Bringing cancer growth to a standstill

Cancer's aggressive growth is associated with a reprogramming of normal metabolic pathways. Shifting metabolism's balance towards normal could dramatically slow cancer growth. Until now, however, this approach has been impossible to test because of the absence of a method to measure this relative balance *in vivo*. **Drs. Lawrence Recht** and **Daniel Spielman**, at Stanford University, California, believe they may have the solution. Their new analytical technique may not only track the effect of anti-cancer drugs, but could lead to a new approach for cancer treatment.

All living tissues need to produce energy to remain alive, a process called metabolism, and which proceeds via several chemical reactions. Interestingly, cancer tissues meet their energetic (and biosynthetic) needs via differential utilisation of these pathways, a phenomenon now termed 'metabolic reprogramming.' If only we could reprogramme them back again, we might be able to halt the uncontrolled growth responsible for the poor prognosis of many cancer patients.

WHY IS CANCER LIKE A SPRINTER?

One key metabolic pathway that differs radically in cancer cells compared to healthy tissue is respiration (the release of energy from food). There are several different pathways of respiration, but the most common in healthy tissue is oxidative phosphorylation, which combines oxygen and glucose to produce water, carbon dioxide and energy. When oxygen is in short supply – for instance in the muscles of a sprinter during a race – cells can employ another form of respiration, glycolysis. Glycolysis is less energy-efficient than oxidative phosphorylation, and produces lactate instead of carbon dioxide and water.

It is this second form of respiration that dominates in cancers, even when oxygen is abundant, a phenomenon described almost a century ago by Nobel laureate, Otto Warburg. Widespread enough to be viewed as a 'hallmark of cancer,' the reason behind the Warburg Effect remains elusive although a recent consensus has been attained wherein most investigators believe the switch to glycolysis makes available carbon skeletons such as lactate that can be directed towards producing biomass. Although it is generally accepted that the preponderance of glycolysis is linked to proliferation and aggressiveness, the converse remains unproven, i.e., whether forcing tumour respiration back towards oxidative phosphorylation will slow growth, thus providing a new paradigm for cancer treatment.

REVERSING THE WARBURG EFFECT

The idea that forms the basis for this project was initiated by a counterintuitive hypothesis proposed by Dr Recht based on clinical observations concerning the actions of the novel therapeutic agent bevacizumab (BEV). Via binding to vascular endothelial growth factor (VEGF), a signalling molecule that stimulates angiogenesis, the growth of blood vessels is arrested, which causes growth arrest.

Because glioblastoma (GBM), the most lethal primary brain tumour, is highly dependent on VEGF, BEV administration

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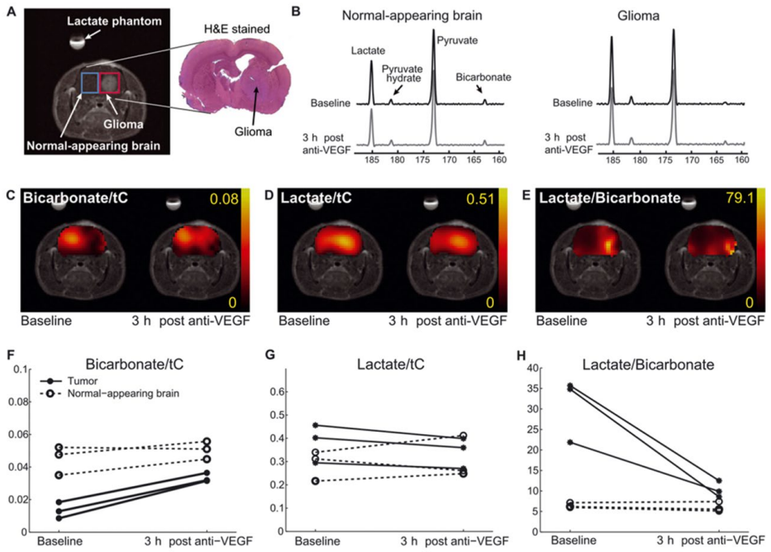
produced an initially extraordinary (albeit temporary) effect that could be quickly seen on neuroimaging. Since the effect on magnetic resonance (MR) appeared too rapid to be explained by inhibition of vessel formation alone, he hypothesised that its initial effects on blood flow redirected the balance of tissue metabolism from a preponderance of glycolysis towards oxidative pathways, thus halting proliferation. What made this hypothesis counterintuitive was that bevacizumab's effect on blood vessels tended to produce hypoxia, which would favour increased glycolysis.

GETTING THE MEASURE OF METABOLISM

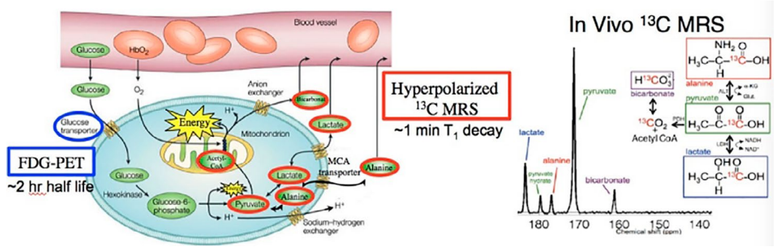
Scientific progress is fuelled by ideas, but ideas themselves really cannot advance understanding without tools to study them. Since this hypothesis requires an analysis of metabolism in the living organism in real time, Dr Recht approached Dr Spielman, who was at the forefront of the development of a novel form of magnetic resonance spectroscopy (MRS) known as dynamic nuclear polarisation magnetic resonance spectroscopy, which provided a crucially non-invasive, radiation free technique to repeatedly measure the constituents of metabolic pathways *in vivo*, without putting patients at risk.

Both glycolysis and oxidative phosphorylation make use of an intermediate molecule called pyruvate. Recht and Spielman 'feed' cancer cells with a special form of pyruvate labelled with 'magnetised' carbon-13 (13C), which is possible through a process call 'dynamic nuclear polarisation' (DNP). This magnetisation makes it highly recognisable to the MRI scanner. The fate of the pyruvate can then be traced, as the hyperpolarised 13C ends up in either of two molecules: lactate, the product of glycolysis, or bicarbonate, a by-product of oxidative phosphorylation. The ratio of hyperpolarised 13C-labelled lactate to bicarbonate provides a quantitative proxy for the relative rates of glycolysis and oxidative phosphorylation – a measure of the Warburg Effect in real time.

This is the crux of Dr Recht and Prof Spielman's current work – identifying a biomarker for the two forms of respiration, and at the same time developing a method by which it can be measured *in vivo*, i.e. in cancer patients.



Above: anti-VEGF therapy results in a marked reduction in lactate/bicarbonate levels as assessed by hyperpolarised $^{13}C_1$-pyruvate magnetic resonance spectroscopy (MRS). From *Curr Oncol Rep 2017.*



Above left: differences between FDG-PET and ^{13}C-pyruvate DNP MRS. FDG measures cellular glucose uptake while $^{13}C_1$-pyr MRS measures the flux of $^{13}C_1$-pyr into other molecules in the metabolic pathways.
Above right: representative acquisition *in vivo* ^{13}C spectrum from a rat heart following the injection of hyperpolarised $^{13}C_1$-Pyr. ^{13}C-Pyr and its products ^{13}C-Lactate, ^{13}C-Alanine, and ^{13}C-Bicarbonate.

'BENCH TO BEDSIDE'

In a ground-breaking five-year study funded by the US National Institutes of Health, Recht and Spielman propose to investigate the action of BEV on aggressive primary brain tumours known as glioblastomas, beginning their studies on laboratory rats. Combining Dr Recht's clinical knowledge with Prof Spielman's advances in medical imaging, the pair acknowledge that their work is a 'high reward/high risk' project, with implications potentially extending to all forms of cancer.

Their immediate aims are to refine and improve the imaging technology, and to develop the capability to quantify additional molecules occupying other, key positions in the glycolysis and oxidative phosphorylation pathways. They will quantify and explore further the impact of BEV upon glioblastomas. Ultimately, the team hope to define an optimal lactate/bicarbonate ratio which can be used to measure the effectiveness of BEV and other anticancer drugs, as well as quantify the aggressiveness of tumours. With this ratio as a goal, patients may in the future be able to enter a regime of repeated imaging, enabling continued adjustment and optimisation of their treatment programme.

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REPROGRAMMING OUR THINKING

Underlying the excitement of dynamic nuclear polarisation MRS and the lactate/bicarbonate ratio as a means to monitor and optimise cancer treatments, Dr Recht's ambition is to challenge the very way we think about cancer therapy. Rather than targeting total tumour eradication, with all the pain, risks and side-effects this may involve, he would like us to envisage cancer alongside other chronic conditions such as diabetes, aiming to manage the condition, not eradicate it. Switching a tumour's metabolism from proliferative, glycolysis based pathways to stable oxidative phosphorylation may be one way to do this. Drs Recht and Spielman's new tools may provide the means to bring this potential paradigm shift to reality.

Q&A

The Warburg Effect has been known for over sixty years. Why do you think no-one else has considered reversing it as a means to cancer treatment?
Many investigators have proposed this; it forms the basis for most 'metabolic therapies'. However, no one has seriously considered the idea of measuring the balance between glycolysis and oxidative metabolism as a way of monitoring these (or any other treatments) because there has, up to now, been no way to assess this in the living organism.

Do you have a theory as to how drugs like BEV are able to force cancer cells to carry out oxidative phosphorylation instead of glycolysis?
I have a theory but it's only that. My feeling is that the cancer tissue has glucose in excess; that's why it can use glycolysis and will do this even in the presence of oxygen. If you restrict 'fuel', however, you 'force' the cells to become more energy efficient, thus diverting these biomolecules for foodstuff.

What is dynamic nuclear polarisation and how is it useful?
Standard MR images water since hydrogen is the most abundant molecule in the body that has an odd atomic number. Because even in a high magnetic field only five hydrogen molecules per million can be magnetised, it is impossible to use this methodology to measure physiology. In nature, other atoms such as C13 (which has seven neutrons) occur at low number of protons but because of their mass, are much harder to magnetise at room temperatures. This constriction is removed at superconductivity temperatures (i.e., 1° K) using specialised lattices (a process called dynamic nuclear polarisation) that allows for a very high percentage of carbon13 molecules to be to be magnetised and viewable on the same MR machine.

What is the importance of non-invasive imaging techniques to developing cancer therapies?
A quick, non-invasive readout might be considered a holy grail in medicine because it allows one to make quick adjustments based on simple quantitative measurement. Consider the blood pressure cuff, wherein one makes important adjustments based on a single reading at a single setting without requiring any special understanding of its underlying pathophysiology. Blood glucose measurement for monitoring diabetes is another example.

What are the benefits of your two labs working together on this project?
Ideas are great but one cannot do much without the right tools. Likewise, tools without applications have limited utility and are unlikely to translate to a large audience. This is an excellent example of how the idea and tool match up as the idea was going nowhere without a proof, while the tool was searching for an application.

Detail

RESEARCH OBJECTIVES

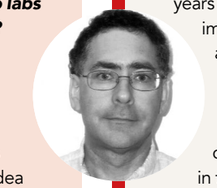
Dr Recht's research explores approaches to treating cancer, primarily glioblastoma, via metabolic approaches.

FUNDING

• National Institutes of Health (NIH)

BIO

Dr Lawrence Recht received his medical degree from Columbia University College of Physicians and Surgeons in New York City. He completed his training as a Resident in Neurology at New York Presbyterian Hospital. Prior to taking up his post at Stanford University, Dr Recht spent 19 years at University of Massachusetts Medical School, caring for patients and researching brain tumours. He relocated to Stanford in 2004 where he has developed the Adult Neuro-Oncology Program.



Dr Daniel Spielman earnt his Masters and PhD in electrical engineering from Stanford University, where he is currently Professor of Radiology. With over 30 years of experience as a medical imaging researcher, Dr Spielman's achievements include the advancement of both MR and spectroscopic imaging methods, which have had a widespread clinical impact. Current research in the Spielman Laboratory explores *in vivo* magnetic resonance imaging and spectroscopy to develop new non-invasive methods of imaging metabolism within the body.

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