As the clinical application of drugs targeting metabolic pathways becomes reality, preclinical tools providing rapid readout of on-target effect could become useful tools for translational drug development.

We have explored the use of mNIS (mouse sodium iodide symporter protein) as a radionuclide imaging reporter gene to provide a direct and rapid readout of tumour cell response to therapy specifically targeting glycolysis and oxidative phosphorylation, the key pathways utilised by tumour cells to regenerate ATP. As a proof of concept, we produced clonal cell lines stably expressing mNIS protein by infecting the HEK293T cells with a 3rd generation lentiviral vector carrying the mNIS sequence under the control of the EF1alpha promoter. Using radioactive mNIS substrates used for PET or SPECT imaging ([^{18}F]tetrafluoroborate (TFB) or [^{99m}Tc]pertechnetate, respectively) and established treatments targeting singly, or in combination, glycolysis and oxidative phosphorylation (2-deoxyglucose and oligomycin A, respectively), we were able to demonstrate a rapid (within 30 minutes) decrease in uptake by the treated mNIS-expressing cells when compared to untreated controls. This robust effect was measurable in advance of the decrease in cell viability or number. Mechanistically, the above phenomenon is the result of the direct dependence of mNIS activity on the sodium gradient across the plasma membrane, generated by Na^+/K^+ ATPase in an ATP-dependent manner.

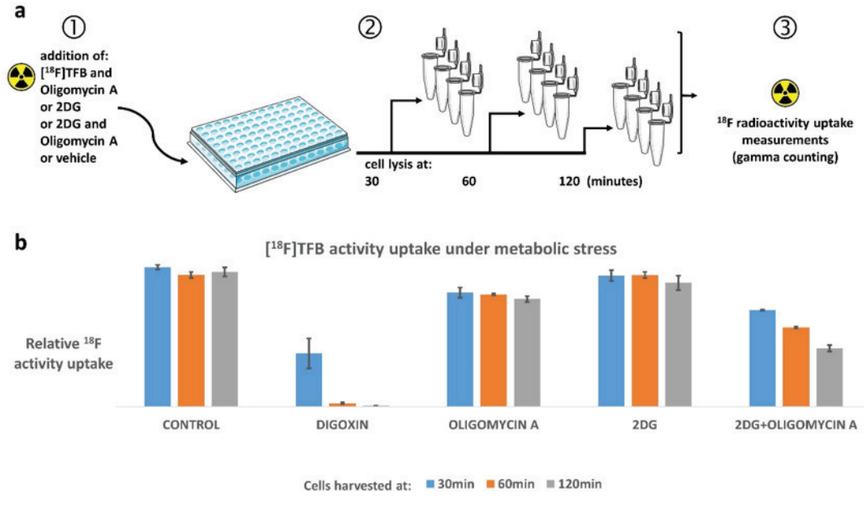

Figure 1
Sodium iodide reporter provides a rapid read-out of metabolic response
(a) HEK293T cells infected with a 3rd generation lentiviral vector PGK-Cre-EF1a-Luc-P2A-NIS. [^{18}F]TFB, and 1μM oligomycin A or 3mM 2DG, or a combination were added. 100nM of the Na/K ATPase inhibitor (Digoxin), was used as a positive control and DMSO was used as negative control. The cells were harvested at 30, 60 or 120 minutes and radioactivity counted.
(b) Modest reduction in ATP-dependent uptake of [^{18}F]TFB was observed following inhibition of glycolysis or oxidative phosphorylation. However, combination therapy produced a significant decrease. Na+/K+ ATPase dependence was demonstrated.

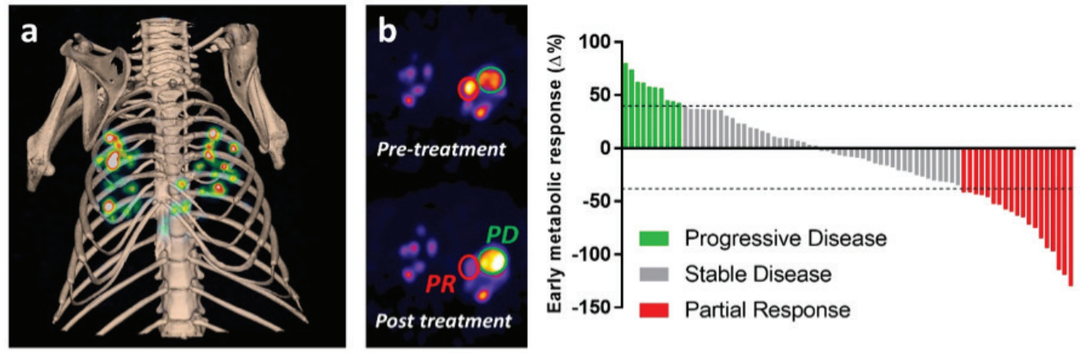

Figure 2
Non-invasive identification of responding and resistant tumour clones using radionuclide imaging
(a) Lung tumour imaging in the KRAS$^{G12D/+}$; p53$^{-/-}$ (KP) mouse with [^{99m}Tc]TcO_4^- SPECT/CT imaging after intranasal lentiviral LV-PGKCre-EF1NIS infection.
(b) Following 1 mg/kg bortezomib, 24% of tumours have early (24 hour) response (PR) while 13% have progressive disease (PD).

Non-invasive identification of clonal resistance using a multi-transgenic vector and tomographic reporter genes

We are entering a new era for some 'hard-to-treat' tumours like lung cancer. Recent clinical trials have shown a proportion of patients with sustained responses to new treatments. Typically only a subset of patients respond; therefore, we need to develop approaches to stratify patients prior to therapy and identify rare responders versus those with innate resistance. We have developed novel vectors for *in vivo* transduction of somatic cells, allowing imaging of spontaneous lung tumourigenesis and single lesion detection of drug responders to investigate the mechanisms underlying heterogeneity in drug response.

We developed a mouse model where we could identify and isolate responders with nanolitre resolution. To be relevant, a mouse model should share several clinical features of lung cancer, including genetic, phenotypic and response heterogeneity. To address these aims in collaboration with Scott Lyons, Cold Spring Harbor Laboratory, we developed two novel lentiviral vectors (LV-PGKCre-EF1SN and LV-PGKCre-EF1LS) to deliver multiple transgenic elements to somatic cells of adult mice with conditional (floxed) oncogenic *Kras* (LSL-Kras$^{G12D/+}$) and *p53$^{fl/fl}$* alleles (KP mice) by intranasal administration. Mice were longitudinally imaged using bioluminescence, radionuclides ([^{18}F]TFB PET and $^{99m}TcO_4^-$ SPECT) and CT. Drug responses were determined using a double baseline and imaging pre- and post treatment. Lesions down to tens of nanolitres could be repeatedly imaged with a coefficient of variation of 11.8%. Drug therapy identified single initiating lesions with significantly reduced $^{99m}TcO_4^-$ SPECT uptake and high CC3 staining not identified by bioluminescence or CT imaging (Fig. 2).

We are exploiting the tomography of radionuclide imaging to track single lesions at nanolitre resolution during cancer therapy and identifying inter- and intratumoral heterogeneity in drug response. These vectors comprise a new platform technology and provide a quantitative, 4D readout of cell viability, critical for monitoring therapeutic efficacy and identifying responders within a heterogeneous tumour.

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