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Imaging brain cancer

Dr Manuel Sarmiento Soto and Dr Sébastien Serres from Prof Nicola Sibson's group

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Oncology

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Pancreatic cancer and the TMPRSS2-ERG fusion gene

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Detection and treatment of brain tumours using molecularly-targeted MRI

by Dr Sébastien Serres

Brain tumours can either originate in the brain (a primary brain tumour) or outside the brain (a secondary brain tumour, also known as brain metastasis). Although brain tumours have been intensively studied, there are still important questions that need to be addressed and this area of investigation has recently been identified by UK charities as a priority research field. Notably, there is urgent need for early detection and efficient treatment, but also for a better understanding of the biology of this disease.

In terms of detection, both primary and secondary brain tumours are diagnosed and monitored clinically using passive (non-targeted) contrast-enhanced magnetic resonance imaging (MRI). Although this method provides reasonable information on tumour size and spatial extent, it often fails to detect small brain metastases or accurately delineate tumour margins, which is an essential criterion for effective surgical resection or treatment with personalised therapeutics.

At the Department of Oncology in Oxford, Prof Nicola Sibson has made ground breaking discoveries in molecularly-targeted MRI, which offers a number of advantages over passive MRI, including early diagnosis and identification of specific molecular processes associated with brain tumour development. Molecularly-targeted MRI, based on a platform of microparticles of iron oxide (MPIO) that are functionalised with surface ligands, can specifically target endothelial markers on tumour blood vessels (e.g. vascular cellular adhesion molecule-1 or VCAM-1) (Figure 1). This approach has enabled early and sensitive detection of brain metastases in mice (1), and is currently being tested in clinical trials. In addition, when brain tumours are sufficiently large to be detected by clinical MRI, this approach could be used to detect upregulation of VCAM molecules in the invasive margins of the tumour. Thus, molecularly-targeted MRI could help refine surgical resection and targeted radiotherapy by offering better spatial delineation of tumour margins and more accurate assessment of tumour pathophysiology.

The efficacy of radiotherapy and chemotherapy in targeting tumour cells and of anti-angiogenic agents in targeting tumour blood vessels is significantly limited by the presence of the blood-brain barrier (BBB), which prevents the access of drugs to the brain, and by abnormal neovascularisation (or angiogenesis) of the tumour. As a consequence, regions of hypoxia can arise within the tumour microenvironment (2). Although it is possible to facilitate drug delivery by permeabilising the BBB at sites of brain metastases (3), important hurdles in tumour treatment remain, the most crucial of which is to improve the abnormal vasculature of brain tumours and thus reduce hypoxia and resistance to treatment (2). One possible improvement could come from imaging probes that detect biomarkers of angiogenesis and hypoxia, which could accurately assess the efficacy of novel therapeutics targeting such micro-environmental factors *in vivo*.

Brain cancers are diverse collection of diseases in terms of tumour biology. Primary brain tumours can now be classified using molecular criteria that better reflect their underlying biological subtypes and response to treatment. Secondary brain tumours are more difficult to classify since they originate from different cancer types. This means that successful therapeutics targeting one tumour subtype will probably not be so successful when applied to others. This is a significant limitation in the targeted treatment of brain cancer.

To overcome this issue, brain tumour research has focused on xenotransplantation of human cell lines into mice using (i) clonal selection (e.g. monolayer in serum containing medium), (ii) selection of cells with stem-cell like phenotype (e.g. neurosphere cultures in serum free medium), or (iii) limited selection of cells (e.g. tissue fragment passaged in immunodeficient animals). Whilst xenografts generated from monolayer cultures display angiogenic growth and well-defined borders (minimally invasive) that resembling normal brain tissue, neurospheres and fragment passage cultures offer numerous improvements, including invasive growth, dilated vessels, angiogenesis and necrosis (4, 5). As a consequence, developing a method that maintains human brain tumours in their original phenotype could have tremendous benefits for basic and translational research focusing on primary and secondary brain tumours.

Overall, molecularly-targeted MRI is an important tool for identifying and characterising brain tumour cells. Translating these developments into the clinic will improve the detection, treatment and understanding of brain tumours, leading to long-term survival benefits for brain cancer patients.

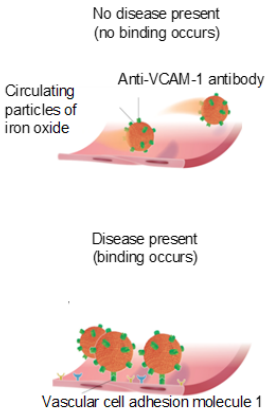

Figure 1: MRI can be used to image specific molecules expressed on endothelial cells using appropriate targeting antibodies conjugated to microparticles of iron oxide (MPIO) (e.g vascular cell adhesion molecule (VCAM)-MPIO targeting endothelial VCAM-1).

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Bone oncology: Targeting the seed and the soil

by
Prof Claire Edwards

The bone provides a unique and supportive microenvironment for a number of solid tumour metastases including breast and prostate cancer, and the haematological malignancy multiple myeloma (1). Once such tumours are detected within bone, treatment is largely palliative and median survival times are short. Therefore it is imperative to understand the cellular and molecular mechanisms involved in disease pathogenesis, in order to develop new and effective therapeutic approaches.

A common feature of these skeletal cancers is the development of a destructive bone disease, associated with pathological fractures and bone pain. This can manifest across a broad spectrum from predominantly osteolytic bone disease with dramatic bone loss, as is seen in myeloma, through to osteoblastic bone disease where bone formation is increased, which is typically seen in prostate cancer bone metastases. The driving force behind the aggressive nature of bone metastases is the reciprocal relationship between the tumour cells (the "seed") and the cells of the bone marrow microenvironment (the "soil"), whereby the tumour cells promote the development of the bone disease and, in turn, these tumour-induced changes in the bone microenvironment promote the growth and survival of the tumour. This relationship results in a "vicious cycle" whereby tumour growth promotes bone disease, and bone disease promotes tumour, thus perpetuating both components of these incurable malignancies. Our research is focused upon understanding how changes within both the tumour cells and the host bone marrow microenvironment promote tumour growth and the associated bone disease.

Historically research has focused upon the usual suspects in bone biology, the bone-resorbing osteoclast and the bone-forming osteoblast (Figure 1). Osteoclasts are well known to be increased in cancer-induced bone disease, and largely responsible for the destructive nature of cancer-induced bone disease. Osteolytic bone disease is exacerbated by a reduction in the number and activity of bone-forming osteoblasts, leading to an inability to repair existing bone lesions. In those tumours associated with osteoblastic bone disease, such as prostate cancer bone metastases, osteoblast number and activity is increased, resulting in an excess of bone formation. In addition to bone cells, the bone marrow microenvironment is comprised of a heterogeneous mixture of cell types, including mesenchymal stem cells, stromal cells, lymphocytes and adipocytes. In recent years, it has become clear that these cells are ideally placed to interact with tumour cells and contribute to disease pathogenesis (2).

Our own studies have identified that changes in bone marrow stromal cells are important in the development of multiple myeloma (3). Using murine models, we have shown that inoculation of a mixture of specific bone marrow stromal cells and myeloma cells results in the development of myeloma, associated with tumour growth within the bone marrow and an osteolytic bone disease. This is all the more striking because inoculation of myeloma cells alone has no effect, and the mice remain free from tumour, highlighting the critical role that the stromal cells play. Our subsequent studies have identified that this is in part due to expression of the Wnt signalling inhibitor Dkk1, which is increased in these specific bone marrow stromal cells, with a reduction in Dkk1 expression resulting in a decrease in tumour burden and bone disease. Taken together, we have identified that stromal-derived Dkk1 is a key contributor to the development of multiple myeloma *in vivo*.

One of the major projects in our lab focuses on the contribution of host-derived adiponectin and obesity in the pathogenesis of cancer-induced bone disease. Using a combination of murine models and patient samples, we have found that host-derived adiponectin is decreased in myeloma-permissive

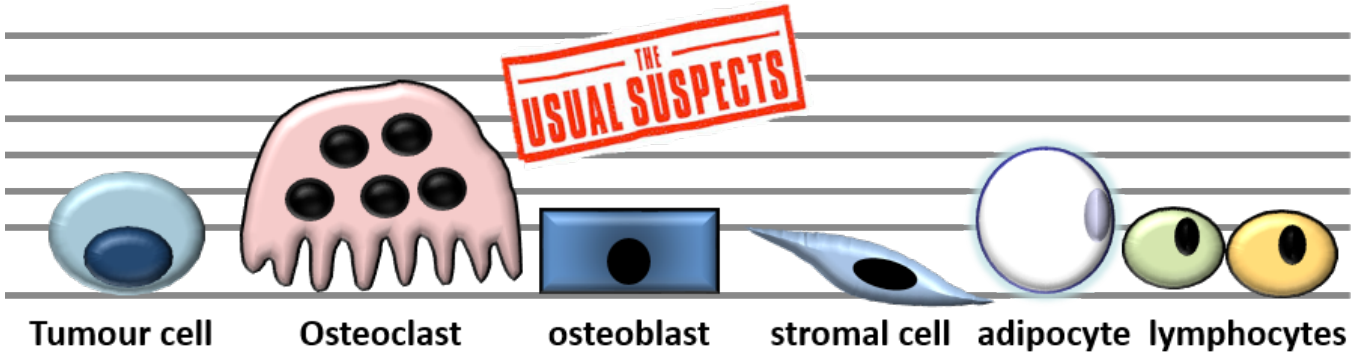


Figure 1: The usual suspects in cancer-induced bone disease. Tumour cells are well known to interact with osteoclasts and osteoblasts to promote cancer-induced bone disease, however the contributions of other cell types, including bone marrow stromal cells, adipocytes and lymphocytes are poorly understood.

environments, either mice that subsequently develop myeloma or patients with the premalignant condition monoclonal gammopathy of undetermined significance (MGUS) that progress to myeloma (4). The strength of our studies comes from the ability to combine genetic manipulation with murine models of cancer-induced bone disease, exemplified by the dramatic increase in tumour burden and bone disease observed in myeloma-bearing adiponectin-deficient mice (5). *In vitro* studies have determined that adiponectin is expressed by bone marrow adipocytes and stromal cells and induces apoptosis in myeloma cells, therefore identifying adiponectin and its downstream signalling pathway as a potential therapeutic target. In support of this, we have shown that pharmacological approaches to increase serum concentrations of adiponectin in a preclinical murine model of myeloma results in a significant reduction in both tumour burden and osteolytic bone disease (Figure 2).

Adiponectin is an adipokine that is expressed by adipocytes and bone marrow stromal cells and is inversely associated with obesity. There is ever-increasing evidence to support an association between obesity and a multitude of disease states, including cancer. With rising obesity becoming a major problem in the Western world, a greater understanding of the critical cellular and molecular mechanisms is required. Within the bone microenvironment – a hugely understudied area – bone marrow adipocytes were widely regarded as passive space-fillers, but are now increasingly recognised as metabolic cells with a multitude of functions. This is a major focus of our laboratory, where we have recently demonstrated the key role that obesity plays in myeloma development (6). By placing mice that normally do not develop myeloma on a high-fat diet prior to inoculation of tumour cells, we were able to promote the development of multiple myeloma, with 90% of mice developing tumour growth within bone, as compared to 10% when mice were placed on a normal diet (Figure 3). Importantly, if the high-fat diet was removed at a time when tumour burden was established within bone, which is reminiscent of the clinical setting, tumour burden was reduced, suggesting the potential for dietary intervention strategies. Our current projects will determine exactly how diet-induced obesity is creating a host environment that is so favourable for myeloma development.

In addition to elucidating the changes in the host microenvironment that promote the development of cancer-induced bone disease, our research also concentrates on changes within the tumour cell that render the cells more capable of surviving within these unique surroundings. Intriguingly, some of these changes alter the phenotype of prostate cancer cells to resemble that of osteoblasts, a concept known as osteomimicry. In collaboration with colleagues at the Target Discovery Institute (University of Oxford) we are undertaking a comprehensive high throughput expression and functional analysis of bone metastatic prostate cancer cells to identify changes in gene and microRNA expression that are important in the key stages of prostate cancer bone metastasis. Once individual or combinations of genes have been identified and validated, these will progress through our pipeline where we will focus on *in vitro* functional investigation, effects on tumour burden and bone disease *in vivo*, and associations with markers of tumour burden and/or bone disease in patient samples.

Despite many recent advances in our understanding of disease pathogenesis, once tumours are established in bone, the disease is largely incurable. Our increased understanding of the changes in the host microenvironment that promote disease development not only reveal potential targets, but also begin to address the challenging question of how to distinguish those patients at greatest risk of disease progression, either from MGUS to myeloma or from primary prostate cancer to prostate cancer bone metastases. By studying the tumour cells and the host bone marrow microenvironment, this maximises the opportunities to hijack the vicious cycle, and ultimately eradicate both tumour growth and bone disease.

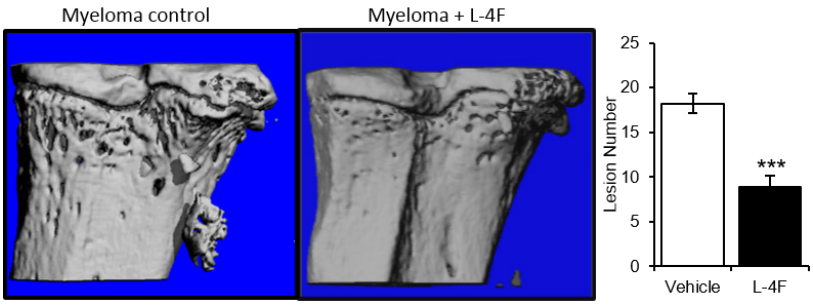
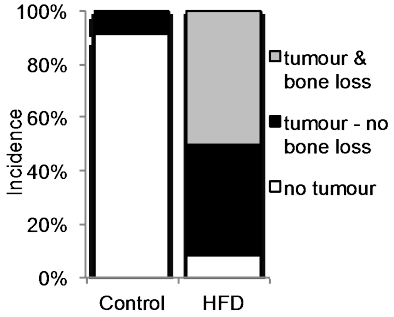


Figure 2: Increasing adiponectin prevents myeloma-induced bone disease. Circulating levels of adiponectin were increased by treatment with L-4F. L-4F treatment resulted in a decrease in tumour burden and osteolytic bone disease. Osteolytic bone lesions were identified by microCT analysis. Adapted from (4).

Figure 3: Diet-induced obesity promotes development of myeloma. Mice were placed on a high-fat diet (HFD) prior to inoculation of myeloma cells. Those mice on a high-fat diet developed evidence of myeloma tumour burden and associated osteolytic bone disease. Adapted from (6).



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The TMPRSS2-ERG fusion gene

by Dr Slav Ovtcharov

It is estimated that 16.2% of men will be diagnosed with prostate cancer (PCa) in their lifetime, and approximately 3% will die of the disease. The origin of PCa, which develops in the prostate gland, is dependent on a variety of different signalling pathways, transcription factors, and hormones. The most prominent hormones involved in the regulation and development of the prostate gland are testosterone and its more potent form, dihydrotestosterone. The interplay of these androgens and androgen-driven signalling has a role in PCa progression. In fact, fusion of the androgen-regulated gene TMPRSS2 (transmembrane protease, serine 2) to the ERG transcription factor (TMPRSS2-ERG) is commonly found in primary tumours and castration resistant cancers.

Fusion genes and cancer

Formation of a fusion gene from two separate genes can naturally occur through translocation, interstitial deletion, or chromosomal inversion. The Philadelphia chromosome, formed through translocation between chromosome 22 and 9, is the prototypic example of a fusion gene (BCR-ABL) associated with malignant transformation in chronic myeloid leukaemia. The discovery of the prostate specific TMPRSS2-ERG fusion gene highlighted the need to study chromosomal aberrations in PCa. TMPRSS2-ERG was detected in more than 50% of patients, but its clinical relevance has not yet been established. However, it is known that the presence of this fusion leads to overexpression of the ERG transcription factor, which promotes migration and invasion in cell models. The overexpression of ERG in mouse models causes the formation of a non-malignant condition known as prostatic intraepithelial neoplasia. These results indicate that the presence of the TMPRSS2-ERG fusion is not sufficient to initiate the disease but occurs as an early molecular event.

> “Correlation between TMPRSS2-ERG expression and [cell] viability”

Standard therapies for localised and advanced tumours

Upon discovery of PCa in patients, clinicians develop an individual treatment plan based on the results from prostate-specific antigen (PSA) testing, digital rectal examination, and biopsies. Certain risk factors such as age, family history, and diet are also taken into consideration. The standard therapy recommended as the initial treatment for patients with metastatic disease is androgen deprivation therapy (ADT). ADT is commonly administrated as an adjunct to radiotherapy for localised tumours, while chemotherapy is recommended for advanced metastatic cancers. However, selecting the subtype of patients that may benefit from either of these therapeutic options, or a combination of them, is not a straightforward process. Given the widespread presence of TMPRSS2-ERG in PCa patients, it is important to define its role in cell response to these treatments.

TMPRSS2-ERG and PCa treatment

Our research group, led by Prof Freddie Hamdy and in collaboration with Prof Ruth Muschel, has been investigating the effect of the TMPRSS2-ERG fusion gene on cell response to ADT, radiotherapy, and chemotherapy. After silencing the expression of TMPRSS2-ERG in cell models expressing the fusion gene endogenously, we evaluate the changes that occur in cell proliferation following radiotherapy or chemotherapy with taxanes. In addition, we seek to understand the effect of androgens in the environment on the cell response to these therapies. So far we have established that ADT can cause a decrease in ERG levels while increasing the levels of androgen receptor and DNA damage response proteins. Further analysis revealed that the combination of ADT with radiotherapy or radiotherapy alone could cause decreased cell proliferation irrespective of TMPRSS2-ERG status. The presence of the latter did not confer proliferative advantage to cells subjected to ADT and/or radiotherapy, whereas untreated cells, harbouring the fusion gene, demonstrated a significant proliferative advantage. When we sought to evaluate the response of these cell models to chemotherapy with taxanes, we identified a correlation between the levels of endogenous TMPRSS2-ERG expression and viability. In other words, cells expressing greater levels of the fusion gene showed significantly higher proliferation compared to those expressing lower levels. This data suggests that the combined administration of ADT and radiotherapy may be adequate for the treatment of prostate tumours harbouring the fusion gene, but further research is required to focus on the effect of different chemotherapeutic drugs, particularly in later stages of the disease.

Dr Slav Ovtcharov completed his DPhil in Prof Ruth Muschel's group in the Oxford Institute for Radiation Oncology

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Oral Cancer Awareness

Head and neck cancer is the sixth most common cancer worldwide, with oral cancers comprising around 85% of cases (1). Oral cancers affect the oral mucosa, lips, nose and throat (Figure 1). They are relatively rare, constituting 2 out of every 100 cancers diagnosed. As with most cancers they are more common in older people, and only around 1 in 8 cases are observed in patients under 50 years of age. Oral cancer is twice more common in men than women.

by Sara Ahrabi and Dr Marzyeh Parvizi

Aetiology

There is no single, clearly defined cause for oral cancer, and it is likely that many factors are involved. However, tobacco and alcohol are the most common risk factors in developing oral cancers. These have a powerful synergistic effect – whereas tobacco itself is carcinogenic due to nicotine-derived *N*-nitrosamines and polycyclic aromatic hydrocarbons contained in tobacco smoke, alcohol enhances the transport of these carcinogens across the mucosal barrier. In alcoholics this mucosal barrier is weak due to epithelial atrophy. Electronic cigarettes (e-cigarettes), recently developed battery-powered vaporizers prescribed to help smokers quit, are gaining attraction. The absence of tobacco in these devices presumably makes them less harmful than cigarettes. However, the results of recently published research (2) reveal high levels of formaldehyde, a well-known carcinogen, in the vapour generated by e-cigarettes when used at high voltages. Although more long-term research is required to draw solid conclusions, e-cigarettes may also be a risk factor for development of oral cancers.

Chronic exposure to sunlight is the major cause for lip cancers. Immunosuppressed people also have a higher incidence of lip cancer, as do people carrying human papillomavirus type 16 (HPV16). Over the past 10 years, there has been a rapid increase in the number of HPV16-positive cases of oral cancer (3). A diet rich in vitamins A, C, E, and selenium may provide protective factors against oral squamous cell carcinoma.

Symptoms

While early stage lesions are often painless, later stage lesions can cause pain, numbness, and difficulty eating and swallowing. The stage at which oral cancer is diagnosed has a significant impact on overall survival. Although signs of oral cancer are visible, 6 out of 10 cases of mouth cancer are found at a late stage due to a lack of awareness among the population. Therefore, as a general rule, any type of mouth lesion – a white or red patch or lump – persisting for longer than two weeks has to be treated with suspicion and reviewed by a dentist or a doctor.

Treatment and challenges

Treatment of oral cancers is uniquely challenging due to their close proximity to critical structures that may be damaged either by the tumour itself or its treatment. The standard therapy includes surgery, radiation therapy, chemotherapy, palliative treatment, or a combination of these approaches. They all come with an array of side effects such as cosmetic defects, functional disabilities (i.e. impairment of speech, chewing or swallowing), sore and dry mouth, and taste and smell disturbances. A treatment plan is based on the likelihood of a cure as well as the resulting cosmetic and functional defects that can have a significant effect on the quality of life.

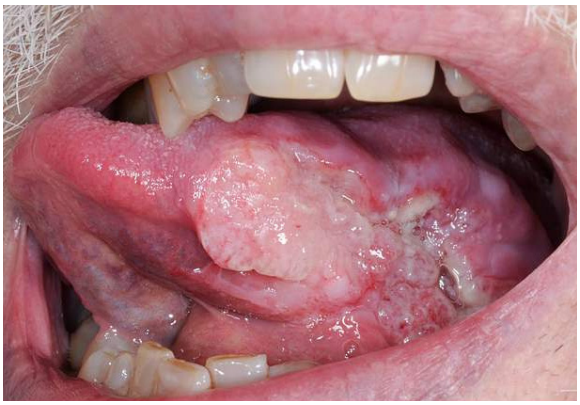

Figure 1: Oral squamous cell carcinoma.

Cancer research

The side effects of standard cancer therapy, which often damages nearby healthy cells, required scientists to seek novel approaches to target cancer cells with higher specificity. Targeted therapy harnesses the small differences between normal cells and cancer cells. Several approaches are currently in clinical trials. Cancer immunotherapy is a promising example of targeted therapy, aiming to take advantage of one's immune system to selectively target and kill cancer cells (4). Importantly, recent research advancements, such as in cancer therapy, will help us better understand the disease and its treatment and hopefully provide benefits to cancer patients in the nearer future.

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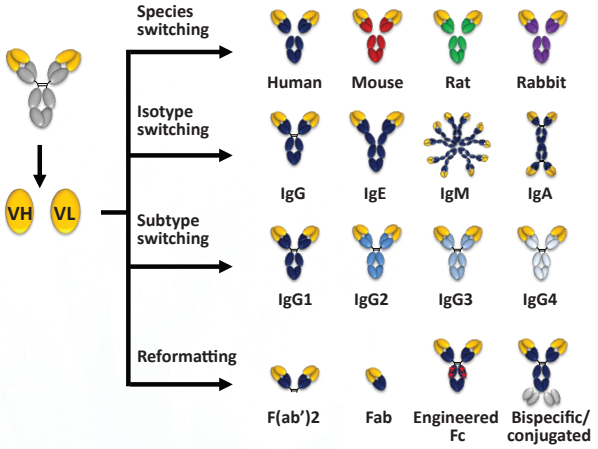

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