SCIENTIFIC REPORT 2012

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Cover image
Dr Andrew Porter, a Post-doctoral Research Fellow in the Cell Signalling Group.

SCIENTIFIC REPORT 2012

Paterson
Institute for Cancer Research

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK | PATERSON INSTITUTE

The Paterson Institute

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DIRECTOR'S INTRODUCTION

Professor Richard Marais
Director of The Paterson Institute for Cancer Research

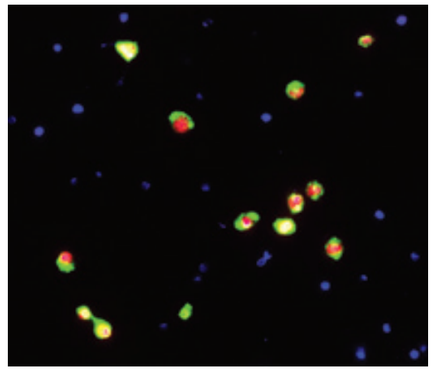
It is a privilege to have taken on the Directorship of the Paterson Institute and my first year in post has been highly enjoyable. This report offers a chance to look back at the developments that have taken place and an opportunity to discuss our ambitions for the future. A major highlight of the year was the successful bid, made with colleagues at the University of Manchester and the Manchester Cancer Research Centre, to the UK Research Partnership Investment Fund which resulted in an award of £12.8m towards our aim of implementing personalised medicine for cancer patients in the North West.

The funds provide £4.1m towards completion of the new Manchester Cancer Research Centre (MCRC) research building which is currently under construction across the road from the Paterson Institute and due for completion in 2014, while the remaining £8.7m will go towards specialised equipment , the majority of which will be housed in the Paterson Institute core facilities and will support research across both buildings. This will help us to progress the basic and translational research that is pivotal to the development of further novel anti-cancer treatments and to accelerate the development of approaches that will lead to improved outcomes for patients. We have made a start on acquiring the necessary technologies with the installation of two next generation sequencing platforms which will greatly expand our capabilities in this area. The remaining purchases will be made over a three year period which will allow us to take advantage of rapidly evolving technology across many of the relevant platforms.

During the summer, Geoff Margison, leader of the Carcinogenesis group, retired from the Institute after an association extending back over forty years. He continues his research with an honorary position at the University of Manchester. Akira Orimo returned to Japan after five years at the Institute as a Junior Group Leader. He will continue to investigate the role that non-cancerous stromal cells play in tumour development in his new post at the Juntendo University School of Medicine in Tokyo. Successful quinquennial reviews were undertaken by Crispin Miller (Applied Computational Bioinformatics and Biology) and Iain Hagan (Cell Division). My own research team focuses on signalling through the RAS/RAF pathway and the role that this plays in the development and progression of melanoma. The group is now established in the Institute with eight new members in addition to five Postdoctoral Fellows who have joined me in moving here from the Institute of Cancer Research in London.

A key objective at the Institute for the coming year is to recruit four new Junior Group Leaders. These new appointments will help strengthen the Institute's portfolio in translational research and will focus on our areas of priority, which include lung cancer, pancreatic cancer, women's cancers and molecular pathology. These themes are largely aligned with the cancer research strategy of our local MCRC partners, the University of Manchester and The Christie NHS Trust, a joint strategy that has been developed over the past year and has personalised medicine at its core. Critically, the priority areas offer exceptional opportunities in Manchester due to local clinical need and existing research activities. Lung cancer is a particular priority on which we wish to focus, building on the recruitment to the Institute of

Development of a pharmacodynamic circulating tumour cell assay: Spiked tumour cells (green) and white blood cells (blue) recovered from a whole blood sample by 'Isolation by Size of Epithelial Tumour Cells' (ISET). A drug target (red) can be quantified, providing a potential pharmacodynamic biomarker for novel targeted therapies. CD45: Blue, Cytokeratin: Green, Drug Target: Red. Image provided by Robert Sloane from the Clinical and Experimental Pharmacology Group.



John Brognard in 2010 and the intense activity in this area within the Clinical and Experimental Pharmacology Group.

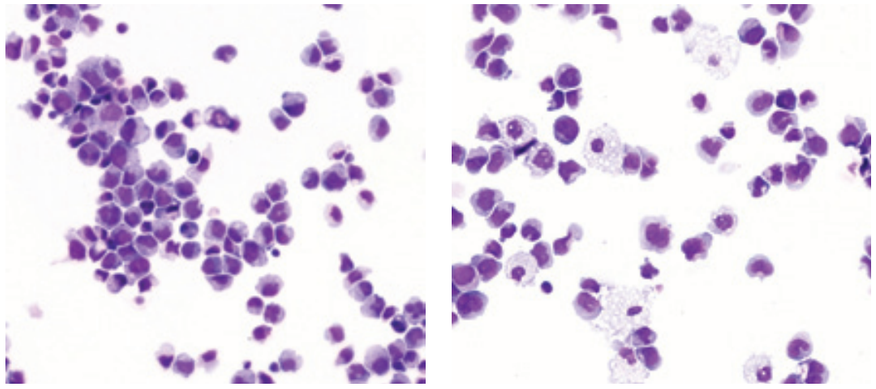
An ongoing goal is to promote translational research at the Paterson Institute. A major activity in this respect is provided by the Clinical and Experimental Pharmacology Group led by Caroline Dive. This year the CEP Biomarker Portfolio has expanded to include a Nucleic Acids Biomarkers (NAB) team, led by CEP Deputy Ged Brady, which is dedicated to developing the current validated CEP blood-based NAB assays. To do so, NAB projects have been initiated to examine miRNA and methylation patterns present in circulating free DNA, RNA profiling and mutation detection in circulating tumour cells. CEP successfully re-negotiated their contract with AstraZeneca, worth £3.1million over three years, to continue their alliance covering blood borne serological biomarker assays. Caroline was awarded the highly prestigious Pasteur-Weizmann/Servier Prize and Tribute. This well-deserved honour is in recognition of the fantastic work by Caroline and her group in developing minimally-invasive biomarker tests for a range of cancers and treatments.

The Drug Discovery Group continue to make good progress and have two active phase projects in collaboration with core Group Leaders (Tim Somervaille and Ivan Ahel) and three pre-projects with core Group Leaders (John Brognard, Ivan Ahel and Karim Labib). The increasing synergy between DDG and CEP was enhanced this year by the joint appointment of an in vivo scientist who will be responsible for progressing the DDG portfolio. The advantages of embedding Drug Discovery within the Institute have been clearly illustrated by another major highlight of the year which was the publication by Tim Somervaille's Leukaemia Biology Group of their discovery of KDM1A as a candidate target for differentiation therapy in one of the most common types of acute myeloid leukaemia. The first author of this study was PhD student Bill Harris who was deservedly the winner of the University of Manchester Institute of Cancer Sciences/PICR postgraduate student of the year award. Bill used chemical inhibitors that had been synthesised by the Drug Discovery Group to down-regulate KDM1A and the success of the approach has resulted in this potential therapeutic strategy progressing towards clinical trials.

The tough financial climate, in which we find ourselves, presented us with significant challenges in 2012 and will continue to do so over the next couple of years. A major focus going forward is to use our core grant from Cancer Research UK to leverage additional funding from external sources in order to carry out the full breadth of research that we wish to undertake. Gill Campbell joined us during the year as a Grants Advisor to help facilitate this. Successful external funding applications increased dramatically in 2012 and successful grants included a Wellcome Trust Senior Investigator Award (Richard Marais) and significant funding from Leukaemia and Lymphoma Research (Georges Lacaud, Tim Somervaille, Peter Stern) and the Pancreatic Cancer Research Fund (Caroline Dive and Ged Brady).

In addition to the major boost provided by the UKRPIF award, there have been further exciting developments in the core research facilities. A successful bid was made to the MCRC for funds to purchase a multi-modal imaging system which will include far-field super-resolution imaging via gated Stimulation Emission Depletion (STED) and two-photon confocal microscopy. We shall be the first site in the UK to have such a capability which will put us at the forefront of super-resolution imaging on live cells and have a major impact on a range of projects across the Institute. We said farewell to Morgan Blaylock after five very successful years building up our Flow Cytometry Facility. Steve Bagley now oversees this service after its merger with Advanced Imaging. Several new members of staff have been recruited who will work across Imaging, Flow Cytometry and Histology to provide an integrated service for users. Significant refurbishment work and updating of equipment has resulted in improvements to Laboratory Services while the Biological Research Unit has had a busy year relocating transgenic services to our new facility at the University which will allow for much needed expansion of our experimental capability.

It is also a pleasure to note some further individual achievements by members of the Institute. Alexia Eliades, a member of the Stem Cell Haematopoiesis Group headed by Valerie Kouskoff, was awarded an EMBO Post-Doctoral Fellowship for her research into the molecular



Addition of a novel KDM1A inhibitor to primary cells from a patient with AML results in differentiation from leukaemic blasts into macrophages. Cells shown in the right hand panel have been treated with the inhibitor whereas those in the left hand panel are untreated.

Figure provided by William Harris and Tim Somervaille from the Leukaemia Biology Group.

mechanisms of haematopoietic specification. Avinash Patel from Cell Division was awarded a short term EMBO fellowship to participate in a highly successful phosphoproteomic collaboration with Boris Maček of the Proteome centre in Tübingen. Allan Jordan, who leads the Chemistry team in the Drug Discovery Group was admitted as a Fellow of the Royal Society of Chemistry (FRSC). The designation FRSC is given to a group of elected Fellows who have made major contributions to chemistry and represents the highest category of membership of the Society. Ali Raaof, also a chemist within Drug Discovery, organised a one day meeting, as part of his activities within the Society of Chemical Industry. The meeting took place at the Paterson Institute and was attended by a mixture of academic and industrial chemists as well as biological scientists. Poster prizes were awarded to Darren Roberts, a postdoctoral scientist who works jointly with Peter Stern and Andrew Renehan, who won the poster prize at the recent 8th World Congress on Peritoneal Surface Malignancies for his work on gene expression profiling and development of a cell culture model of Pseudomyxoma peritonei. Romina Girotti from the Molecular Oncology Group won a poster prize at the South American Spring Symposium in Signal Transduction and Molecular Medicine meeting for her work on mechanisms of resistance to BRAF inhibitors in melanoma.

The past year has provided us with many opportunities to engage with Cancer Research UK's supporters whose generosity allows us to carry out our research. Our annual Open Day was a great success and attended by over 100 visitors who enjoyed research demonstrations by Institute scientists in several laboratories. The guests included Dr Elspeth Russell, who is the daughter of Edith and Ralston Paterson, two pioneering doctors, after whom the Institute takes its name. Our School's Day was also highly successful with a number of sixth form students spending a day in the lab. Scientists from the Institute attended a number of events to meet supporters including Race for Life, Relay for Life and to give talks at fundraising committee annual general meetings. It was also great to see our staff getting involved with their own fundraising efforts which included setting up an impromptu hairdressing salon for a day, running in the Manchester 10K race, cake sales, sponsored walks and participating in the Manchester Shine event.

The coming year promises to be very exciting with the introduction of further innovative technologies and the strengthening of our research portfolio through the recruitment of new Group Leaders. I look forward to the challenges ahead and to continuing to develop the Institute in a way that helps us to contribute to CR-UKs goals of finding new ways to diagnose and treat cancer.

RESEARCH HIGHLIGHTS

In this section we are highlighting some research publications from 2012 which report significant advances in specific areas. The selected papers demonstrate the breadth and the quality of the research being undertaken by the groups at the Institute.

Harris WJ, Huang X, Lynch JT, Spencer GJ, Hitchin JR, Li Y, Ciceri F, Blaser JG, Greystoke BF, Jordan AM, Miller CJ, Ogilvie DJ, Somervaille TC.
The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. ***Cancer Cell***, 2012, 17;21(4):473-87.

Drugs targeting aberrant epigenetic mechanisms are under intense investigation within the pharmaceutical industry as candidate novel therapies in cancer. The Leukaemia Biology Laboratory has been assessing the potential of targeting deregulated epigenetic mechanisms in the treatment of acute myeloid leukaemia (AML) because few effective therapies currently exist for this disease.

Their study identified the histone demethylase, KDM1A, as an enzymatic target which, when inhibited either by knockdown or pharmacological inhibition, induced differentiation of murine leukaemia stem cells. In collaboration with the Drug Discovery Unit, potent inhibitors of KDM1A active in the nanomolar range were synthesised in-house, and these too were found to abrogate clonogenic potential and induce differentiation of both murine AML cells and primary human patient leukaemia cells. Significantly, the functional potential of normal haematopoietic stem and progenitor cells was largely unaffected by these compounds, providing strong evidence for a potential therapeutic window. Thus KDM1A (also known as LSD1) is a candidate target for differentiation therapy in MLL mutated AML, a common cause of both childhood and secondary, treatment-associated AML, which tend to have a poor clinical outcome.

This study also revealed intriguing preliminary evidence that KDM1A inhibition could be a therapeutic strategy in other molecular subtypes of myeloid malignancies. Further lab-based studies are required to characterise KDM1A as a therapeutic target in AML, to optimise inhibitor dosing strategies in vivo and to determine whether it is optimally effective in combination with other differentiation inducing agents such as retinoic acid. This work also provides a basis for early-phase clinical trials of KDM1A inhibitors.

Weston R, Peeters H, Ahel D.
ZRANB3 is a structure-specific ATP-dependent endonuclease involved in replication stress response. ***Genes and Dev***, 2012, 26:1558–1572.

During DNA replication, cells are frequently confronted with the challenge of overcoming potentially dangerous DNA lesions. When such damage is encountered, a cell faces a choice of repairing the damage, or bypassing it without the actual repair. The mechanism which cells employ to repair DNA damage, and thus ensure continuity of DNA replication on an undamaged DNA template, is not well understood. Recent work by the DNA Damage Response Group published in the journal 'Genes and Development' provides new insights into this process.

The DNA Damage Response Group has identified a novel replication associated protein ZRANB3 (Zinc finger, RAN-Binding domain containing 3), and proposed a role for it in the repair of replication-blocking lesions. ZRANB3 localises at DNA replications sites and interacts with the components of the replication machinery. It is recruited to damaged replication forks by multiple mechanisms, which involve interactions with the replication factor PCNA, K63-polyubiquitin chains, and branched DNA

structures. Most importantly, the authors have shown that ZRANB3 acts as a unique structure-specific endonuclease, whose activity is dependent on ATP hydrolysis. It cleaves branched DNA structures with unusual polarity, generating a break in the leading strand DNA template. Collectively, these data support a role for ZRANB3 in the replication stress response and suggest how DNA repair might be coordinated with DNA replication to maintain genome stability.

Mack NA, Porter AP, Whalley HJ, Schwarz JP, Jones RC, Khaja AS, Bjartell A, Anderson KI, Malliri A.
β2-syntrophin and Par-3 promote an apicobasal Rac activity gradient at cell-cell junctions by differentially regulating Tiam1 activity. ***Nat Cell Biol***, 2012, 11:1169-1180.

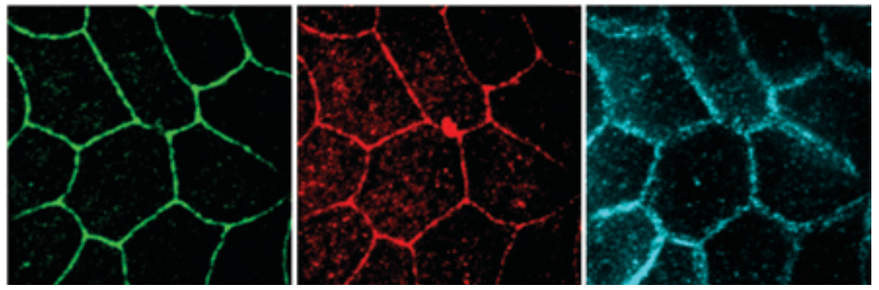
Epithelial cells are attached through junctional complexes spaced along their lateral membranes. The most apical junctions, tight junctions, block the free passage of molecules between cells and the diffusion of molecules from apical to basolateral membrane domains. The assembly and disassembly of junctional complexes is modulated during development, wound healing and tissue homeostasis. Deregulated intercellular adhesion is a hallmark of cancer cells. The Rho-family member Rac, and its activator Tiam1, have been implicated in the regulation of junctional complexes.
This study now furthers our understanding of the spatiotemporal control of Rac signalling at junctional complexes. The Cell Signalling Group identified β2-syntrophin, part of a larger cell adhesion complex, as a binding partner of Tiam1. Furthermore, they demonstrated that β2-syntrophin promotes the activation of Rac via Tiam1 and also limits the diffusion of Tiam1 and Rac towards the apical domain. In parallel, Par-3, associated with tight junctions, inhibits Tiam1. The combined actions of Par-3 and β2-syntrophin establish a gradient of Rac concentration and activity required for correct formation and function of tight junctions and for the generation of apicobasal polarity.

Interestingly, reduced membrane β2-syntrophin was found to correlate with human prostate cancer progression. Therefore, reduced membrane β2-syntrophin and/or overexpression of Tiam1 and Rac, all frequently observed in tumours, could contribute to the deregulation of adhesion and polarity and promote tumourigenesis.

De Piccoli G, Katou Y, Itoh T, Nakato R, Shirahige K, Labib K.
Replisome stability at defective DNA replication forks is independent of S phase checkpoint kinases. ***Mol Cell***, 2012, 45(5):696-704.

Defects in DNA replication are thought to drive the early stages of cancer development and are retained in some mature tumours. It is therefore very important to understand the pathways that allow cells to survive such 'DNA replication stress', as drugs targeting these pathways might kill cancer cells preferentially. The S-phase checkpoint pathway is central to this regulation and is conserved from yeasts to humans. Defects in DNA replication lead to activation of the ATR kinase, which then activates the downstream kinase Chk1. Acting together, these kinases preserve the functional integrity of the DNA replication machinery in ways that are still understood poorly. Previous work indicated that the checkpoint kinases were needed to preserve the stability of the replisome at DNA replication forks. In contrast, this study in *Molecular Cell* shows that replisome stability is actually independent of the S-phase checkpoint pathway. It now seems likely that the checkpoint kinases regulate replisome function rather than replisome stability, and the underlying mechanisms will be an important focus for future work in this area.

Grallert A, Connolly Y, Smith DL, Simanis V, Hagan IM.
The S. pombe cytokinesis NDR kinase Sid2 activates Fin1 NIMA kinase to control mitotic commitment through Pom1/Wee1. ***Nat Cell Biol***, 2012, 14(7):738-45.



The coordinated action Par3 (green) and Syntrophin (blue) on Tiam1 (red) sets up a gradient of Rac activity along the cell membrane, and it is this gradient which is crucial for the correct formation of tight junctions, disruption of which is linked to the development and progression of tumours. The Cell Signalling Group has been able to identify and subsequently disrupt this Rac gradient by interfering with the expression of Syntrophin, a novel regulator of Tiam1, and a potential marker for prognosis in prostate cancer. Image taken 6 hours after formation of the tight junctions.

Image provided by Andrew Porter from the Cell Signalling Group

A multitude of signalling pathways influence the timing of cell division by regulating the balance of Wee1 kinase and Cdc25 phosphatase activities towards the Cdk1/Cyclin B complex that promotes commitment to mitosis. While the identity of most of the key players has been known for over 20 years, the "how", "where", "when" and "why" of this activation remains unclear. This study describes how two highly conserved protein kinases control Wee1 activity to regulate the timing of mitotic commitment. The NDR kinase Sid2/Mob1 is activated roughly two thirds of the way through the G2 phase that precedes mitosis to promote the activity of the NIMA kinase, Fin1, towards a "Cell Geometry Network" (CGN). The CGN inhibits Wee1's repression of Cdk1/Cyclin B. The involvement of Sid2/Mob1 in controlling mitotic commitment was unanticipated as this kinase and its budding yeast counterpart have only been linked to the control of mitotic exit and cytokinesis in previous work. The links between the equivalent kinases (Nek2, Lats/Mob1) and cancer make translation of these findings from this model system into the more complex controls of human cell division an appealing proposition.

Hou JM, Krebs MG, Lancashire L, Sloane R, Backen A, Swain RK, Priest LJ, Greystoke A, Zhou C, Morris K, Ward T, Blackhall FH, Dive C.
Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. ***J Clin Oncol***, 2012, 30(5):525-32.

Lung cancer is the leading cause of cancer-related death worldwide. Small Cell Lung Cancer (SCLC) accounts for 10-15% of lung cancer cases and is characterised by early development of widespread metastasis. Patients with this disease initially respond well to conventional chemotherapy but fatal disease relapse is almost universal and often rapid. There are few biomarkers to guide patient management and serial tumour biopsies are very challenging. Circulating Tumour Cells (CTCs) are thus appealing as a readily sampled source of tumour cells before, during and after treatment. We posit that understanding CTC biology will aid efforts to combat this disease. This study explored the presence and prognostic value of CTCs in 97 SCLC patients. Using the CellSearch technology platform, we showed that CTCs were present in 85% of patients and that CTC number was reduced after one cycle of therapy mirroring the initial patient response. With careful consideration of biostatistics, we showed that patients with ≥ 50 CTCs in a 7.5ml blood sample at baseline had significantly worse progression free and overall survival than those with < 50 CTCs. We also showed that apoptotic CTCs were evident in 57% of patients; circulating tumour microemboli (CTM, clusters of CTCs) were present in 32% of patients and just one CTM in a patient's blood sample heralded worse prognosis. The authors hypothesise that tumour cells within CTM might have a survival advantage as they have not detected apoptosis in CTM. Moreover, unlike the majority of viable single CTCs, cells within CTM are not proliferative, based on Ki67 staining implying a relative resistance to the conventional chemotherapeutics used to treat SCLC. This translational research formed the basis for ongoing studies where the team are now making strides to perform single CTC and CTM molecular analysis with a view to the discovery of new drug targets and predictive biomarkers.

Lancrin C, Mazan M, Stefanska M, Patel R, Lichtinger M, Costa G, Vargel O, Wilson NK, Möröy T, Bonifer C, Göttgens B, Kouskoff V, Lacaud G.
GFI1 and GFI1B control the loss of endothelial identity of hemogenic endothelium during hematopoietic commitment. ***Blood***, 2012, 120(2):314-22.

Recent studies have established that most, if not all, blood cells are generated from specific types of endothelial cells with haematopoietic potential, i.e. haemogenic endothelium cells. The transcription factor RUNX1 is a frequent target of gene rearrangements and mutations in human acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). Consistent

with its initial implication in leukaemias, RUNX1 is critical for normal haematopoietic development and in particular for this process of endothelial to haematopoietic transition (EHT). However the precise identities and specific roles of RUNX1 downstream effector genes remain largely unknown.

In this study, the Stem Cell Biology Group reported the identification of the transcriptional repressors GFI1 and GFI1b as critical targets of RUNX1 and establish their crucial function in regulating this trans-differentiation from endothelial to blood cells. GFI1 and GFI1B are able to trigger, in the absence of RUNX1, the down-regulation of endothelial markers and the formation of round cells, a morphological change characteristic of this cell fate switch. Conversely, in *Gfi1* and *Gfi1b* deficient embryos, the first generated blood progenitors maintain the expression of endothelial and cell adhesion molecules. These cells are therefore hampered in their ability to be released in the vasculature and to be disseminated in the yolk sac and in the embryo-proper.

The group demonstrates a critical and specific role of the GFI1 transcriptional repressors in the generation of haematopoietic progenitors from haemogenic endothelium. The results suggest that the EHT could be decoupled firstly into repression of the endothelial identity controlled by Gfi1s repressors and in parallel into activation of the haematopoietic cell fate programme. These new insights could result in new strategies to generate blood cells for regenerative medicine from embryonic stem cells or from induced pluripotent stem cells.

Latypov VF, Tubbs JL, Watson AJ, Marriott AS, McGown G, Thorncroft M, Wilkinson OJ, Senthong P, Butt A, Arvai AS, Millington CL, Povey AC, Williams DM, Santibanez-Koref MF, Tainer JA, Margison GP.
Atl1 Regulates Choice between Global Genome and Transcription-Coupled Repair of O^6-Alkylguanines. ***Mol Cell***, 2012, 47(1):50-60.

Alkylating agents are mutagenic, carcinogenic and toxic and we are exposed both exogenously, for example during certain types of cancer chemotherapy, and endogenously, the origins of which are currently not defined. One of the most genotoxic lesions that is generated by these agents is O^6-alkylguanine. In humans, and many other organisms, this damage is repaired by O^6-alkylguanine-DNA alkyltransferase (AGT), which stoichiometrically reverses the damage by transferring the alkyl group to a cysteine residue in the active site of the protein. Recently, the Carcionogenesis Group used the human AGT as a model to interrogate a *Schizosaccharomyces pombe* database and discovered a protein that resembles AGT, but without the critical cysteine residue. Originally, they demonstrated that this alkyltranserase-like (ATL) protein, Atl1, binds to O^6-alkylguanines and flags them for processing by the nucleotide excision repair (NER) pathway. Now the team show that binding affinity varies according to the nature of the alkyl group and the relative ease of dissociation of Atl1 from DNA containing some O^6-alkylguanines allows completion of NER, whereas strong binding of Atl1 to other O^6-alkylguanines stalls transcription and diverts the damage to the transcription-coupled NER pathway. These findings raise the question of whether or not O^6-alkylguanine lesions that are poor substrates for human AGT might, by analogy, signal such lesions for repair by NER.

Castro FV*, McGinn OJ*, Krishnan S, Marinov G, Li J, Rutkowski AJ, Elkord E, Burt D, Holland M, Vaghjiani R, Gallego A, Saha V, Stern PL.
5T4 oncofoetal antigen is expressed in high risk of relapse childhood pre-B acute lymphoblastic leukemia and is associated with a more invasive and chemotactic phenotype. ***Leukemia***, 2012, 26(7):1487-98.

Although the overall prognosis in childhood acute lymphoblastic leukaemia (ALL) is good, outcome after relapse is poor. Recurrence is frequently characterised by the occurrence of disease at extramedullary sites such as the central nervous system and gonads. Subpopulations of blasts able to migrate to such areas may have a survival advantage and give rise to disease recurrence. In this collaboration between the Immunology and Children's Cancer groups, gene expression profiling of diagnostic pre-B-ALL bone marrow samples revealed higher 5T4 oncofoetal antigen transcript levels in cytogenetic high-risk subgroups of patients. Flow cytometric analysis determined that bone marrow from relapse patients have a significantly higher percentage of 5T4 positive leukaemic blasts than healthy donors. 5T4 has also been shown to regulate CXCL12 chemokine and Wnt signalling pathways which are important in leukaemia growth and spread. For example, CXCL12 is naturally produced by various tissues of the body and controls the distribution of different types of immune cells but can also act as a "magnet" for leukaemia cells. Only 5T4+ve B-ALL cells show CXCL12 specific chemotaxis in vitro and this can be blocked by a monoclonal antibody (mAb) to 5T4 but not HLA.

In a xenograft model 5T4+ve, compared to 5T4-ve, B-ALL cells showed differential spread to the omentum and ovaries following intraperitoneal inoculation; this could also be blocked by mAb5T4. Consistent with this, the 5T4+ve B-ALL cells show increased invasion in vitro concordant with increased LFA-1 and VLA-4 integrin expression, adhesion to extracellular matrix and secretion of matrix metalloproteases (MMP-2/-9) compared with their negative counterparts. The xenograft model system was used to show that 5T4+ve B-ALL are susceptible to 5T4 specific superantigen antibody-dependent cellular toxicity providing support for targeted immunotherapy in high risk pre-B-ALL patients. The results of this study are consistent with the hypothesis that 5T4 is a marker of leukaemic cells which are relatively resistant to chemotherapy including through an increased ability to migrate to tissue sites which provide for disease relapse following treatment. If 5T4 marks the most drug resistant B-ALL then 5T4 directed therapies could provide a rational and effective way to treat such patients.

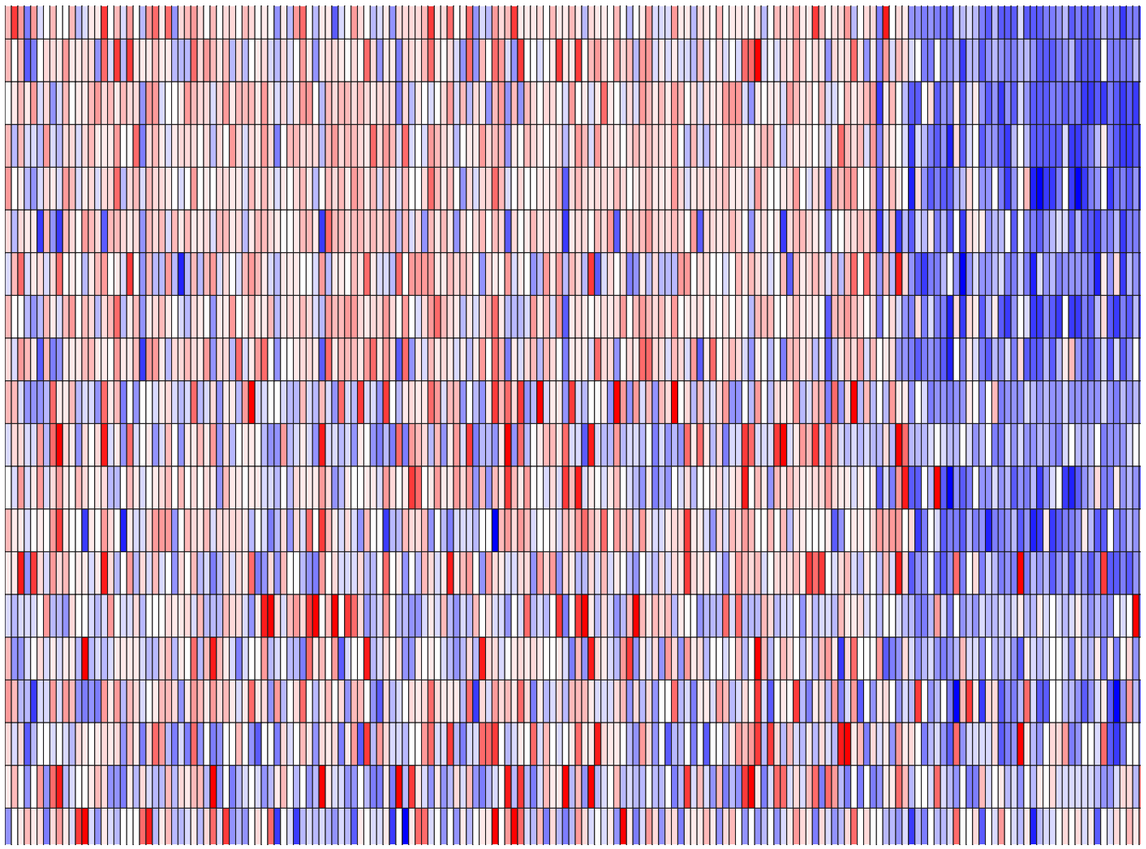
Dovedi SJ, Melis MH, Wilkinson RW, Adlard AL, Stratford IJ, Honeychurch J, Illidge TM.
Systemic delivery of a TLR7 agonist in combination with radiation primes durable anti-tumor immune responses in mouse models of lymphoma. ***Blood***, 2012, Oct 18, doi: 10.1182.

Radiotherapy plays an important part in the local control of many lymphomas and leads to extremely high response rates even in patients that are refractory to conventional chemotherapy approaches. The cell death caused by radiation therapy has the potential to stimulate immune responses against the cancer cells. However, these immune responses tend to be weak and insufficient to improve a patient's outcome. Combining radiation therapy with a drug that can stimulate the immune system has the potential to generate durable and effective anti-cancer immune responses capable of eradicating widespread malignant disease and reducing disease recurrence.

Using a synthetic agonist of TLR-7 which activates a systemic immune response by mimicking a viral infection, the Targeted Therapy Group found that the anti-tumour efficacy of radiation therapy can be enhanced in pre-clinical models of lymphoma. Combination therapy but not single-agent treatment resulted in long-term clearance of tumour and enhanced survival. This response was

dependent upon the activation of CD8+ cytotoxic T-cells, as depletion of these immune cells rendered the combination ineffective.

Moreover, the CD8+ T cells provided a durable response capable of protecting against disease recurrence. These data reveal that combination therapy with radiation and a TLR7 agonist is able to generate tumour-specific immunological memory. These findings demonstrate the potential for novel therapeutic combination approaches involving radiotherapy and immunotherapy for the treatment of cancer.



Transcriptional profiling was used to identify genes up-regulated upon activation of stress signalling. The heat map depicts the expression of stress-responsive genes in a panel of breast tumours versus normal tissue samples, (blue colour indicates under-expression). The impaired expression of this set of genes occurs in a range of tumour types, suggesting a suppressive role for this pathway in tumour development.

Image provided by Steve Lyons from the Cell Regulation Group.

THE PATERSON INSTITUTE FOR CANCER RESEARCH

RESEARCH GROUPS

APPLIED COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

www.paterson.man.ac.uk/bioinformatics

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[3] joint with Stem Cell Biology
[4] joint with Stem Cell Haematopoiesis
[5] joint with Cell Division
[6] embedded by Drug Discovery
[7] joint with IT department

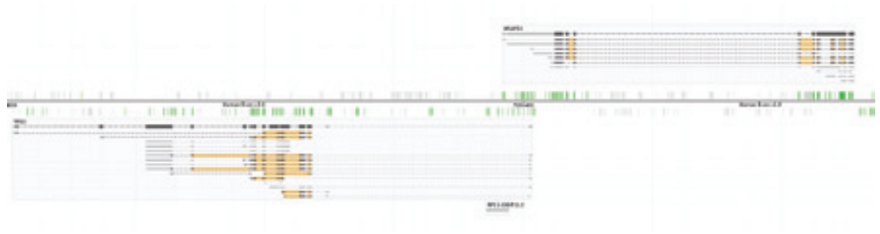
We are interested in how genes influence the way tumours grow and develop, and how the behaviour of genes differs between tumour and normal cells. Whilst most genes encode protein sequences, nearly forty percent of human genes are transcribed but never translated into proteins. Our research is focussed on developing a better understanding of how these non-coding genes function, and how their activity is altered in cancer cells. We are doing this by applying a mixture of computational biology, bench- and clinical-science, in an interdisciplinary programme centred on high throughput genomics technologies that include deep sequencing and tandem mass spectrometry.

In parallel, the group collaborates with many other groups in the Institute and the wider Manchester Cancer Research Centre (MCRC) to provide computational input into their research programmes (see for example the work by Harris *et al* in the Leukaemia Biology group described on p36). This last year has seen a significant increase in demand for support as groups exploit the power of our microarray, deep sequencing and mass spectrometry platforms. These fields are all moving rapidly, and the complexity of the datasets they generate often demands novel analyses, resulting in many projects requiring substantial research-level computational biology. We have met this demand with the development of a model in which Postdoctoral Fellow-level analysts develop extended collaborations with other research groups, allowing them to become immersed in the research question, and leading to a much deeper contribution than would be possible with a more traditional 'analysis-as-service' approach. This allows us not only to make use of the latest techniques emerging from the computational biology research community, but also to develop the novel algorithms and software tools we need to perform these analyses (Figure 1). We are active contributors to the Bioconductor project – an international collaboration to develop open source software packages for the analysis of biological data, and this is the primary route by which we make our software available to the wider community. Underpinning all of our research is a computational platform that includes a High Performance Computing (HPC) Linux cluster and associated Lustre file system. Programmers in the group have developed pipelines to process our deep sequencing and proteomics data, and Bioconductor packages that help support our downstream analysis. The goal is to take novel methods developed as part of our research, and turn them into tangible software tools when they will be used to support frequent analyses across multiple datasets. Since much of our research is directed at generating a better understanding of the less well-characterised regions of the genome (see below), the major computational focus of the group is on developing tools that allow fine-grained representations of gene structure to be integrated with statistical methods (Figure 2). This then allows these annotations to be used to provide detailed context when interpreting high throughput genomics datasets, and to help bring the data that emerges from different technologies together into a single integrated analysis. The group therefore sits at the interface of biology, mathematics and computing, and comprises a highly interdisciplinary team that incorporates both 'wet' and 'dry' science.

Novel non-coding regulators of gene expression

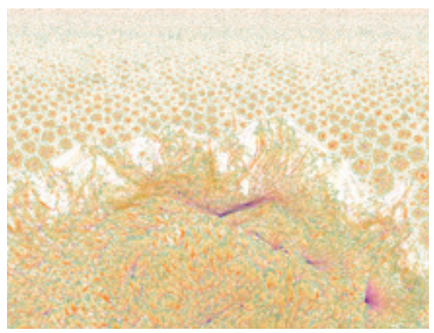
Current research in the group is investigating the role of non-coding RNAs in regulating gene expression, and builds on previous work using *Schizosaccharomyces pombe* (fission yeast) in which we identified sets of *cis*-acting non-coding RNAs that are differentially expressed as fission yeast undergoes meiosis. We used strand specific sequencing of total RNA first to reannotate the genome to include accurate representations of each gene's untranslated regions (UTRs), and then to explore how patterns of RNA abundance changed over the course of meiosis. When we did this, we identified a set of non-coding RNAs opposite protein coding genes that are critical regulators of sexual differentiation, leading us to speculate that they might act to control the activity of these key proteins. In collaboration with Cell Division (p20), we were able to show that this was indeed the case: over-expression of an Antisense Regulatory Transcript (ART) integrated ectopically into the genome phenocopied a deletion of the corresponding protein-coding gene. We also showed that their function was dependent on components of the RNA interference (RNAi) pathway, an aspect of the fission yeast genome that is conserved with *H. sapiens*, and suggesting that similar mechanisms might occur in human cells. Many of these transcripts arise from overlapping 3' UTRs of convergent adjacent gene pairs transcribing towards one another, and we were able to confirm that genes in this configuration can indeed co-regulate to modulate the expression of their neighbours.

Figure 1
Annmap (annmap.picr.man.ac.uk) is a genome browser based on the Google Maps API. Each grey box represents a gene, with individual transcripts shown as horizontal tracks within. Coding exons are black, UTRs, grey. Regions encoding protein domains are identified in yellow. Green lines represent target locations for Affymetrix Exon 1.0ST microarrays. Associated with the genome browser is a Bioconductor package that provides programmatic access to the same annotation data, allowing it to be brought into the statistical context provided by Bioconductor and the R programming language.



Nearly 40% of known human genes are non-coding, and whilst the majority have yet to be assigned a function, an increasing body of research is showing that they can interact with DNA, with proteins and with other RNAs to modulate and control their function. Although it is not clear what proportion of these newly discovered non-coding RNAs are functional, rather than simply corresponding to background 'chatter' in the genome, their prevalence, and their ability to act through a diversity of mechanisms, raises the possibility that they may have a profound impact on our understanding of genes and gene expression. We are using high throughput genomics tools, including deep sequencing and quantitative protein mass spectrometry, to identify novel non-coding RNAs, to investigate the function of existing ones, and to search for those with behaviour that is altered in tumour cells. This work therefore integrates experimental and computational approaches and makes use of both in vitro and clinical datasets.

Figure 2
Underpinning much of our work are genome annotation databases that link genes to the RNA and protein molecules they encode. The figure shows just a tiny subset of the millions of relationships we need to record. We use computer software to help interpret these networks.



Analysis of archival material

Archival Formalin Fixed Paraffin Embedded (FFPE) tissue is an immensely valuable source of information pertaining to cancer. Unfortunately, the preservation process damages RNA, making it hard to use these samples as a source for systematic analysis of gene expression profiles, and difficult, therefore, to use this material in global genomic studies. The development of successful methods for measuring global RNA abundance in these samples, and for performing strict Quality Control, would be extremely beneficial. We have been collaborating with Translational Radiobiology (p58), to develop methods to support the analysis of RNA from FFPE samples. We have previously shown that it is possible to generate meaningful gene expression data from archival material, however in some samples, the quality of RNA is too poor to be amenable for further study. Recently we showed that miRNAs, a type of short non-coding RNA, are less susceptible to the effects of preservation in FFPE than longer mRNA transcripts, and can be used to generate meaningful data from FFPE samples even when the mRNA has deteriorated beyond the point of utility (Hall JS, Taylor J *et al.* 2012 *British Journal of Cancer* 107(4):684-694). These, plus other advances in both biochemistry and bioinformatics, are raising the prospect of generating useful classifiers and biomarkers from archival material, further unlocking the potential of this enormously valuable resource.

Publications listed on page 68

CARCINOGENESIS

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[1] left in 2012

[2] Jointly with Dr Andy Povey, Health Sciences group at the University of Manchester

The consumption of red meat is a risk factor in human colorectal cancer (CRC) causation, possibly by enhancing the nitrosation of bile acid conjugates, which rapidly convert to DNA-damaging carcinogens. Indeed, the toxic and mutagenic DNA adduct, *O*^{6}-carboxymethylguanine (*O*^{6}-CMG), is frequently present in human DNA, increases in abundance in people with high levels of dietary red meat and may therefore be an important factor in CRC. Using synthetic oligodeoxyribonucleotides containing *O*^{6}-CMG, we now show that, contrary to our previous suggestion, the human DNA repair protein, *O*^{6}-methylguanine-DNA methyltransferase (MGMT), is very effectively inactivated by *O*^{6}-CMG in vitro and this involves the transfer of the carboxymethyl group to its active site cysteine residue. *O*^{6}-CMG is therefore an MGMT substrate and hence MGMT is likely to be an important protective factor in CRC.

Background

Most sporadic colorectal cancers arise through an adenoma-carcinoma sequence, the molecular pathways of which have been well characterised. Known risk factors for CRC include dietary red and processed meat and while the mechanisms by which these factors modify CRC risk remain to be fully elucidated, one possibility is that such diets increase *N*-nitrosation reactions within the colon. *N*-nitrosation of compounds containing amino groups, such as bile acid conjugates, can result in the formation of alkylating agents that can be potent mutagens, clastogens and carcinogens. These genotoxic effects of alkylating agents can be attributed largely to their ability to alkylate DNA and the biological properties of some of the DNA adducts formed, especially *O*^{6}-alkylguanine lesions, are well-characterized. Thus *O*^{6}-methylguanine (O^6-MeG) is a known toxic, mutagenic and carcinogenic base modification in DNA which, in the absence of repair, can induce GC→AT transition mutations and recombination events in the form of sister chromatid exchanges. More than a decade ago, we showed that almost all human colorectal DNA samples contain *O*^{6}-MeG, the levels of which vary 100-fold, with the highest occurring in the sigmoid colon and rectum, where most sporadic tumors occur. In addition, others have shown that exfoliated colon cell DNA contains *O*^{6}-CMG, probably arising from *N*-nitrosation of the bile cojugate, glycocholic acid, which also generates *O*^{6}-MeG in DNA. That the levels of *O*^{6}-CMG in DNA from exfoliated colonic cells is increased by diets high in red meat diet strongly implies its role in CRC.

Processing of mutagenic lesions in DNA

O^6-MeG is eliminated from DNA by the DNA repair protein, *O*^{6}-methylguanine DNA methyltransferase (MGMT), in a stoichiometric process that results in the transfer of the methyl group to Cys145 in the active site of the protein. In human colorectal mucosa, MGMT activity is highly variable, partly a consequence of MGMT polymorphisms, which have been found to modify CRC risk depending upon the diet.

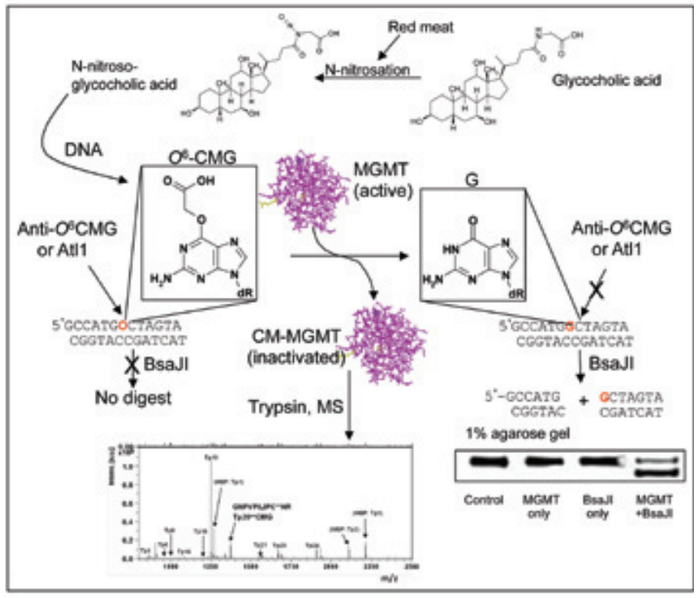

Figure 1
Strategies for demonstrating that *O*^{6}CMG is a substrate for MGMT. At the top, glycocholic acid is nitrosated to N-nitrosoglycocholic acid, a process that is enhanced by red meat consumption. N-nitrosoglycocholic acid is metabolised by cytochrome P_{450} enzymes and gives rise to a carboxymethylating agent that damages DNA to produce *O*^{6}-CMG (and *O*^{6}-MeG, not shown). When present in short ODN, this can be detected by binding with anti-*O*^{6}-CMG antibodies or Atl1 (results not shown) and also blocks digestion with BsaJI. Purified active MGMT (in pink) removes the carboxymethyl group to restore guanine (G) in the ODN, which now becomes digestible by BsaJI giving rise to two small fragments that can be resolved from undigested ODN using agarose gel electrophoresis. The repaired ODN no longer binds anti-*O*^{6}-CMG antibodies or Atl1. Finally the inactivated carboxymethylated MGMT is digested with trypsin and the active site peptide, GNPVPILIPCHR, is shown to contain carboxymethylcysteine based on its mass to charge ratio.

Furthermore, colorectal tumours often occur in gastrointestinal regions expressing low MGMT activity, and low activity in normal colon tissue has been associated with the presence of K-*ras* GC→AT transition mutations in colorectal tumours. In addition, cytosine-methylation of CpG islands within the promoter region of the MGMT gene is associated both with reduced MGMT expression and with an increased frequency of GC→AT transition mutations in K-*ras* in CRCs. Indeed, adenomas containing a K-*ras* GC→AT mutation have lower MGMT levels (relative to adjacent normal tissue) than adenomas without this mutation. As MGMT removes *O*^{6}-alkylguanine lesions from DNA, these observations strongly support the hypothesis that alkylating agents are involved in the aetiology of at least a proportion of CRC.

While MGMT is known to have very broad substrate specificity, our previous work using cell-free extracts of *E. coli* overexpressing the *E. coli O*^{6}-alkylguanine-DNA alkyltransferase-encoding genes, *ada* or *ogt* and a human cell line expressing endogenous MGMT suggested that alkyltransferases do not act on *O*^{6}-CMG in DNA (Shuker and Margison GP. *Cancer Res.* 1997, 57: 366-369). Given the compelling evidence for a role for MGMT in protecting against CRC, this would suggest that *O*^{6}-CMG is unlikely to be a significant factor in CRC risk, despite the observation that its abundance increases in high-risk diet situations.

Revisiting the substrate specificity of MGMT

To address this apparent inconsistency, in collaboration with David Williams at the University of Sheffield, we have synthesised short oligodeoxyribonucleotides (ODNs) containing *O*^{6}-alkylguanines and used these as substrates for purified *E. coli* Ogt and MGMT proteins in the strategies outlined in Figure 1. We found that an *O*^{6}-MeG-containing ODN inactivated both alkyltransferases with similar efficiency. However, while the *O*^{6}-CMG-containing ODN was a very poor substrate for Ogt, it was a very effective inactivator of MGMT, with an IC50 (1.8nM) very close to that of the *O*^{6}-MeG-containing ODN (0.93nM). That MGMT actually removed the carboxymethyl group from the *O*^{6}-CMG-containing ODN was shown in enzyme-linked immunosorbent assays (ELISAs) in which ODN were bound via terminal biotin linkers to streptavidin-coated microtitre plates. The *O*^{6}-CMG lesions were detected using polyclonal antibodies that we raised to this adduct, and also using the *S. pombe* alkyltransferase-like protein Atl1, which we have previously described to bind strongly to all DNA-*O*^{6}-alkylguanines that we have so far assessed, including *O*^{6}-CMG. We confirmed that the carboxymethyl group had been removed from the *O*^{6}-CMG by exploiting our observation that this lesion blocked digestion by the restriction endonucleases, BsaJI and PstI, when located in the recognition sequences of these enzymes. Incubation of these ODN with MGMT converted the lesion to guanine, and allowed digestion by these enzymes, clearly demonstrating that repair was by damage reversal. Finally, in collaboration with Clare Eyers at the University of Manchester, mass spectrometric analysis of tryptic digests of the MGMT following incubation with the *O*^{6}-CMG ODN showed the presence of the CM group in S-carboxymethylcysteine within the active site cysteine residue contained in a tryptic peptide. Control experiments showed methyl group transfer from ODN containing *O*^{6}-MeG.

These findings clearly demonstrate that *O*^{6}-CMG is as good a substrate for MGMT as *O*^{6}-MeG, and connect increased red meat consumption with low levels of MGMT as combined critical factors in human colorectal carcinogenesis.

Publications listed on page 68

CELL CYCLE

www.paterson.man.ac.uk/cellcycle

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[1]left in 2012

Our group studies the mechanisms and regulation of chromosome replication and cytokinesis, defects in which drive cancer development. Drugs targeting these pathways are effective against a variety of tumour cells, so that a better understanding of these areas of cell biology should aid the development of novel anti-cancer treatments.

A highlight of our work with chromosome replication this year was the identification of a novel role for the Mcm10 protein during the initiation of chromosome replication. The Cdc45-MCM-GINS DNA helicase is needed to unwind the parental DNA duplex, and we discovered a novel Mcm10-dependent step during the activation of Cdc45-MCM-GINS at DNA replication origins. In our cytokinesis studies, we showed that the Inn1 protein is an essential activator of chitin synthase in budding yeast. Our work suggests how novel anti-fungal therapies could be developed against important human pathogens such as *Candida albicans*, which affect immuno-compromised people including cancer patients.

Mcm10 defines a novel step during activation of the essential DNA helicase at eukaryotic DNA replication origins

In all eukaryotic cells, the major regulated step during the initiation of chromosome replication is the assembly at replication origins of the 11-subunit DNA helicase known as Cdc45-MCM-GINS, which unwinds the parental DNA duplex at DNA replication forks. The Mcm2-7 complex forms the catalytic core of the helicase, and is loaded around double strand DNA at origins during the G1-phase of the cell cycle, as an inactive double-hexameric ring. When cells enter S-phase, activation of cyclin-dependent kinase (CDK) and Cdc7 kinase (also known as Dbf-dependent kinase or 'DDK') leads to the recruitment of Cdc45 and the four-protein GINS complex to the pre-loaded Mcm2-7 double-hexamer, producing the Cdc45-MCM-GINS helicase that unwinds the origin DNA and allows the establishment of two replication forks (Figure 1). By developing a novel 'degron' allele of budding yeast Mcm10, Frederick van Deursen showed that origin unwinding was blocked very efficiently upon inactivation of Mcm10, but assembly of Cdc45-MCM-GINS was just as efficient as in control cells. It thus appears that Mcm10 is dispensable for assembly of Cdc45-MCM-GINS and instead defines a novel step during helicase activation at replication origins.

Future work will be needed to establish exactly how Mcm10 helps activate Cdc45-MCM-GINS, but we found that Mcm10 interacts preferentially with the loaded double-hexamer of inactive Mcm2-7, and an exciting possibility would be that Mcm10 modulates the loaded Mcm2-7 complex in some way during the initiation reaction. Work from other groups indicated that Mcm2-7 is initially loaded around double-strand DNA at origins, but it seems likely that it subsequently encircles single-strand DNA at replication forks. This suggests that the Mcm2-7 ring must transiently open and close during the activation of the Cdc45-MCM-GINS helicase, and Mcm10 is an excellent candidate for a factor that facilitates this step. Intriguingly, earlier studies searched for mutations in yeast genes that could suppress defects in Mcm10 function, and identified mutations in the Mcm2 subunit of the Mcm2-7 complex, at sites predicted to be at an interface with adjacent subunits. It is possible that these mutations weaken an inter-subunit interface in such a way as to mimic the action of Mcm10 during the initiation of chromosome replication.

Inn1 regulates chitin synthase during cytokinesis in budding yeasts

We originally discovered the budding yeast Inn1 protein as a novel factor that is essential for cytokinesis. In animal cells and in yeasts, cytokinesis is mediated by a contractile ring of actin, type II myosin and many other factors, which assembles under the plasma membrane at the cleavage site and is activated at the end of mitosis, causing ingression of the plasma membrane and thus producing division of the cytoplasm. Unlike animal cells, however, the plasma membrane in yeasts is surrounded by a cell wall, which means that contraction of the actomyosin ring and ingression of the plasma membrane must also be coupled to the deposition of primary and secondary septa. When cytokinesis is complete, digestion of the primary septum induces cell separation and the formation of two independent daughter cells. Inn1 associates with the actomyosin ring and is required for membrane ingression and septum formation. We showed previously that the key to Inn1 function during cytokinesis is the recruitment to the bud-neck of an essential 'C2-domain' at the amino terminus of the Inn1 protein, since mutations in the C2-domain block cytokinesis, whereas fusion of the C2-domain to other cytokinesis factors supports cell proliferation in the absence of the endogenous *INN1* gene. The critical issue was thus to define how the C2-domain acts.

C2-domains are formed by a sandwich of two Beta-sheets, one side of which has protruding loops that interact with lipid or protein targets. We found that lysine 31 in 'loop 1' of the C2-domain of Inn1 is conserved in all orthologues of Inn1 and is essential for cytokinesis, suggesting that this site makes an important contact with a key target of Inn1. To identify this factor, Asli Devrekanli screened during her PhD studies for mutations in other budding yeast genes that could suppress the lethal effects of the *inn1-K31A* mutation (Figure 2). In this way she identified a large number of dominant mutations that are extremely efficient suppressors of *inn1-K31A*, allowing cells to grow in a very similar fashion to control cells. Remarkably, all these suppressor mutations mapped to the *CHS2* gene, which encodes the essential chitin synthase that makes the primary septum during cytokinesis in budding yeast. Most importantly, the suppressor mutations map to two adjacent sites in the catalytic domain of Chs2, which flank a predicted binding site for the substrate, UDP-N-acetylglucosamine. These findings suggested that the mechanism of suppression might involve an alteration in the catalytic activity of Chs2, further suggesting that Inn1 might normally act by regulating the catalytic activity of Chs2 during cytokinesis.

Unlike Chs3 that makes the majority of the chitin in the yeast cell wall, Chs2 is normally inactive when assayed in purified cell membranes, indicating that its function is carefully regulated in vivo and must be stimulated during cytokinesis by a previously unknown mechanism. Critically, we found that the suppressor alleles of Chs2 are constitutively active in vitro. Moreover, we showed that Inn1 associates with Chs2 from yeast cell extracts. Overall, these data indicate that the C2-domain of Inn1 regulates Chs2 activity during cytokinesis, in order to stimulate the formation of the primary septum behind the contracting actomyosin ring. Our findings also show how cell-based screens could be developed for small-molecule inhibitors of Inn1 function. Such molecules should block cytokinesis in wild type budding yeast cells, but should be much less effective against the suppressor alleles of *CHS2*. Both Inn1 and Chs2 are conserved in other budding yeasts including *Candida albicans* and *Cryptococcus neoformans*, indicating that small-molecule inhibitors of the Inn1-Chs2 interface would provide a novel therapy that should be effective against these important human pathogens.

Publications listed on page 69

Figure 1

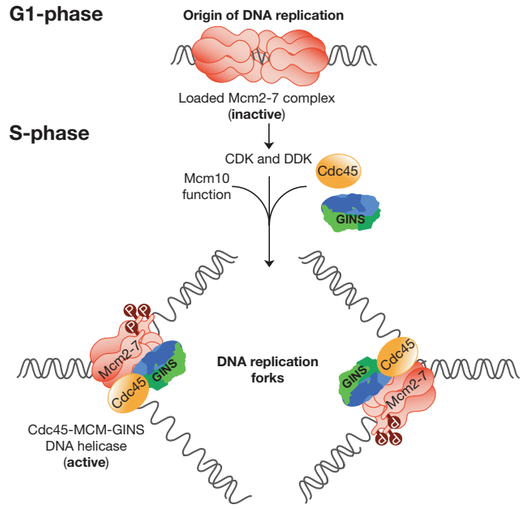

Figure 2

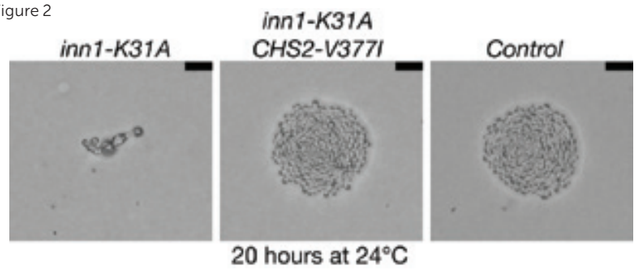

Figure 1
Mcm10 is required for activation of the Cdc45-MCM-GINS DNA helicase during the initiation of chromosome replication. The Mcm2-7 complex is loaded at replication origins during G1-phase as an inactive double hexamer, which is then activated in situ when cells enter S-phase. Previous work showed that the initiation step involves recruitment of GINS and Cdc45 to form the Cdc45-MCM-GINS complex. Our work indicates that Mcm10 is dispensable for Cdc45-MCM-GINS assembly, but is needed for a novel step during activation of the Cdc45-MCM-GINS helicase at replication origins.

Figure 2
Inn1 activates Chs2 during cytokinesis in budding yeasts. Mutation of lysine 31 of Inn1 in the C2-domain blocks cytokinesis, so that germinating spores die as chains of a few cells. Mutations at a predicted substrate-binding site in *CHS2* suppress the lethal phenotypes of *inn1-K31A*, allowing cells to grow in a very similar fashion to the control. The scale-bars denote 20μm.

CELL DIVISION

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[2]joint with ACBB

Errors in chromosome transmission alter the balance between tumour suppressor and tumour promoting genes. This imbalance favours changes in genome composition in the ensuing cell divisions that can lead to cancer.

Chromosome segregation during mitosis is initiated by the attachment of the microtubules of the mitotic spindle to the chromosomes. Once all chromosomes have become attached to both spindle poles the chromosomes split into two identical chromatids that then move to opposite poles. Because the regulatory networks that regulate mitotic commitment and progression are highly conserved, studying the complexities of cell division in the relatively simple unicellular yeasts greatly accelerates the analysis of the more complex issue of cell division control in man.

We use the fission yeast *Schizosaccharomyces pombe* to study cell division because it is a simple, unicellular organism with excellent genetics that is cheap to grow and divides rapidly. Our major activity asks how cells take the decision to divide? We also collaborate with the Applied Computational Biology and Bioinformatics Group to use fission yeast as a model organism in which to develop approaches for the interrogation of genome function on a global scale with a particular focus on the role played by the function of non-coding RNAs in regulating cellular differentiation and stress responses.

Mitotic commitment

Commitment to mitosis is regulated by the activity of a protein kinase called MPF. MPF is composed of a Cdk1 catalytic subunit and a regulatory subunit Cyclin B. Prior to mitosis MPF is inhibited via phosphorylation on a residue (tyrosine 15) within Cdk1's ATP binding pocket. This phosphate is removed by Cdc25 phosphatase. The balance of Cdc25 and Wee1 activities determines when MPF will be activated to drive mitotic commitment. Once a critical threshold level of MPF is reached a positive feedback loop is triggered that boosts Cdc25 and suppresses Wee1 activities, thereby driving full-scale commitment to mitosis. Fully activated MPF then activates a number of highly conserved kinases including members of the Polo, Aurora and NIMA families of kinases.

Cut12, the spindle pole and mitotic commitment

Our studies of the spindle pole body (SPB) component Cut12 have uncovered a critical role for events on the spindle pole in mitotic control. Specifically, they suggest that the MPF amplifying positive feedback loop is primed from the SPB. The foundations for this view lie in the reciprocal genetic interactions between *cut12* and *cdc25*. The *cut12.s11* gain of function mutation suppresses loss of function mutations in *cdc25*. Conversely, enhancement of Cdc25 activity suppresses loss of Cut12 function.

In seeking ways to understand how an SPB component could compensate for loss of Cdc25, we drew upon the observation that removal of Wee1 function enables cells to survive without Cdc25 as there is no requirement for a phosphatase to remove a phosphate from Cdk1 if the kinase that puts this phosphate there is absent. Because Polo kinase plays a key role in the MPF positive feedback loop, we considered the hypothesis that Cut12 suppresses ablation of Cdc25 as it inappropriately prompts Polo to shut down Wee1. We found a direct relationship between Polo activity and Cut12 status; Polo activity is elevated when Cut12 function is enhanced and severely reduced when Cut12 function is compromised (Figure 1).

We are now exploiting a range of approaches to identify the means by which a structural component of the spindle pole can exert such a strong impact upon the mitotic commitment

Figure 1

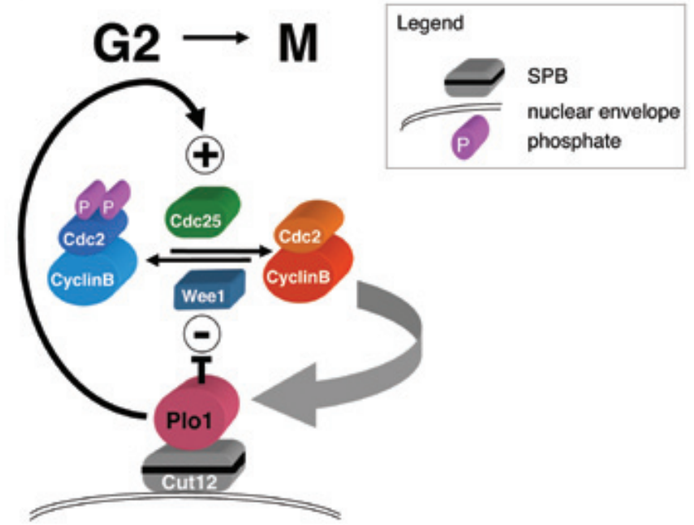

Figure 2

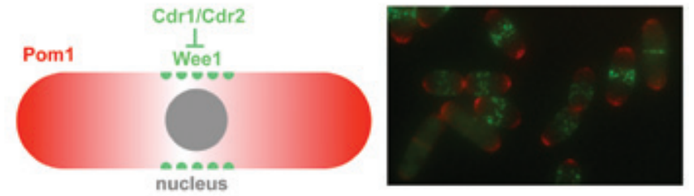

Figure 1 - Cut12, Polo and the mitotic commitment switch. The dephosphorylation of Cdc2/Cyclin B that promotes mitotic commitment is accelerated through phosphorylation by the polo kinase Plo1. This active Plo1 also inhibits the Wee1 kinase that puts these phosphates onto Cdc2. Crucially, this entire control only operates once Cdc2/CyclinB is active, making it a feedback control that ensures a rapid and complete transition from interphase into mitosis. Recruitment of Plo1 to the spindle pole by Cut12 appears to be critical for this control.

Figure 2 - The Cell Geometry Network (CGN). Sequestration of the DRYK (Dual-specificity tyrosine-regulated kinase) Pom1 to cell tips (red) establishes a gradient of Pom1 activity. This gradient can inhibit the Cdr1/Cdr2/Wee1 complexes that accumulate on centrally positioned nodes (green) more efficiently in shorter cells than it can in longer cells. Thus, the CGN couples mitotic commitment to increased cell length.

switch. We have used mass spectrometry to identify 25 sites of phosphorylation on Cut12 and are following this up by assessing the consequences of blocking or mimicking phosphorylation on these sites. We are also collaborating with the group of Boris Maček of the Proteome Center, Tübingen, to use global phosphoproteomic approaches to identify sites of phosphorylation that are reduced by loss of polo function or enhanced in a *cut12.s11* background. We have also been assessing the consequences of targeting active Cdc2 or Plo1 to different locations within the cell. Encouragingly, we can only trigger mitotic commitment when we target either kinase to the spindle pole and no other location tested.

Work from the group of Professor Jon Pines (Gurdon Institute, Cambridge) has shown that active MPF first appears on human centrosomes, strongly suggesting that the networks we are studying in yeast occur in human cells. In other words, key decisions about whether to divide or not do not arise from the gradual accumulation of a "pro mitosis" state, rather, they are taken at a discrete location, the spindle pole. This concentration of signalling to a limited subset of molecules at a discrete location facilitates rapid and highly sensitive cross talk between different signalling networks.

Control of mitotic commitment by a "mitotic exit" kinase

We have been studying the function of the fission yeast NIMA kinase, Fin1, in the control of the activity of the "Cell Geometry Network" (CGN). This network ensures that cells do not inhibit Wee1 kinase to promote cell division before they have reached a critical cell size. Pom1 kinase inhibits the activity of the Cdr1 and Cdr2 kinases towards Wee1 so that the ultimate impact of Pom1 kinase is to delay mitotic commitment by delaying time at which inhibition of Wee1 by Cdr1 and Cdr2 is relieved. Pom1 accumulates at cell tips, while the Cdr1/Cdr2/Wee1 kinases associate with cortical nodes at the cell equator. In short cells the gradient of Pom1 kinase activity reaches and inhibits Cdr1/Cdr2 at the nodes thereby blocking commitment to mitosis. When growth moves the Pom1 gradient away from the nodes, Pom1 inhibition of Cdr1/Cdr2 is relieved and they inhibit Wee1 to promote mitosis (Figure 2).

Fin1 activity restrains CGN activity towards Wee1, resulting in an acceleration of the timing of mitotic commitment. Fin1 activity towards the CGN is promoted by a protein kinase called Sid2. Sid2 is a member of the NDR (nuclear Dbf2-related) kinase family. Prior to our study, the activity of Sid2 had only been associated with events during mitotic exit where it controls cytokinesis (the splitting of one cell into two). This demonstration that a key component of the mitotic exit machinery also controls mitotic commitment suggests that there maybe a greater level of co-ordination between mitotic entrance and exit pathways than has been appreciated previously.

Lessons from yeast

The ability to manipulate genes at will in a simple organism whose primary purpose is simply to grow and divide is enabling us to explore the finer points of the pathways that co-ordinate growth with spatial and environmental cues. This information informs studies in higher systems that, in turn, raise models that can be most readily tested in yeast. This re-iterative cycle of comparative studies ensures that great strides are being made in understanding the molecular basis of cell division and growth.

Publications listed on page 69

CELL REGULATION

www.paterson.man.ac.uk/cellregulation

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The Cell Regulation Lab is addressing molecular biological questions centred around MAP kinase signalling pathways and downstream transcription factors, in particular members of the AP-1 family. Over the years our group has identified and characterised components of these pathways involved in development, homeostasis as well as cellular response to growth and stress inducing stimuli. We also found that these pathways have important and diverse roles in the initiation of tumorigenesis as well as in the response to therapeutic treatment. We have addressed these functions using biochemical and genetic approaches and have employed genetic model systems including mouse and fission yeast. Importantly, these diverse model systems have shown a remarkable degree of conservation in specific signalling pathways and have provided a powerful tool to address fundamental questions about stress signalling.

MAP kinases of the JNK and p38 family are active in response to cytotoxic and genotoxic stimuli, including ionising radiation, ROS, and chemotherapeutic drugs. JNK and p38 can activate apoptosis by directly modulating the mitochondrial apoptotic pathway as well as by regulating the activities of downstream transcription factors, including members of the AP-1 family. AP-1 is a dimeric complex composed of proteins belonging to the JUN, FOS, ATF, and MEF families of B-ZIP transcription factors. Different AP-1 complexes regulate a vast number of target genes involved in cell growth, differentiation, but also cell cycle arrest and apoptosis. The transcription factors ATF2 and ATF7 are highly homologous members of the AP-1 family. Increasing evidence shows that JNK- and p38-dependent pathways are frequently modulated in tumours. Our long term goal has been to identify key biological activities that are mediated by the JNK-ATF2/7 and p38-ATF2/7 signalling axes during cellular stress and oncogenic transformation.

Suppressive functions of ATF2/7 in oncogenic Ras mediated cellular transformation

In tumorigenesis, ATF2 can exert pro-tumorigenic but also anti-tumorigenic activities depending on the tumour type (Gozdecka and Breitwieser, 2012). Therefore one focus of our research has been to decipher these context dependent activities. In a current project we adapted a mouse model of hepatocellular carcinoma (HCC), whereby oncogenic HRas transformed hepatoblasts, proficient or deficient in ATF2/7 functions, are transplanted orthotopically and develop primary liver tumours. As a result, we found that ATF2/7 double mutant hepatoblasts show a significantly stronger tendency to develop into HCC in recipient livers compared to ATF2 active controls. We also found that active ATF2 induces cell death in transformed hepatoblasts in culture and reduces colony formation in soft agar. These activities appear to be dependent on the activation of an upstream kinase (JNK) because expression of a phosphorylation deficient form of ATF2 (ATF2-AA) fails to rescue the ATF2 loss of function mutant. Furthermore, we find that expression of active JNK had similar suppressive activities during oncogenic transformation of hepatoblasts and that these activities strictly require ATF2/7. Therefore, tumour suppressive functions involving JNK mediated pathways are at least in part dependent on ATF2/7 transcriptional activities. Using microarray analysis we identified ATF2 target genes that may be involved in tumour suppression. In meta-analysis of tumour expression data we find that down-regulation of many ATF2/7 targets correlates with tumour status.

ATF2/7 roles in Myc transformed B cells and B lymphoma

In samples of human B cell lymphoma cell lines, we found that ATF2 as well as JNK MAP kinase are significantly up-regulated compared to normal human B cell lines. This was particularly the case in Burkitt's lymphoma and other lymphoma types, typically associated with the activation of the c-Myc transcription factor. To analyse the potential interaction between activated c-Myc and the JNK-ATF2 signalling pathway we developed a mouse model in which ATF2 and 7 were specifically deleted in the B cell compartment and tested this in the presence of B cell specific overexpression of c-Myc (Eμ-Myc). As with the human B lymphoma samples, we found that mouse lymphomas induced by Myc overexpression also showed strongly enhanced activation of JNK as well as of ATF2. Furthermore, B cell specific loss of ATF2/7 resulted in a significant acceleration of Eμ-Myc induced lymphoma onset suggesting that the JNK-ATF2/7 pathway is engaged in tumour suppressive activities in a Myc overexpression context.

To further characterise these activities we derived cell lines from Myc induced primary lymphomas and induced the deletion of ATF2 in vitro using Cre/loxP mediated recombination. Here we found that while loss of ATF2/7 in B lymphoma cells did not affect growth in culture, they showed remarkably reduced levels of spontaneous apoptosis as well as reduced levels of apoptosis induced by growth factor withdrawal or treatment with genotoxic drugs, e.g. doxorubicin or mitoxantrone. It has previously been shown that while forced Myc expression induces proliferation of lymphoma and other cells it also sensitises cells to stress induced apoptosis. Our findings therefore suggest that JNK and ATF2/7 may be at least partially responsible for the apoptotic arm of Myc activities and that this could underlie the observed tumour suppressive activities of ATF2/7 in the mouse B lymphoma model. In an analysis of ATF2/7 dependent transcription regulation we showed that transcriptional targets include other AP-1 factors, including c-Jun and ATF3 as well as at least one member of BH3 domain containing apoptotic regulators, Hrk (Walczynski et al., accepted for publication in Oncogene, 2013).

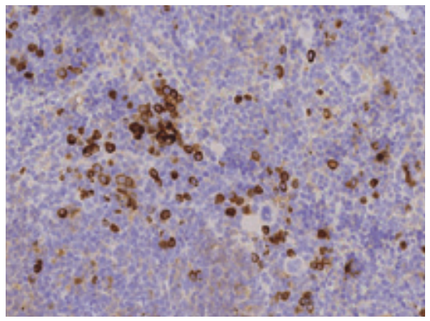

Figure 1
Apoptotic (Caspase 3) staining of Myc induced mouse lymphomas.

Oncogenic Ras regulated microRNAs involved in suppression of transformation and drug induced apoptosis

Over the past few years it has become apparent that non coding RNAs display important functions in the regulation of gene expression and modulation of signalling pathways. In an approach to identify new mechanisms involved in oncogenic Ras-mediated signalling, we identified a number of microRNAs that were specifically down regulated in their expression in response to activated HRas. Upon further characterisation we found that a number of these miRs, including miR99 and miR335 are involved in the suppression of Hras mediated transformation, for example by reducing colony formation in soft agar. Furthermore, ectopic expression of miR335 leads to enhanced apoptosis in response to genotoxic drugs such as cisplatin. To evaluate this role for miR335 in a clinically relevant setting we analysed ovarian tumour cell lines for the expression of miR335 and found that while a number of these show strong resistance to cisplatin induced cell death they also have very low expression of miR335. In contrast re-expression of miR335 strongly re-sensitises cisplatin resistant cell lines to the cisplatin-induced apoptosis. A typical mode of evasion to drug sensitivity by tumour cells is by modifications in the methylation status of the genome. Interestingly, we found that treatment of drug resistant cells with demethylating agents led to enhanced expression of miR335 in correlation with their increased sensitisation to cisplatin treatment. We therefore aim to further test the possibility that at least one mechanism of acquired drug resistance in tumours is by silencing the expression of microRNAs including miR335. We are also exploring the targets of mir335 involved in cisplatin induced cellular responses.

Publications listed on page 69

CELL SIGNALLING

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Tumour initiation and progression result from the inappropriate activity of intracellular signalling cascades. Rho-like GTPases are molecular switches in signalling pathways that regulate cell morphology, adhesion, motility, as well as cell cycle progression and survival. Data has emerged to directly implicate Rho proteins in tumourigenesis. We investigate the mechanisms by which certain regulators of the Rho-like GTPase Rac control cell cycle progression and cell adhesion and how their activities, as well as activity of Rac itself, are controlled.

Rac1 cycles between a GDP- and a GTP-bound state. When GTP-bound, it interacts with various effector molecules that elicit downstream responses. Multiple mechanisms control Rac1 activity including control of nucleotide binding and hydrolysis by Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs) respectively, regulation of subcellular localization and modulation of Rac1 protein levels. More recently, regulation by post-translational modification has emerged as a significant means of regulating Rac activity.

Post-translational modifications of Rac1 during cell migration

To gain further insight into the regulation of Rac during cell migration, we performed a screen for proteins that interact with Rac following treatment of cells with a motility-inducing factor, Hepatocyte Growth Factor (HGF). This revealed the small ubiquitin-like modifier (SUMO) E3-ligase, PIAS3, as a novel Rac interacting protein. PIAS3 interacts better with GTP-bound Rac and is required for increased Rac activation and optimal cell migration in response to HGF. Subsequently we demonstrated that Rac1 can be conjugated to SUMO-1 in response to HGF and that the GTP-bound form of Rac is a better substrate for SUMOylation. Furthermore, we identified non-consensus sites within the polybasic region of Rac1 as the main location for SUMO conjugation. We demonstrated that PIAS3-mediated SUMOylation of Rac1 controls Rac1-GTP levels and the ability of Rac1 to stimulate lamellipodia, cell migration and invasion (Castillo-Lluva *et al.* Nat Cell Biol. 2010; 12:1078).

Rac1 activity is also regulated through ubiquitylation and subsequent degradation. However, the E3 ubiquitin ligase responsible for Rac1 degradation following activation by a migration stimulus was unknown. Recently, we identified this to be the tumour suppressor HACE1. We showed that HACE1 and Rac1 interaction is enhanced by HGF signalling and that HACE1 catalyses the poly-ubiquitylation of Rac1 at lysine 147 following its activation by HGF, resulting in its proteasomal degradation. HACE1-depletion is accompanied by increased total Rac1 levels and accumulation of Rac1 in membrane ruffles. Moreover, HACE1-depletion enhances cell migration independently of growth factor stimulation, which may have significance for malignant conversion. These findings identified HACE1 as an antagonist of cell migration through its ability to degrade active Rac1 (Castillo-Lluva *et al.* Oncogene 2012). Jointly the above two studies suggest that SUMOylation and ubiquitylation of Rac1 act coordinately to fine-tune Rac1 activity in migrating cells, promoting Rac activity at sites where the cell membrane is advancing, while antagonising Rac1 at sites where membrane protrusion needs to cease.

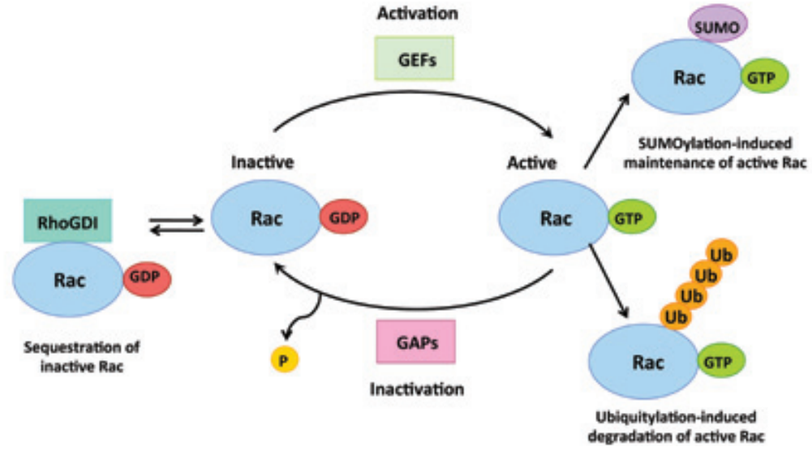

Figure 1
Multiple mechanisms exist to regulate Rac activity. The Rac GTPase cycles between inactive GDP-bound and active GTP-bound states. Rac activation is facilitated by the action of GEFs (such as Tiam1), which promote GDP dissociation from Rac and allow GTP to bind instead. Through the association with GAPs the intrinsic GTPase activity of Rac is accelerated thereby inactivating Rac. Through association with RhoGDIs Rac can be sequestered in its inactive state. Activated Rac can also be removed through ubiquitylation-induced degradation (mediated by HACE1 following a migration stimulus) or it can be maintained following its modification by SUMO (mediated by PIAS3).

Role of the Rac activator Tiam1 in cell-cell adhesion.

Tiam1 (for T-lymphoma invasion and metastasis protein) is a Rac specific GEF. Mice deficient for Tiam1 are resistant to the formation of skin tumours. However, the few tumours arising in these mice progressed more frequently to malignancy (Malliri *et al.*, Nature 2002; 417: 867). One mechanism by which Tiam1 and Rac suppress malignant progression is through promoting cell–cell adhesion. Over-expression of activated Rac or Tiam1 promotes the formation of adherens junctions (AJs) and an epithelial-like phenotype in a number of mesenchymal cell lines (Malliri & Collard, Curr Opin Cell Biol 2003; 15: 583). Moreover, Tiam1 is required for both the formation as well as maintenance of cadherin-based adhesions (Malliri *et al.*, J Biol Chem 2004; 279: 30092). The oncoprotein Src, a non-receptor tyrosine kinase implicated in malignant progression, potently induces epithelial–mesenchymal transition (EMT) by targeting AJs for disassembly. We recently showed that Src phosphorylates Tiam1 on tyrosine 384 predominantly at AJs during the initial stages of Src-induced EMT triggering the localised degradation of Tiam1 at AJs by calpain proteases. Abrogating Tiam1 phosphorylation and degradation suppressed Src-induced AJ disassembly and inhibited cell migration (Woodcock *et al.* Mol Cell. 2009; 33: 639).

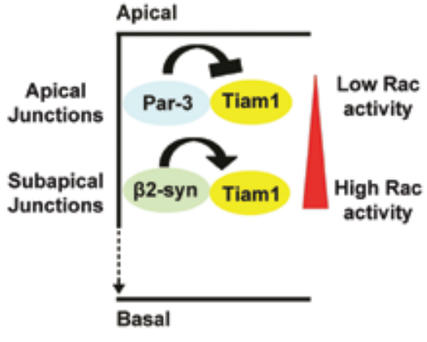

Figure 2
Model depicting the differential localisations of Par-3 and β2-syntrophin and their differential effects on Tiam1-Rac activity at cell-cell junctions.

To better our understanding of the contribution of Tiam1-Rac signalling to tumourigenesis, we further investigated its function at cell-cell adhesions. A screen we performed for Tiam1 interacting proteins identified β2-syntrophin as one of its binding partners. β2-syntrophin is a component of the dystroglycan adhesion complex. Our study (Mack *et al.* Nat Cell Biol. 2012; 14: 1169) unearthed a novel role for this complex in regulating the assembly of adherens junctions (AJ) and tight junctions (TJ) and the generation of apicobasal polarity through controlling Tiam1-Rac signalling. The mechanism we uncovered entails the generation of a Rac activity gradient in the membrane region encompassing these junctions, with lower Rac activity at apical TJ and higher Rac activity sub-apically. This gradient depends upon the ability of β2-syntrophin to stimulate Tiam1-Rac signalling at the sub-apical end and of the polarity determinant Par3 to inhibit Tiam1-Rac signalling at the apical end. By targeting constitutively active Rac to TJs which disrupts the gradient, we demonstrated that the gradient of Rac activity is required for optimal TJ assembly and the generation of apicobasal polarity. Finally, we found that reduced membrane β2-syntrophin correlates with human prostate cancer progression. We conclude that β2-syntrophin and Par-3 finely-tune Rac activity along cell-cell junctions controlling TJ assembly and the establishment of apicobasal polarity. Furthermore, we propose that deregulation of β2-syntrophin, Par-3, or Tiam1, would disrupt the Rac activity gradient, and in turn disrupt TJs and apicobasal polarity, thereby promoting tumourigenesis.

Publications listed on page 70

CLINICAL AND EXPERIMENTAL PHARMACOLOGY

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CEP validates and implements pharmacodynamic, prognostic and predictive biomarkers working in tandem with the Christie Cancer Treatment Centre that incorporates one of the largest early clinical trials units worldwide. This year we have focussed on enumeration and molecular analysis of circulating tumour cells; developed a suite of circulating nucleic acids biomarker assays, examined a large panel on angiogenesis associated circulating proteins at baseline in patients receiving drug regimens containing Avastin and the associated requirement of biomarker statistics.

We have also built an expanded pipeline of ongoing clinical trials in lung cancer patients incorporating circulating biomarkers. The highlight of 2012 was the award to Professor Dive of the prestigious Pasteur-Weizmann/ Servier International Research Prize and Tribute for her laboratory's research on non-invasive biomarkers to aid the treatment of cancer patients.

Building a portfolio of lung cancer trials incorporating biomarkers (Lead: Fiona Blackhall)

We have expanded our lung cancer trial portfolio this year to evaluate newly validated biomarker assays and in particular we have initiated new collaborations within the CR-UK Experimental Cancer Medicine Centre (ECMC) network (see Table 1). In collaboration with Corrinne Faivre-Finn, we are examining the impact of radiotherapy on circulating tumour cell (CTC) numbers in the CONVERT and MEKRT trials. Having demonstrated the high prevalence of CTCs in small cell lung cancer (SCLC) - Hou *et al* JCO 2012, see research highlights - we are now evaluating their utility in several clinical trials. In a new exciting collaboration with Immunogen and with a validated assay to measure CD56 (a neuroendocrine marker) in SCLC CTCs, we are deploying this assay on the North Trial evaluating a CD56 targeted therapy. Exciting data are emerging in collaboration with Dr Phil Crosbie on the detection of CTCs in the pulmonary vein of patients with resectable non-small cell lung cancer (NSCLC).

The translational research in the CTC team is increasingly integrated with that in the Nucleic Acids Biomarkers team as we seek to undertake molecular profiling of purified CTCs.

The Nucleic Acids Biomarkers (NAB) Team

The CEP Biomarker Portfolio has expanded to include a Nucleic Acids Biomarkers (NAB) team led by CEP Deputy Ged Brady. Over the last year, the NAB team has established a range of circulating biomarker assays including miRNA profiling of plasma samples and RNA profiling of single isolated cells. NAB assay development takes into consideration the requirement for simple sample processing amenable to upcoming multi-site clinical trials. Projects using next generation sequencing (NGS) of paired lung cancer patients' blood and tumour samples are underway within CEP and in collaboration with Peter Campbell at the Cancer Genome Project group at the Wellcome Trust Sanger Institute. A major focus of the NAB team has been to establish routine molecular analysis of CTCs that complements and expands on CTC analytical approaches established within CEP. Currently CEP makes extensive use of the Veridex CellSearch system which delivers reliable and informative enumeration of EpCam and Cytokeratin positive CTCs from patient blood samples. Over the last few months the NAB team established a single cell isolation and whole genome amplification (WGA) approach applicable to individual CTCs enriched by the Veridex CellSearch system. New collaborations have also been established with companies

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Project No	Project Name and Description	Biomarkers Studied
CEP073	**CONVERT** (Concurrent ONce-daily VErsus twice-daily RadioTherapy): A 2-arm randomised controlled trial of concurrent chemo-radiotherapy comparing twice-daily and once-daily radiotherapy schedules in patients with limited stage small cell lung cancer (SCLC) and good performance status.	DNA analysis from whole blood samples. CTC enumeration with assessment of Ki-67 and BCL-2 expression. Cytology on tumour biopsies.
CEP074	**CHEMORES** (Tumour Chemotherapy Resistance): Biomarker analysis of Molecular mechanisms of drug resistance in lung cancer.	Genomic and proteomic analysis of patient tumour and serum/plasma samples. Enumeration of CTCs.
CEP153	**RADAR** (Resistance and Damage to Radiotherapy in Lung Cancer): Lung Cancer Biomarkers of Response and Toxicity to Radiotherapy.	Measurement of the following by ELISA in patient plasma specimens: Ang1, Ang2, FGFb, HGF, PDGFbb, VEGFA, VEGFC, IL8, KGF, PlGF, VEGFR1, VEGFR2, Tie2, M30, M65, EGF, E Selectin, VCAM1, IL1b, Il6, IL10, IL12, TNFa, OPN, CA-IX, CYRFA. Measurement of Ki-67 in FFPE tumour blocks by IHC.
CEP155	**MEKRT**: Phase I trial of the MEK Inhibitor AZD6244 in combination with thoracic radiotherapy in NSCLC.	Measurement of K-RAS, pMAPK, & Ki-67 in tumour biopsy specimens using IHC. Assessment of circulating free DNA in blood specimens for K-RAS mutations. Measurement of the following in patient plasma specimens using ELISA: M30, M65, OPN, VEGF, PlGF, VEGF-R1 & R2.
CEP209	An investigation of blood-borne biomarkers of early stage lung cancer using differential analysis of pulmonary venous and arterial blood (with Dr Philip Crosbie).	Enumeration of CTCs.
CEP238	**STOMP**: Small cell lung cancer Trial of Olaparib (AZD2281) as Maintenance Programme.	Enumeration of CTCs. Plasma biomarkers (to be confirmed). cfDNA (to be confirmed).
CEP241	**VanSel-1**: A Cancer Research UK Phase I dose escalation trial of the oral VEGFR and EGFR inhibitor, Vandetanib in combination with the oral MEK inhibitor, Selumetinib in solid tumours (dose escalation) and NSCLC (expansion cohort).	Enumeration of CTCs. Depending on tissue available, measurement of the following markers in CTCs and/or tumour biopsy specimens: Phospho ERK, Total ERK, Ki67, Phospho EGFR, Total EGFR, Phospho AKT, Total AKT. Measurement of the following by ELISA in patient plasma specimens: VEGFR2, VEGF-A, M30, M65. Assessment of cfDNA for mutations in K-RAS & EGF-R.
CEP263	**MAPPING**: Double blind randomised phase III study of maintenance Pazopanib versus placebo in NSCLC patients non progressive after first line chemotherapy.	Enumeration of CTCs.
CEP264	ImmunoGen **NORTH** trial: The NORTH clinical trial is designed to assess the impact on patient outcomes of adding the experimental agent, IMGN901, to standard treatment for newly diagnosed SCLC.	Enumeration of CTCs with assessment of CD56 expression.

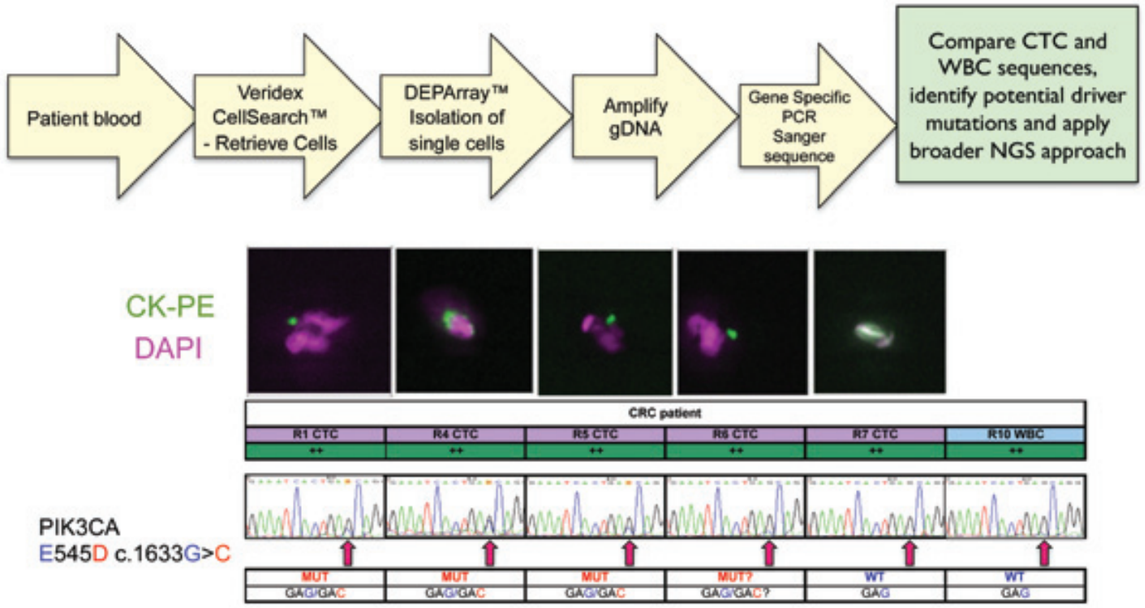

Figure 1
Schematic of the workflow used for isolation and genetic analysis of individual CRC CTCs. Images are of 5 putative CTCs stained for cytokeratin (CK-PE) and DAPI. Sanger sequencing data obtained from each of the 5 putative CTCs and WBCs from the same patient is shown where red arrows indicate the location of the heterozygous PIKC3A mutation observed.

developing novel CTC/blood biomarker technology platforms; in particular the acquisition of the Silicon Biosystems DEParray platform, which purifies single CTCs, has added enormous strength to our ability to deliver our ambitious goals. The single cell isolation approach has been applied to over 20 clinical samples and has led to the identification of mutations found in CTCs and not in healthy white blood cells (WBC) from the same patient (Figure 1). Currently the single cell WGA approach is being adapted to allow Next Generation Sequencing (NGS) of a large number of clinically relevant tumour 'driver' genes.

Predictive biomarkers in ovarian cancer for the anti-angiogenic drug Avastin

CEP works in tandem with the translational angiogenesis (TAG) group led by Professor Gordon Jayson. One of the most important conundrums regarding anti-angiogenic therapies such as Avastin is the absence of a predictive biomarker(s) to identify patients most likely to benefit, and to avoid drug-induced toxicity in those patients where benefit is unlikely. ICON 7 was a randomised two-arm Phase III trial in which patients with advanced ovarian cancer were treated either with bevacizumab or placebo, in addition to standard chemotherapy. Using the Aushon Biosystems (Boston, US) multiplex ELISA platform and CEP validated assays, we examined 15 proteins associated with angiogenesis (Ang1, Ang2, FGFb, GCSF, HGF, IL8, KGF, PDGFbb, PlGF, Tie2, VEGFA, VEGFC, VEGFD, VEGFR1 and VEGFR2) collected at baseline from a subset of 91 ICON7. Real progress with biomarker statistics has been made this year by Dr Andrew Renehan and Dr Cong Zhou in CEP along with the recent recruitment to the University of Manchester of Professor Carlo Benzuini. Together they conducted a thorough statistical analysis and identified Ang1 and Tie2 as candidate predictive biomarkers for Avastin in this patient cohort. The information provided by these two biomarkers translates to a clinical decision strategy that Avastin should be added to conventional chemotherapy for patients with high Ang1 levels and low Tie2 levels at baseline leading to

Debbie Burt and Jakob Chudziak using the DEPArray™ platform for CTC detection and manipulation.

significantly longer progression free survival (PFS) than seen with conventional treatment alone. The data also suggest that patients with high levels of both Ang1 and Tie2 at baseline should not be given Avastin. Further prospective trials are now under discussion to confirm the predictive biomarker utility of Ang1 and Tie2 for Avastin treatment and the TAG team will conduct 'reverse translation' research to investigate the molecular mechanism(s) that underpin these clinical observations.

The CEP/AstraZeneca Serological Alliance

Since 2006 an Alliance has been in place between AstraZeneca (AZ) and CEP to advise on, analyse, report, and interpret data obtained on circulating biomarker assays validated for clinical use in CEP's Good Clinical Practice Laboratories (GCPL). The AZ/CEP Alliance has grown yearly to encompass a broad range of biomarkers (cell death, invasion, angiogenesis, CTCs, cfDNA) supporting the exploratory biomarker requirements of AstraZeneca's oncology portfolio. Over the past two years, in addition to an ongoing high throughput biomarker analysis component, the Alliance has drawn upon the wider expertise of CEP to deliver several bespoke biomarkers for clinical trial deployment. As one example we are developing a multi-marker immunofluorescence assay to investigate the levels of androgen receptor (AR) protein expression together with epithelial and mesenchymal markers in prostate cancer CTCs to evaluate CTC heterogeneity and inform on CTC utility for AR directed therapy. A second example is the development of serum biomarker assays to support development of a pan-Erb inhibitor. Highly sensitive ELISAs are enabling circulating levels of the ErbB ligands to be measured in cancer patients' plasma with the hypothesis that the baseline levels of these ligands may predict for response to inhibitors of Erb pathway signalling.

Our biomarker alliance was expanded and renewed in September 2012 (£3.1M) under the guidance of Carl Barrett (AZ Boston). The new Alliance remit allows a more diverse range of biomarkers to be investigated, from nucleic acid based work with Ged Brady's NAB team, through tissue biomarkers, to further bespoke biomarker work including preclinical projects that map to our focus on SCLC.

Publications listed on page 70

DNA DAMAGE RESPONSE

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Many cancer therapy procedures, such as radiotherapy and some types of chemotherapy, work by overwhelming the capacity of the cell to repair DNA damage, resulting in cell death. Most rapidly dividing cells - cancer cells - are preferentially affected by such treatments, providing the opportunity to use DNA damaging agents to selectively kill cancer cells. In addition, genomic instability is the driving force of cancer development, which requires multiple DNA mutations resulting in loss of cellular growth control. In order to accelerate the accumulation of genetic changes, cancers often sacrifice specific DNA repair pathways. This can make cancer cells additionally susceptible to DNA damaging agents and/or to inhibitors that block alternative repair pathways. For these reasons, studying the protein components involved in the repair of damaged DNA has proven to be a valuable strategy in searching for novel approaches and targets in cancer therapy.

Poly(ADP-ribosyl)ation in regulation of DNA repair

Poly(ADP-ribosyl)ation is a post-translational protein modification that controls several nuclear processes known to be important for genome stability, including DNA repair, regulation of chromatin structure, cell cycle checkpoint activity, transcription, apoptosis and mitosis. Poly(ADP-ribose) is a highly negatively charged polymer that is formed from repeating ADP-ribose units linked via glycosidic ribose-ribose bonds, and is synthesised by the poly(ADP-ribose) polymerase (PARP) family of enzymes using a vital cellular cofactor NAD+ as a substrate. The reversion of poly(ADP-ribosyl)ation is performed by the hydrolytic action of an enzyme called poly(ADP-ribose) glycohydrolase (PARG), which specifically targets ribose-ribose bonds and cleaves poly(ADP-ribose) into ADP-ribose monomers. The role of poly(ADP-ribosyl)ation is best understood in the regulation of DNA repair, which is controlled by the three PARPs responsive to DNA strand breaks (PARP1, PARP2 and PARP3). Poly(ADP-ribosyl)ation arising at the sites of damaged DNA serves as a platform for specific recruitment and scaffolding of DNA repair complexes. In addition, the damage-induced poly(ADP-ribosyl)ation has a role in relaxation of chromatin structure and in apoptotic signalling. The recent development of potent PARP inhibitors provides powerful tools to study pathways regulated by poly(ADP-ribose), as well as providing a promising novel class of drugs for cancer treatment. Specifically, selective inhibition of the DNA break repair pathway using permeable PARP inhibitors has proven highly effective against certain breast and ovarian cancers (Bryant *et al*, Nature 2005). Thus, understanding the molecular basis of poly(ADP-ribose)-dependent DNA repair processes is likely of vital importance for selecting and developing efficient therapies.

Identification and characterisation of novel poly(ADP-ribose)-regulated factors

Our laboratory is particularly interested in identification of novel DNA repair pathways and protein functions regulated by poly(ADP-ribosyl)ation in order to identify components of these pathways that can be exploited as targets for cancer therapy. For this, we have been screening for proteins that have the ability to respond to DNA damage in a manner that is blocked by treatment with clinically relevant PARP inhibitors such as olaparib. Our goal is to characterise some of the obtained candidate proteins and elucidate their exact biochemical functions in DNA repair, as well as their mode of regulation in response to DNA damage.
Recently, in screening for proteins with the ability to bind poly(ADP-ribose), we discovered a poly(ADP-ribose)-binding zinc finger motif (PBZ). PBZ is a structurally distinctive, atypical type of zinc finger that is associated with several proteins involved in response to DNA damage (Ahel *et al*, Nature, 2008). One of the human proteins containing a PBZ motif is a protein called Checkpoint protein with FHA and RING domains (CHFR). CHFR is an ubiquitin ligase frequently inactivated in human epithelial tumours, which acts as a key regulator of the poorly understood early mitotic checkpoint that transiently delays chromosome condensation and nuclear envelope breakdown in response to a variety of stresses. The elucidation of the function of the PBZ motif gave us a vital clue to discover that the CHFR-dependent checkpoint is regulated by PARPs and that the PBZ motif in CHFR protein is critical for checkpoint activation. Another PBZ-regulated protein we are studying is a protein called Aprataxin-PNK-like factor (APLF). APLF uses tandem PBZ repeats for direct interaction with poly(ADP-ribosyl)ated PARP1, which allows APLF's timely localisation to the sites of DNA damage. We recently discovered that the role of APLF is to act as a histone chaperone to modulate chromatin structure and facilitate DNA repair reactions in response to poly(ADP-ribose) signalling (Mehrotra *et al*, Mol Cell, 2011).

Another class of DNA damage response proteins on which we focus our research is the macrodomain proteins. The macrodomain is another evolutionary widespread module with the capacity to bind poly(ADP-ribose) and we recently identified several human macrodomain protein factors that are recruited to broken DNA ends in a poly(ADP-ribose)-dependent manner. These include a histone H2A variant called MacroH2A and several other uncharacterised macrodomain proteins.

Structural and functional analysis of poly(ADP-ribose) glycohydrolase (PARG) and its validation as a target for cell-permeable inhibitor design

Available data indicates that inhibiting PARG might offer a promising and beneficial approach in the treatment of cancer and cardiovascular conditions. However, unlike the case of PARP inhibitors, progress in developing permeable, small-molecule PARG inhibitors has been limited, partly due to the lack of functional and structural data for the human PARG protein. Recently, we solved the crystal structures of several PARG enzymes from bacteria and lower eukaryotes, which gave the first insight into the basic principles of PARG structure and its mechanism of catalysis (Slade *et al*, Nature, 2011; Dunstan *et al*, Nat Commun, 2012) (Figure 1). These structures revealed that the PARG catalytic centre is a diverged type of macrodomain and demonstrated that they are likely to prove useful in guiding structure-based discovery of new classes of PARG inhibitors. Despite these advances, structural information on human PARG is still lacking. Our goal is to solve the structures of human PARG in complex with substrate analogues and inhibitors which in combination with solution and cell biology studies, should address the mechanism, structure and regulation of human PARG, as well as providing a foundation for the development of small, cell-permeable PARG inhibitors.

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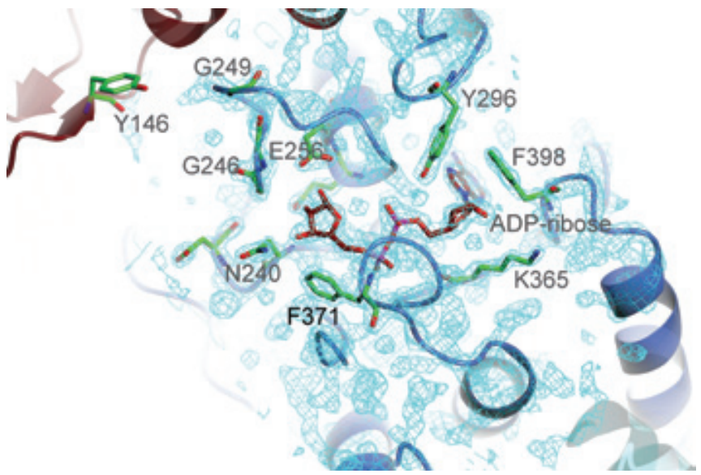
Figure 1
Active site of *Tetrahymena thermophila* PARG enzyme with bound ADP-ribose (in red).



DRUG DISCOVERY

www.paterson.man.ac.uk/drugdiscovery

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[1]joined in 2012

[2]joint with ACBB

During 2012 we have further enhanced the Drug Discovery team in key areas, specifically by developing our bioinformatics platform and strengthening our chemistry efforts. Most significantly, we have progressed one of our DNA repair projects into the lead identification stage, while the rest of our portfolio has also advanced. In addition we currently have four hit-to-lead projects aimed at a variety of cancer targets. These activities are underpinned by multiple collaborations both within and beyond the Manchester Cancer Research Centre.

People

One of the most challenging aspects of drug discovery is target selection. We have therefore strengthened our links to Crispin Miller's Applied Computational Biology and Bioinformatics group by jointly recruiting Phil Chapman, a skilled bioinformatician with drug hunting experience. Phil will focus on the target selection process and will help build our target-related interactions with groups inside the Paterson Institute and with other Cancer Research UK funded drug discovery groups. As our portfolio expands we have further strengthened our synthetic and medicinal chemistry team by welcoming Kristin and Colin, two new team members with significant industry experience. We have also said goodbye to Laura, one of our synthetic chemists who left us to pursue a different career path.

Infrastructure

In terms of infrastructure, the biggest change has been the addition of an Access Workstation to our existing Echo acoustic dispensing platform. The Access system (shown in Figure 1) has allowed us to fully automate compound and reagent dispensing for our in vitro and cellular assays. The advantages of this increased efficiency are several fold; first the accurate dispensing allows us to save on valuable compounds and expensive reagents, secondly it gives us the ability to effectively screen medium sized (15-20K) compound libraries. Most importantly, the system frees our highly skilled biologists from routine tasks allowing them to concentrate on more challenging biological issues. All of this combines to make us more efficient and effective in our search for new drugs delivering patient benefit.

Drug discovery targets

We continue to review many cancer drug target opportunities and, during 2012, have worked closely with several Paterson Institute Group Leaders (John Brognard, Tim Somervaille, Ivan Ahel and Karim Labib) in the identification, validation and prosecution of putative drug targets. In 2012 we have also focussed on expanding our interaction with industrial drug hunting companies. By collaborating early with large and medium-sized pharmaceutical companies and within the Biotech arena we hope to find partners who will help us take our projects through the development stage and deliver benefit to patients more quickly, leveraging extra value from our existing funding.

Hit finding

Once a target has been selected for drug discovery the next stage is to try and identify prototype small molecules, or "hits", that interact with the target molecule. However, not all interesting oncology targets are amenable to interaction with small molecules. Whilst certain target classes are known to be hard to drug, such as transcription factors, for many of the targets we discuss with local group leaders, there is no prior precedent in terms of anticipated "ligandability", i.e. whether a small molecule can interact with the target in a potent and selective fashion in order to cause the desired biological response.

Figure 1
Nicola Hamilton working at the Echo access station

To address this question, and to investigate possible early hit matter, we have invested time in developing a fragment-based ligandability assessment of novel targets. In this approach, we screen our fragment library of around 1200 compounds (kindly donated by our sister drug discovery unit at the Beatson Institute) against novel targets in a representative biological assay system. The resultant hit profiles allow us to assess the likelihood of finding new hit matter against the target. Those with higher hit rates and with a unique "fingerprint" of hits (as depicted in Figure 2) are proposed to be more amenable to finding potent and selective hits through additional hit finding approaches such as High Throughput Screening (HTS). Through this we intend to judge the relative chemical risk of new projects, compared to those already under active investigation. We have spent some time building confidence in this approach by comparing our ligandability assessments against our historical successes (and failures) with HTS and are ready to deploy this platform, to make real decisions as to whether new targets displace those in our existing portfolio, early in 2013.

Project portfolio

Our current portfolio includes one lead identification project (DNA repair) and four hit identification projects against metabolic, epigenetic, redox modulation and oncogene signalling targets. For the most advanced project, HTS screening has identified hit compounds whose in vitro potency has been improved a thousand fold by our chemists. The pharmacology of these more potent compounds is currently being explored in cells, both internally and through key expert collaborators. Additionally, another of these projects is close to meeting our requirements for progression to the lead identification stage. In support of all of our projects, we have initiated a range of target biology and technology-related collaborations both within and beyond the MCRC.

Cancer Research UK

We remain actively involved in the broader Cancer Research UK drug discovery activities and interact with most of the Cancer Research UK drug discovery units. During the summer of 2012 we underwent a successful annual review with the Drug Discovery Advisory Group.

The future

During 2012 we have expanded our team and progressed our project portfolio into the lead identification stage. Increasingly, new project opportunities are arising out of our collaborations with Paterson Institute scientists. In 2013 we look forward to progressing at least one more internal project to the next stage of the drug discovery process and maintaining our focus on the ones most likely to deliver patient benefit We would also hope to announce at least one partnership with a large pharmaceutical company.

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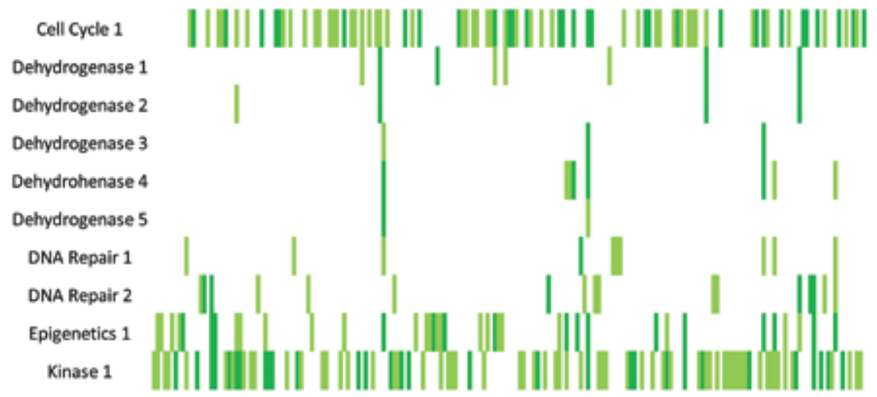
Figure 2
"Ligandability" profiling of our current active and early portfolio. Each bar in the heatmap represents a fragment "hit" against the target. Light green bars represent weaker hits and darker bars represent the stronger hits. This allows a fast, visual representation of hit patterns, to determine outline "ligandability". For example, closely matching our experimental observations, our epigenetics and DNA repair targets appear to be more chemically tractable than those in our dehydrogenase areas of interest.



IMMUNOLOGY

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[3] with Andrew Renehan

We have established that 5T4 oncofoetal glycoprotein expression is associated with epithelial mesenchymal transition, CXCL12 chemokine and Wnt signalling pathway modulation in embryonic and human cancer cells. 5T4 promotes CXCL12/CXCR4 chemotaxis but when absent, CXCL12 uses the alternative receptor CXCR7 which promotes proliferation or anti-apoptosis. 5T4 also inhibits Wnt/β-catenin canonical while concomitantly activating the non-canonical Wnt signaling pathway associated with increased motility. It is likely that the integrated 5T4 regulation of these pathways acts to promote cancer spread as well as functional migration in development. New 5T4 antibody based therapies are in development to target this functional tumour associated molecule.

5T4 and CXCR4/ CXCR7 mediated biological responses in human cancer cells

CXCL12 is a pleiotropic chemokine capable of eliciting multiple signal transduction cascades and functions, via interaction with either CXCR4 or CXCR7. Factors that determine CXCL12 receptor preference, intracellular signalling route and biological response, are poorly understood but are of central importance in the context of therapeutic intervention of the CXCL12 axis in multiple disease states. We have recently demonstrated that 5T4 oncofoetal glycoprotein facilitates functional CXCL12/ CXCR4 mediated chemotaxis in mouse embryonic cells. Using wild type (WT) and 5T4 knockout (5T4KO) murine embryonic fibroblasts (MEFs), we have now established that CXCL12 binding to CXCR4 activates both the ERK and AKT pathways within minutes, but while these pathways are intact they are non-functional in 5T4KO cells treated with CXCL12. Importantly, in the absence of 5T4 expression, CXCR7 is upregulated and becomes the predominant receptor for CXCL12, activating a distinct signal transduction pathway with slower kinetics involving transactivation of the EGFR, eliciting proliferation rather than chemotaxis. Consistent with these observations we have identified 5T4/ CXCR7 reciprocity in human small cell lung carcinoma (SCLC) cell lines with the expression of 5T4 versus CXCR7 predictive of specific biological responses to CXCL12. Furthermore 5T4 KD in 5T4 positive SCLC cells led to loss of chemotactic responsiveness (McGinn *et al* 2012). Alteration of the receptor preference equilibrium of CXCL12 could dramatically change cellular behaviour in response to the chemokine. In a primary tumour, 5T4 surface expression by cells at the periphery would provide for chemotaxis to CXCL12 secreting endothelial cells and metastatic spread whereas in the centre of the tumour, preferential CXCR7 expression could detect lower levels of chemokine and promote cell growth.

5T4 and CXCR4 trafficking

Investigation of the trafficking mechanisms of 5T4 and CXCR4 in E6/E7 immortalised MEFs suggested that the two molecules employ distinct trafficking pathways for transport to the cell surface, constitutive and ligand induced endocytosis/turnover. 5T4 shows dependence on intact microtubules and actin cytoskeleton for endocytosis, whereas CXCR4 requires intact microtubules for expression at the cell surface. By overexpressing suitably tagged 5T4 and CXCR4 molecules in 293T cells, FRET techniques were used to investigate their molecular interactions. Together with the results from a proteomics study it seems likely

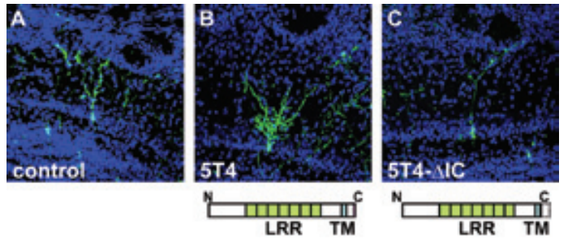

Figure 1
The 5T4 intracellular (IC) region is necessary for the dendritic arborisation. *A-C*, Lentiviral vectors carrying EYFP (*A*), 5T4-IRES-EYFP (*B*) and 5T4-ΔIC-IRES-EYFP (*C*) under the control of CMV promoter and expressed in interneurons in the olfactory bulb. Expression of the 5T4 construct gave more branched dendrites in the interneurons than the control EYFP, while expression of the IC domain-deletion construct (5T4-ΔIC) showed a similar level of branching numbers in the dendrites to the control EYFP. IRES; internal ribosome entry site, EYFP; enhanced yellow fluorescent protein.

that functional CXCR4 and 5T4 interactions may be achieved via additional molecules, likely including actin binding proteins. Consistent with this, 5T4 is found extensively in clathrin-coated pits, where it could form such a functional complex with CXCR4 prior to ligand stimulation with CXCL12. 5T4 influence on chemotaxis was further investigated exploiting 5T4 positive (Sup5T4) and negative (Sup) sub-lines established from the SupB15 B-acute lymphoblastic leukaemia. Only the Sup5T4 cells exhibit chemotaxis to CXCL12 and this correlated with the ability of Sup5T4 cells to desensitise and internalise CXCR4 more slowly and to a lesser extent than Sup cells, leading to prolonged signalling from the receptor (Castro *et al* 2012).

5T4 inhibits canonical but favours non-canonical Wnt signalling in human cancer cells

In the Wnt canonical pathway, a Wnt ligand binds to a Frizzled receptor/LRP5/6 complex leading to translocation of β-catenin from the membrane to the nucleus where it regulates the expression of target genes involved in cell cycle regulation through partnerships with TCF/LEF. This pathway is often mutated in cancer, leading to oncogenic transformation and increased proliferation. The non-canonical pathways do not involve β-catenin signalling and are activated by Wnt ligands binding to the Frizzled or ROR family receptors, producing a calcium flux in the $Ca2^+$ pathway and/or activating downstream effectors such as JNK in the planar cell polarity (PCP) pathway. The non-canonical pathway regulates gene expression through NFAT and actin cytoskeleton rearrangement leading to increased motility. Activation of non-canonical signalling, through the archetype non-canonical ligand Wnt5a or through disregulation of the canonical Wnt suppressor Dkk-1, is hypothesised to lead to a more invasive cancer phenotype; increased serum levels of these proteins has been correlated with poor cancer survival outcomes. We have previously shown in zebrafish embryogenesis that 5T4 expression acts as an inhibitor of the canonical Wnt pathway in Wnt-receiving cells (Dev. Cell 21: 1129-1143, 2011). 5T4 interacts with LRP6 and interferes with Wnt-dependent internalisation of LRP6, without affecting LRP/Frizzled interaction. 5T4 competition with Dkk1 for LRP6 binding provides Dkk1 to help activate the non-canonical Wnt/PCP pathway.

We have now shown that in 5T4 positive SKOV-3 human ovarian tumour cells, the canonical Wnt pathway is inhibited and the non-canonical Wnt pathway is active, potentiated by Dkk-1. In SKOV-3 5T4 KD cells, the levels of secreted Dkk-1 and phosphorylated JNK are reduced whereas β-catenin-dependent signalling and levels of phosphorylated LRP6 are increased. We have developed a mouse model which will enable us to determine whether this perceived "switch" in Wnt pathway activity has an effect on in vivo tumour growth and metastasis. Since the 5T4 intracellular domain is necessary and sufficient for the sensory input-dependent dendritic shaping in the $5T4^+$ interneuron granule cells of the olfactory bulb (Figure 1), we speculate that it may interact with Wnt signalling molecules to regulate the dendritic arborisation (Yoshihara *et al* 2012).

Gene expression profiling and development of a cell culture model of pseudomyxoma peritonei (PMP)

PMP is a rare neoplastic process characterised by progressive intra-abdominal dissemination of mucinous tumour, and generally considered resistant to systemic chemotherapy. In collaboration with Sarah O'Dwyer and Andrew Renehan (Peritoneal Tumour Service) and also supported by NORD/Christie Trust Charitable funds, we have determined the gene expression profile of PMP in comparison to normal colonic mucosa and established immortalised cell lines suitable for laboratory based investigations into PMP. Primary cultures of cells from PMP biopsies were established and subsequently immortalised with a SV40 T antigen lentiviral vector. They demonstrate epithelial morphology, expression of mucin 2, cytokeratin 20 and other PMP markers. For expression profiling, cells from PMP and normal colonic epithelium were harvested from each of three fresh frozen surgical biopsies using laser capture microscopy. Isolated RNA was hybridised to Affymetrix Human Exon 1.0 ST arrays and differentially expressed genes identified. In comparison to normal colonic mucosa, 34 and 27 genes were significantly altered, at least two log2 fold lower or higher in PMP respectively ($p< 0.05$ after adjustment for multiple testing). Many of these down- or upregulated genes have been associated with cancer biology (20, including 5 tumour suppressors), metabolism/ion transport (11) or cell migration (5). Several of these genes are current targets for drug development. The PMP gene profiles are being validated by various expression analyses in primary biopsies as well as by using our immortalised PMP cell lines. This work has provided important tools for preclinical development of improved therapies for PMP.

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INOSITIDE LABORATORY

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[1]left in 2012

[2]co-supervised with Tim Somervaille

Phosphoinositides are a family of lipid second messengers that are regulated in response to environmental changes by a network of kinases and phosphatases. Alterations in phosphoinositide levels can regulate many different cancer-relevant pathways including cell survival, proliferation, migration, cell substratum interactions and transcription. In cancer cells PtdIns(4,5)P_2 is at the heart of phosphoinositide signalling and can be synthesised by two different kinase pathways (Figure 1). De-regulation of the PIP5K and PIP4K pathway leads to different outcomes in cancer signalling suggesting that each pathway also controls specific downstream targets.

PIP5Ks and PtdIns(4,5)P_2

PtdIns(4,5)P_2 is present in the plasma membrane and in the nucleus and can be synthesised by two different families of kinases utilising two different substrates (Figure 1). There are three active isoforms of PIP5K, α, β and γ which all localise to the plasma membrane, although specific isoforms also localise to other subcellular compartments, such as the golgi (PIP5Kβ), the nucleus (PIP5Kα and γ), focal adhesions (PIP5Kγ) and the cytokinetic furrow (PIP5Kα). The targeting and regulation of PIP5Ks are still not exactly understood. Previously, we identified the small molecular weight G protein Rac and the plasma membrane level of PtdIns(4,5)P_2 as critical components that coordinate the localisation of PIP5K to the plasma membrane. Mass spectrometric analysis of PIP5Kβ identified twelve phosphorylation sites on PIP5Kβ, two of which are on tyrosine residues, nine on serine and one on a threonine residue revealing potential novel pathways that regulate PIP5Kβ. All the identified sites are conserved between human and mouse PIP5Kβ while six are present in Drosophila but only one is present in the yeast homologue. The majority of phosphosites that we detected are present in the C-terminal domain of the PIP5Kβ isoform. We developed an antibody that specifically recognises S413 phosphorylation and together with mutants that mimic or block the phosphorylation of S413, we showed that S413 phosphorylation is increased in response to treatment of cells with phorbol ester (a PKC activator), after energy depletion or hydrogen peroxide (H_2O_2) treatment. Specific inhibitors of kinase pathways defined a stress dependent pathway that requires the activity of the cellular energy sensor AMP-Kinase (AMPK) and PKC to regulate S413 phosphorylation. Furthermore we discovered that PKC can directly phosphorylate S413. Studies using phosphomimetic mutations revealed that unlike Rac and PtdIns(4,5)P_2, which regulate the localisation of PIP5Kβ, S413 phosphorylation decreases PIP5Kβ activity both in vitro and PtdIns(4,5)P_2 synthesis in vivo. Our studies suggest that S413 phosphorylation is a critical switch which regulates PtdIns(4,5)P_2 synthesis in response to the cells energy status and in response to oxidative stress (Figure 2).

PIP4K and PtdIns5*P*

There are three isoforms of PIP4Ks of which α is cytosolic, β is cytosolic and nuclear and γ localises to internal membrane compartments. We developed a specific antibody to PIP4Kβ and interrogated tissue microarrays of advanced human breast tumour samples. We show that down-regulation of PIP4Kβ correlates with worse overall patient survival (with Professor Goran Landberg, University of Manchester). Studies in human breast tumour cell lines show that down-regulation of PIP4Kβ expression decreases the expression of the tumour suppressor protein E-cadherin, which regulates cell adhesion, and increases the cells propensity to undergo an epithelial to mesenchymal transition (EMT). This transition is important for tumour cells to leave the primary tumour site and to metastasise to distant organs. In other cell types targeted RNAi studies indicate that decreased expression of PIP4Kα specifically reduces tumour cell growth, while having a relatively minor effect on normal cell growth. Our studies are aimed at unravelling how expression of different isoforms of PIP4K controls cell fate and if specific inhibitors of PIP4K might be useful to target cancer cell growth.

Figure 1

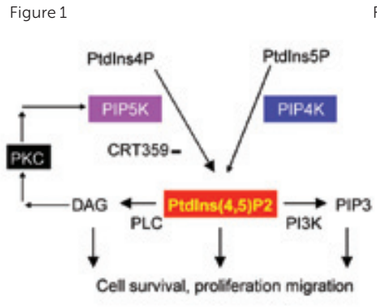

Figure 2

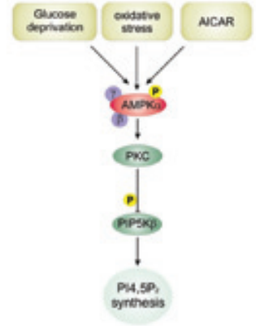

Figure 3

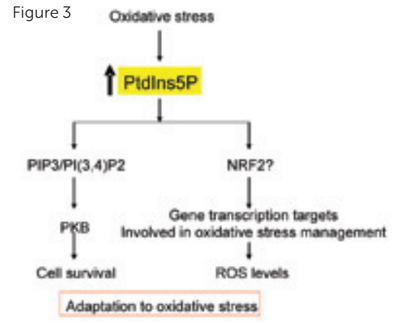

Figure 1
The phosphatidylinositol-5-phosphate kinase (PIP4K) and phosphatidylinositol-4-phosphate kinase PIP5K pathway contribute to phosphatidylinositol(4,5) bisphosphate (PtdIns(4,5)P_2) synthesis, however the major synthetic pathway is likely through PIP5K. PIP4K regulates the levels of phosphatidylinositol-5-phosphate (PtdIns5*P*). Diacylglycerol (DAG) activates protein kinase C (PKC) which regulates the phosphorylation and intracellular activity of PIP5K. We expect that an inhibitor of PIP5K will inhibit both the PI3K and the Phospholipase C (PLC) pathway.

Figure 2
Diagram showing how glucose deprivation and oxidative stress regulate S413 phosphorylation leading to a decrease in the kinase activity of PIP5Kβ. PKC directly phosphorylates PIP5Kβ. AICAR is a direct activator of AMPK leading to enhanced S413 phosphorylation.

Figure 3
Oxidative stress rapidly and reversibly increases the levels of PtdIns5*P*. PtdIns5*P* stimulates the levels of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 which in turn control PKB activation and cell survival. PtdIns5*P* also enhances the transcription of genes that control the accumulation of toxic reactive oxygen species (ROS). This may be through regulation of the NRF2 protein. Together these responses represent an adaptive change to oxidative stress.

How PIP4Ks signal is still not clear, and we hypothesised that PtdIns5*P* is a likely key signalling intermediate controlled by PIP4K to regulate specific downstream pathways. Oxidative signalling plays a key role in aging and cancer, and in response to oxidative stress, cells engage adaptive responses in order to control cell fate. We discovered that oxidative stress leads to a rapid and reversible increase in the levels of PtdIns5*P* and that PtdIns5*P* signalling impinges on the survival capacity of cell. The PKB, FOXO and NRF2 pathways are evolutionarily highly conserved regulators that control responses to oxidative insults. Oxidative stress induced increases in PtdIns5*P* maintain PKB activation and increase cell survival. Increased PtdIns5*P* also stimulates the expression of genes downstream of the NRF2 pathway that modulate the accumulation of detrimental reactive oxygen species (ROS) in response to oxidative stress. We found that cells that have higher levels of PtdIns5*P* have lower accumulation of toxic ROS. Stress induced PtdIns5*P* appears to regulate both cell survival and management of ROS levels, possibly through the regulation of two distinct subcellular targets: PKB in the cytoplasm and in the nucleus, NRF2-mediated gene transcription.

Using lipid affinity purification and mass spectrometry we have identified proteins that interact with and therefore function as downstream targets for PtdIns5*P* signalling such as PHD (Plant Homeo Domain) finger containing proteins. The PHD finger is a cross-braced Zinc finger that can interact with, and decode changes in, histone tail modifications to regulate transcription and chromatin structure. We found that the PHD finger of TAF3, a component of the basal transcription complex that regulates transcription and cell differentiation, interacts with phosphoinositides. We identified mutants that no longer interact with PtdIns5*P* and used these to demonstrate that changes in nuclear PtdIns5*P* target the transcription of a subset of TAF3 regulated genes through its interaction with the PHD finger. Mutants that do not interact with PtdIns5*P* are still able to interact with components of the basal transcription machinery and to interact with modified histone tails. We are using TAF3 as a model to understand how changes in nuclear PtdIns5*P* directly regulate gene transcription.

Measuring phosphoinositides and their interaction with proteins

The Inositide Laboratory has developed strategies to be able to measure phosphoinositides in mammalian cells and tissues and in model organisms such as zebrafish and Drosophila. In collaboration with Professor Martin Lowe (University of Manchester) we showed that reduced expression of the OCRL gene led to an increase in PtdIns(4,5)P_2 in fish. Mutations in OCRL, a phosphoinositide phosphatase, in humans lead to a rare X-linked recessive disorder with life expectancy rarely exceeding 40. The development of these techniques will enable further analysis of phosphoinositides in cancer relevant zebrafish models. Over the years we have also developed novel techniques to analyse the interaction of phosphoinositides with protein domains. This year we collaborated with Dr. Mark Petronski's group (LRI, Clare Hall Laboratories) who identified a novel protein that controls cell abscission during mitosis. The C2 domain within this protein targets it to the membrane and mutations in the C2 domain that ablate membrane interaction also attenuate progression through mitosis. We showed that the C2 domain confers interaction with the phosphoinositides PtdIns4*P* and PtdIns(4,5)P_2 and that mutation within this domain ablates its interaction with phosphoinositides.

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LEUKAEMIA BIOLOGY

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[1]joined in 2012

[2]left in 2012

[3]co-supervised with Nullin Divecha

High throughput sequencing analysis of haematological malignancy genomes has revealed that mutations in genes coding for epigenetic regulators recur frequently. These and other observations have emphasised the critical importance of the structure and function of chromatin in the biology of blood cancers, and hold promise for the development of epigenetic therapies. Using microarray data from a mouse model of human acute myeloid leukaemia (AML), we identified the histone demethylase LSD1 as a candidate regulator of leukaemia stem cell (LSC) potential. In collaboration with the Drug Discovery Unit, we synthesised small molecule inhibitors of LSD1 and demonstrated their in vitro and in vivo efficacy in inducing LSC differentiation of both murine and primary human leukaemia cells.

LSD1 (also known as KDM1A) (Figure 1) was the first histone demethylase discovered and is a member of the flavin adenine dinucleotide (FAD)-dependent amine oxidase family of demethylases. It is found associated with protein complexes that function as repressors of transcription, including CoREST and NuRD and demethylates monomethyl- and dimethyl-histone H3 lysine 4 (H3K4Me1 & H3K4Me2) marks, which are associated with active transcription states. It is also found in complexes associated with active transcription and may demethylate monomethyl- and dimethyl-histone H3 lysine 9 (H3K9Me1 & H3K9Me2) marks, which are associated with repressed transcriptional states. Having identified expression of *Lsd1* as correlated with LSC potential in a mouse model of *MLL* leukaemia, William Harris performed *Lsd1* knockdown experiments in murine MLL-AF9 AML cells and discovered that leukaemia cells underwent differentiation and apoptosis whereas normal haematopoietic stem and progenitor cells (HSPC) were spared. These results were phenocopied using the monoamine oxidase inhibitor tranylcypromine, which inhibits the activity of LSD1 at an IC_{50} of approximately 20uM. Tranylcypromine is a licensed therapy for depression that has been in the formulary for some decades. However, its relatively low selectivity and potency versus LSD1 render it inappropriate for clinical trials.

To address this issue, and in collaboration with the Drug Discovery Unit at the Paterson Institute, we synthesised some novel tranylcypromine analogues recently reported in the patent literature by Oryzon Genomics, a Spanish biotechnology company. These compounds exhibit significantly higher potency and selectivity versus LSD1 than the parental compound tranylcypromine. We found that in vitro and in vivo pharmacologic targeting of LSD1 in AML cells using these analogues, which are active in the nanomolar range, phenocopied *Lsd1* knockdown in both murine and primary human AML cells exhibiting *MLL* translocations. By contrast, once again, the clonogenic and repopulating potential of normal HSPC was spared. Our data establish LSD1 as a key effector of the differentiation block in MLL leukaemia which may be selectively targeted to therapeutic effect in myeloid leukaemias exhibiting translocations targeting the mixed lineage leukaemia gene. This work, which was published in April 2012 in Cancer Cell, lays the groundwork for early phase clinical trials of LSD1 inhibitors in AML at The Christie NHS Foundation Trust, an option now being actively explored by our group in a formal collaboration with Oryzon Genomics.

Figure 1 - Domain structure of LSD1
A) Linear representation and B) surface structure of LSD1 highlighting the SWIRM domain (orange), the amine oxidase domains (green) and the tower domain (blue). Image (B) was generated using PyMOL (v1.5) based on X-ray structure PDB 2DW4.

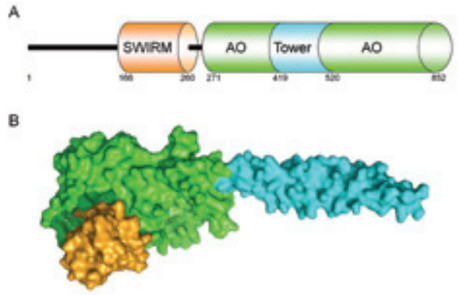

Figure 1

Figure 2 - Protein components of the EP400-centred complex and interacting proteins
Chr – chromodomain; Br – bromodomain; SANT – histone binding module; SWI/SNF – ATPase nucleosome remodelling activity; PHD – plant homology domain; HAT - histone acetyltransferase; PI-3K – phosphoinositide-3-kinase.

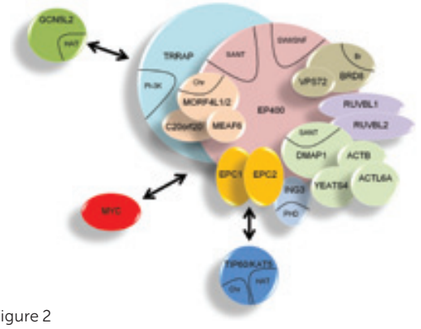

Figure 2

Identification of genes and cellular pathways which are required for the proliferation or survival of LSC, but which are of less importance in normal HSPC is key to the development of novel leukaemia-selective therapies. In separate work, and in keeping with the increasing appreciation of the critical role in leukaemia of proteins that regulate the structure and function of chromatin, Xu Huang performed a lentiviral screen in human AML cells, targeting some 280 genes coding for chromatin regulators for knockdown. In addition to MLL itself and menin, which is a critical oncogenic co-factor for MLL oncogene mediated transformation, the screen identified EPC1 and EPC2 (for enhancer of polycomb) as essential to prevent apoptosis of AML stem cells. *EPC* is conserved and essential in yeast, fly and mouse and its gene product forms part of the EP400 chromatin regulatory complex, variants of which include the TIP60 histone acetyltransferase complex and a MYC binding complex. Given the lack of information as to any role for EPC in normal or malignant haematopoiesis, we investigated this novel dependency further. Intriguingly, while knockdown of *Epc1* or *Epc2* induced apoptosis of LSC, the functional potential of *Epc* knockdown normal HSPC was spared. Analyses of *Epc* knockdown AML cells using exon arrays, performed with the assistance of the Molecular Biology Core Facility, revealed a transcriptional signature of MYC activation prior to induction of apoptosis. This prompted analysis of MYC protein levels in *Epc* knockdown AML cells which revealed robust accumulation of MYC 48 hours following initiation of knockdown. MYC is a key transcription factor that acts genome wide as an amplifier of the transcription of expressed genes. Next, to determine whether regulation of MYC turnover was dependent solely on EPC, or is instead dependent on multiple members of the EP400 complex, knockdown of other complex members was performed. In each case there was robust accumulation of MYC protein followed by induction of AML cell death. These data indicate a previously unappreciated and perhaps counterintuitive role for the EP400 complex in leukaemia cells: it prevents excess accumulation of MYC in transformed AML cells through promotion of MYC turnover.

To determine why this was observed in AML cells and not normal HSPC, we performed chromatin immunoprecipitation followed by next-generation sequencing (ChIPseq) using antibodies raised against EPC1 and MYC. These studies were performed with the assistance of Yvonne Hey in the Molecular Biology Core Facility, and Yaoyong Li and Crispin Miller in the Applied Computational Biology and Bioinformatics Group. The two proteins exhibited strong genome wide co-localisation at promoters in proportion to gene expression in AML cells, but not in normal HSPC, suggesting a strong physical co-localisation in the former, but not the latter, cell setting. In separate ChIPseq analyses we have also discovered a defect in histone H3 and H4 tail acetylation at promoter regions in AML cells which is not observed in normal HSPC. Indeed the genome wide distribution of EPC1 in AML cells was a virtual mirror image of that of the histone H4 lysine 5 acetylation mark, raising the possibility that EP400 complex components act at promoters in MLL leukaemia cells to inhibit histone acetyltransferase activity. This is relevant because, when EPC1 is knocked down, the version of MYC that accumulates is significantly acetylated. Indeed, double knockdown of EPC1 and the histone acetyltransferase GCN5 reduced accumulation of MYC and ameliorated apoptosis.

Our data establish EPC1 and EPC2 as components of a complex which serves to prevent MYC accumulation in myeloid leukaemia cells and their sensitisation to apoptosis, thus sustaining oncogenic potential (Figure 2). Therapeutic targeting of the EPC/EP400 complex may facilitate restoration of the natural tumour suppressor mechanisms that prevent cellular transformation by MYC. This work will be published in 2013.

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MOLECULAR ONCOLOGY

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Our group aims to develop new therapeutic strategies for cancer based on improved understanding of cancer biology. In particular, we focus on melanoma and use a range of approaches including biochemistry, cell biology, molecular biology, mass spectrometry and next generation sequencing. We also make extensive use of model systems and tumour samples to understand the biology of each tumour so that we can begin to develop tailored treatments for individual patients. This approach is called "personalised medicine" because treatment is tailored to each patient's tumour, rather than using a one size fits all approach. Thus, our aim is to implement a personalised medicine approach for melanoma to improve treatment outcomes for patients and we anticipate that the lessons we learn in melanoma will be applicable to other cancer types.

Melanoma is a potentially deadly form of skin cancer. In the UK, melanoma affects over 12,000 new patients and kills over 2,000 people each year. Work over the last decade has demonstrated that the RAS-RAF-MEK-ERK signalling pathway plays a critical role in this disease. RAS is a small G-protein that is activated downstream of receptor tyrosine kinases and RAF, MEK and ERK are cytosolic protein kinases that control cell growth and survival (Figure 1). There are three *RAS* (*HRAS, KRAS, NRAS*) and three *RAF* (*ARAF, BRAF, CRAF*) genes in humans. Notably, *NRAS* is mutated in about 20% of melanomas, and *BRAF* is mutated in a further 45% of cases.

We have found that the different genetic forms of melanoma display different biochemical properties. For example, the anti-diabetic drug metformin drives the growth of melanoma in which BRAF is mutated, but inhibits the growth of melanoma in which NRAS is mutated. This is because metformin increases the production of vascular endothelial growth factor (VEGF) in BRAF mutant, but not in NRAS mutant melanoma cells. Critically, VEGF encourages the development of new blood vessels into the growing tumours, increasing the flow of oxygen and nutrients and allowing the tumours to grow more rapidly. Agents that target VEGF overcome this effect and cooperate with metformin to suppress the growth of the BRAF tumours. These data highlight the importance of understanding the biology of each tumour if effective therapies are to be developed for individual patients.

Melanoma develops from specialised cells in the skin called melanocytes. These cells provide skin and hair tone, but more importantly, they protect us from the damaging effects of ultraviolet (UV) light radiation. UV light is present in sunlight and is produced by tanning devices, and it is the only known environmental risk factor for melanoma. The most common form of melanoma occurs on hair bearing skin that is intermittently exposed to UV light (recreational sun exposure) and we have developed transgenic models for melanoma that allow us to investigate gene-gene and gene-environment interactions that drive melanoma development. We are currently using these models to identify the genes that interact with BRAF and NRAS to drive melanoma development and to examine the role of UV light.

Figure 1
The RAS-RAF-MEK-ERK pathway is depicted. NRAS is activated downstream of receptor tyrosine kinases (RTK) and it activates BRAF, which in turn activates MEK and MEK activates ERK, driving cell growth and survival.

As an alternative approach to improving our knowledge of melanoma, we are also using next generation sequencing to reveal the landscape of mutations that occur in individual human melanoma samples. Over the last year, we have focussed on acral melanoma in particular, a rare form of melanoma that develops on the non-hair bearing skin of the hands and feet. These sites were thought to be protected from the damaging effects of UV light, but our sequencing revealed that some acral melanomas present a UV light DNA-damage "signature". This suggests that UV light also appears to play a role in the aetiology of some acral melanomas. We are also expanding our studies to examine the genetics of other rare forms of melanoma to allow us to gain further insight into melanoma biology so that we can develop new therapeutic strategies for the treatment of all forms of this disease.

A major breakthrough in melanoma treatment occurred with the development of drugs that inhibit BRAF. These drugs inhibit the RAS-RAF-MEK-ERK pathway in cells in which BRAF is mutated. Importantly, these drugs can achieve impressive clinical responses in patients whose tumours express the mutant forms of BRAF, but are ineffective in patients whose tumours express wild-type BRAF. Curiously, one of the unexpected side-effects of BRAF drugs is that they activate rather than inhibit the RAS-RAF-MEK-ERK pathway when RAS is mutated. This effect occurs because RAF drugs stabilise the formation of complexes between ARAF, BRAF and CRAF in the presence of active RAS. The complexes contain drug-bound, and drug-free RAF molecules and the drug-bound partners hyper-activate the drug-free partners, thereby hyper-activating the signalling pathway and stimulating the growth of the cells (Figure 2). Thus, while BRAF drugs inhibit the signalling pathway in cells in which BRAF is mutated, they activate the pathway in cells when NRAS is mutated and this effect is called the "*RAF-inhibitor paradox*".

We have shown that the RAF-inhibitor paradox underlies the development of non-melanoma skin lesions (keratoacanthomas and squamous cell carcinomas) in about a third of patients treated with these drugs. Notably, this is not because the BRAF drugs act as tumour promoters *per se*; rather they act by accelerating the growth of pre-existing, pre-malignant tumours in susceptible patients. The drugs in effect place a growth-selective advantage on these pre-existing tumours, and this knowledge led us to discover that anti-proliferative agents such as 5-fluorouracil can be used to treat these lesions in patients for whom surgery is not an option.

Although BRAF drugs have provided a paradigm shift in the treatment of melanoma, unfortunately the responses to these drugs are generally short-lived and most patients will fail on treatment after a relatively short period of disease control. Furthermore, about 20% of patients do not respond to BRAF drugs despite the presence of a BRAF mutation. We have therefore continued to develop new BRAF drugs and are testing if these are effective in patients whose tumours are resistant to the pre-existing drugs. We are also studying how resistance develops in patients undergoing treatment.

My Group relocated to Manchester in October 2012. We are a multi-disciplinary team that provides an excellent training environment for scientists and clinicians alike. Our philosophy is that patient care should be based on in-depth knowledge of cancer biology and we work at the basic-clinical interface to translate our basic research findings into patient benefit. At the Paterson Institute for Cancer Research we aim to continue to investigate the biology of melanoma and to use the knowledge from those studies to develop and implement a platform for personalised medicine for melanoma patients.

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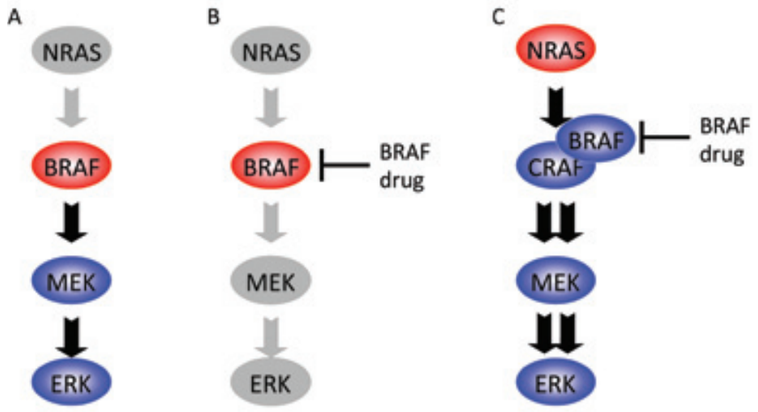
Figure 2
The RAF paradox. (A) In the presence of mutated BRAF, the MEK/ERK pathway is hyper activated even though RAS is not active. (B) In BRAF mutant cells, BRAF drugs block BRAF activity and inhibit the activity of MEK and ERK. (C) When cells in which NRAS is mutated are treated with BRAF drugs, although BRAF is inhibited, it is driven into a complex with CRAF and hyper-activates CRAF, thereby driving paradoxical hyper-activation of MEK and ERK.



SIGNALLING NETWORKS IN CANCER

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Signalling pathways dictate a range of important cellular outcomes ranging from cell death, to replication, to cellular migration. Genetic lesions that skew the balance of these pathways towards abnormal growth, proliferation, and cell survival are the fundamental mechanisms that cause normal cells to become premalignant. Kinases are the key regulators of signalling pathways (similar to transistors in a circuit) and dictate the activation or amplification of a given signal that ultimately leads to cellular fate decisions. When these kinases are hyperactivated or inactivated by genetic mutations they become the main drivers of tumorigenesis and thus serve as primary targets for the development of small molecule inhibitors. Cancer genomic sequencing studies and genome-wide siRNA screens are highlighting the amazing diversity in the kinases required to maintain tumorigenic phenotypes or permit drug resistance and emphasise the importance that neglected or understudied kinases play in the development and maintenance of a tumour. Thus a major goal of our lab is to identify and elucidate novel kinase drivers that are essential for tumour development, required for maintenance of tumorigenic phenotypes, or play a role in therapeutic resistance, focusing primarily on lung cancer.

Genetic Drivers of Lung Cancer

Approximately 70% of all non-small cell lung cancer (NSCLC) patients present with late stage disease (stage III-IV) and there is a pressing need to develop better therapies for these patients. This remains a major challenge as the underlying genetic causes of nearly half of all NSCLCs remain unknown. Recent success with therapies targeting mutationally activated EGFR and constitutively active EML4-ALK serve as proof of principle that drugs targeting genetically activated drivers of lung cancer result in better, more durable therapeutic responses in patients. The lab utilises three strategies to identify genetic drivers of lung cancer. In the first approach we use bioinformatic tools to evaluate the functional impact of somatic mutations in novel or understudied kinases identified in lung cancer genomic screens. We use the following bioinformatics applications to assess the functional impact of somatic mutations: CanPredict, PMUT, polyphen2, mutationtaster, snpeffect, puppasuite and SNPs and go. Kinases where a majority of mutations are predicted to be "likely cancer", "pathological" or likely to have a "high" functional impact are further evaluated in the lab. Additionally, we model many of the mutations that score highly in our analysis to determine the structural consequences of the putative driver mutations concomitant with experiments in the lab (Figure 1 and 2). We have found that this approach works extremely well in identifying loss of function (LOF) mutations and candidate tumour suppressing kinases.

Figure 1

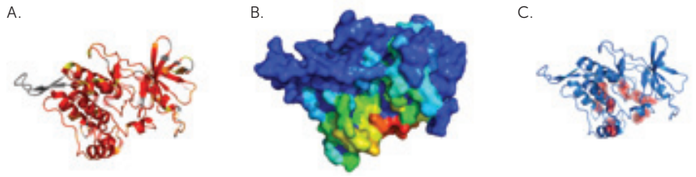

Figure 2

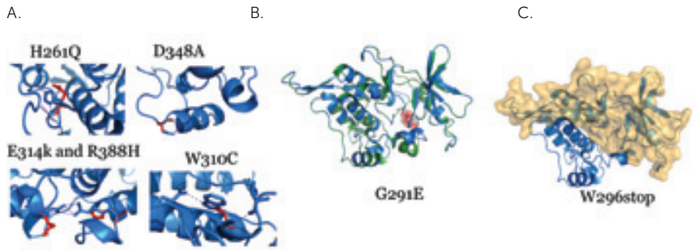

Figure 1
Structural analysis of mutations in a candidate tumour suppressing kinase.

MLK4 is a novel kinase that is frequently mutated in lung and colon cancer. A homology model of the MLK4 kinase domain was produced using SwissModel, based on the structure of MLK1 (Fig 1A; coloured based on homology). Comparison of binding site predictions (Fig 1B) and the location of mutations (Fig 1C) show most mutations occur close to areas predicted to be involved in substrate binding.

Figure 2
Analysis of the structures of MLK4 mutants shows that most mutants interrupt polar contacts within the kinase domain (Fig 2A) and are predicted to be loss-of-function mutations. Interestingly, mutant G291E causes a large perturbation of the structure close to a region required for coordinating binding to ATP (Fig 2B). Mutant W296stop removes a large proportion of the substrate binding region (Fig 2C).

In a second approach we use genetic dependency screens to identify mutationally activated drivers of lung cancer. In this approach we deplete cells of all somatically mutated genes in an effort to identify gain-of-function (GOF) mutations that are likely to be robust drivers of lung tumorigenesis and potentially druggable targets. In our knockdown screen we monitor for alterations in cell survival and proliferation under the premise that depletion of a mutationally activated driver will result in robust increase in cell death and inhibition of proliferation. Top targets are further characterised in detail, initially monitoring impacts of mutations on kinase activity. This approach has worked well to date and we have identified three novel kinases with GOF mutations in lung cancer, and have expanded these initial results and observe that two of these kinases are mutationally activated in 3-5% of all lung cancers. This screening approach has the potential to identify both therapeutic targets and biomarkers.

Our final strategy involves an unbiased kinome wide evaluation of all kinases focusing on amplification and somatic mutations in squamous cell lung cancer. Utilising online data portals such as cBio the lab hones in on kinases that are both amplified and somatically mutated in an effort to identify kinases where increased expression or mutational activation drives lung tumorigenic phenotypes. We start with the kinome wide approach, a candidate kinase is identified that is frequently amplified or mutated in lung cancer, and regions of mutations are highlighted. This kinase is then studied in depth in the laboratory to determine if it is required to maintain lung cancer cell survival and proliferation and to elucidate what downstream mechanisms are utilised to promote these phenotypes.

To characterise the mutant kinases, our general strategy is to first assess the functional consequences of somatic mutations on overall kinase activity utilising in vivo and in vitro kinase activity assays. We compare the activity of the kinases harbouring cancer mutations (engineered through site-directed mutagenesis) to WT, kinase dead (KD) and hyperactivated forms of the kinase. Next we determine phenotypic effects of expressing the WT, KD and mutant forms of the target kinase on proliferation, survival and transformed properties of appropriate tumour and normal cell lines. We then verify the function of the kinase using si/shRNA and evaluate the role of the endogenous kinase in regulating cellular phenotypes associated with tumorigenesis. We also investigate the molecular mechanisms utilised by the cancer mutants to promote tumorigenesis. For example, if the mutation is an activating mutation, we will identify cancer relevant substrates that are phosphorylated by the cancer mutants to promote tumorigenesis. Finally we will assess the consequences of somatic mutations utilising cell lines that harbour endogenous mutations in the target kinase. The overall goal of these studies will be to identify common and convergent pathways utilised by cancer cells to promote lung tumorigenesis and identify convergent and essential targets that could be exploited for the development of novel therapeutics for the treatment of lung cancer patients.

This next generation of personalised medicine is becoming a reality in NSCLC. Normal cellular growth relies on the interaction of networks of kinases that turn cellular processes 'on' and 'off'. A small percentage of NSCLC patients have a specific genetic change that generates an "always on" version of a kinase (EML4-ALK is an example of a genetically activated kinase). Cells with this mutated kinase are unable to turn 'off' certain cellular processes and therefore grow out of control to form a tumour. Patients with this defective kinase benefit significantly from treatment with a small molecule inhibitor (Crizotinib) that targets the activated kinase to turn 'off' the pathway. The major aim of our research is to identify new druggable targets by screening lung cancers to find the next set of hyperactivated genes that represent an Achilles heel for tumours of the lung and can result in better therapies for lung cancer patients.

Publications listed on page 74

STEM CELL BIOLOGY

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Transcription factors bind to specific sequences in DNA and control how genes are transcribed into RNA and, as a consequence, indirectly control the translation of RNA into functional proteins. Genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFβ, are frequently rearranged or mutated in human leukaemias such as acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its implication in leukaemias, RUNX1 has also been shown to be critical for haematopoietic development. Similarly the transcriptional co-activator MOZ is involved in three independent myeloid chromosomal translocations fusing *MOZ* to the partner genes *CBP, P300* or *TIF2* in human leukaemia. Our group studies the function of RUNX1 and MOZ in haematopoietic development and maintenance in order to better understand how alterations of these functions lead to leukaemogenesis.

Novel functions of GFI1 transcriptional repressor in the generation of blood cells

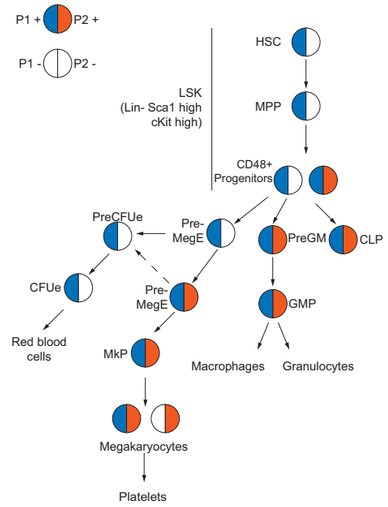
There is a worldwide shortage of matched donors for blood stem cell transfer of leukaemia or lymphoma patients. The generation of blood cells upon in vitro differentiation of embryonic stem (ES) cells or induced pluripotent stem (iPS) cells could represent a powerful approach to generate the autologous cell populations required for these transplantations. In this context, it is important to further understand the development of blood cells.

The earliest site of blood cell development in the mouse embryo is the yolk sac where blood islands, consisting of haematopoietic cells surrounded by a layer of angioblasts, develop at approximately day 7.5 of gestation. The parallel development of these two lineages in close association provided the basis for the hypothesis that they arise from a common precursor, a cell called the haemangioblast. A conflicting theory however associates the first haematopoietic cells to a phenotypically differentiated endothelial cell with haematopoietic potential, i.e. a haemogenic endothelium.

We have recently established a new model of haematopoietic development based on the in vitro differentiation of embryonic stem cells. We demonstrated that haematopoietic cells are generated from the haemangioblast through the formation of a haemogenic endothelium intermediate. During this process, the haemogenic endothelial cells loose their endothelial identity by altering their flat, adherent appearance into the characteristic round shape of mobile haematopoietic precursor cells. This haemogenic endothelial cell population is transiently generated during these blast cultures and is also detected in gastrulating embryos. Further experiments showed that the transcription factor RUNX1/AML1 is critical for generation of haematopoietic cells from this haemogenic endothelium.

These results suggest that RUNX1 regulates the expression of a set of genes critical for the development of the haematopoietic system from the haemogenic endothelium. To identify these genes, we have compared gene expression in cell populations generated from either *Runx1* deficient or *Runx1* competent ES

Figure 1
Model of Activities of P1 and P2 Promoters in Adult Haematopoiesis.



cells. We identified the *Gfi1* and *Gfi1b* genes as direct targets of RUNX1. *Gfi1* and *Gfi1b* genes encode two highly homologous nuclear zinc finger proteins that function as transcriptional repressors. A C-terminal domain containing six C_2-H_2-type zinc finger motifs mediates the DNA binding activity of these proteins while their repressional activity requires the N-terminal SNAIL/GFI-1 (SNAG) domain. GFI1 deficiency leads mainly to neutropenia and reduction in self renewal capacity of the haematopoitic stem cells. In contrast, its paralogue *Gfi1b* is mostly expressed in erythroid and megakaryocytic lineages and *Gfi1b* knockout results in embryonic lethality at E14.5 due to a deficiency in erythroid and megakaryocyte development. Several studies have shown that the expression of *Gfi1* and *Gfi1b* are controlled by negative feedback loops and that GFI1 and GFI1b can repress expression of each other in a cross regulatory fashion.

We first validated the differential expression of both *Gfi1* and *Gfi1b* in the presence/absence of RUNX1 and then demonstrated a direct binding of RUNX1 to these loci by chromatin immunoprecipitation (ChIP) and Chip-seq. To investigate the potential functions of GFI1 and GFI1b in the endothelial to haematopoietic transition, we evaluated their ability to rescue defects observed in *Runx1*$^{-/-}$ differentiation cultures. We demonstrated that in the absence of RUNX1, GFI1 and GFI1B are able to trigger the loss of endothelial identity of haemogenic endothelium. Upon *Gfi1* or *Gfi1b* expression, these cells down-regulate the expression of endothelial genes and undergo the morphological changes observed in the transition from endothelium adherent cells into haematopoietic round cells. However these cells are unable to acquire the full competence to generate haematopoietic colonies. Conversely, we established that fully committed blood progenitors generated in *Gfi1* and *Gfi1b* double knockout embryos maintain the expression of endothelial genes and cannot be released from their cell layer within the yolk sac and therefore fail to disseminate into embryonic tissues. Taken together, our findings strongly suggest a new and unexpected role of GFI1 and GFI1B transcription factors in mediating the loss of endothelial identity in the generation of haematopoietic progenitors from the haemogenic endothelium.

RUNX1 isoforms

Previous studies have indicated a critical requirement for RUNX1 at the onset of haematopoietic development and that RUNX1/AML1 is expressed as multiple, naturally occurring spliced isoforms that generate proteins with distinct activities on target promoters. Deletion of *Runx1* in adult haematopoietic cells results in significant defects, including an expansion of peripheral blood monocytes and granulocytes, a reduction in lymphocytes and thrombocytopenia. Stringent regulation of *Runx1* expression during haematopoiesis is therefore vital. The transcription of *Runx1* is under the control of 2 promoters: the *Distal* (P1) and *Proximal* (P2) promoters, encoding isoforms RUNX1C and RUNX1B respectively. RUNX1B and RUNX1C differ solely in their N-terminal domains, RUNX1C being 14 amino acids longer and beginning with MASDS and RUNX1B beginning with MRIPV.

To study their expression during adult haematopoiesis, we utilised a dual *P1-GFP/P2-hCD4* reporter mouse line to sort adult haematopoietic cell populations by flow cytometry and investigate their haematopoietic potential. We found that *P1-GFP* is expressed in all Lin$^-$cKit$^+$ progenitors as well as mature macrophages, granulocytes, B cells, subsets of T cells and immature erythroblast populations. By comparison, *P2-hCD4* expression is highly heterogeneous, being upregulated in subsets of Lin$^-$cKit^{high} Sca1^{high} (LSK) CD48$^+$ progenitors and in lymphoid and granulocyte-monocyte (GM) progenitors. The pre-megakaryocyte-erythroid progenitor (PreMegE) can also be separated into *P2-hCD4*$^-$ "pro-erythroid" and *P2-hCD4*$^+$ "pro-megakaryocyte" subsets.To elucidate the functional roles of the *Runx1* isoforms, we utilised *P1* null mouse models. The absence of RUNX1C results in aberrant T cell development, reduced long-term competitive repopulation and an alteration of myelo-lymphoid potential in engrafted bone marrow cells. These data support a hypothesis of distinct roles for the two RUNX1 isoforms during adult haematopoiesis.

Publications listed on page 74

STEM CELL AND HAEMATOPOIESIS

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At the early stages of vertebrate ontogeny, blood and endothelial cells develop from a common mesodermal progenitor, the haemangioblast. Upon commitment, the haemangioblast generates blood precursors through populations of endothelial cells with haemogenic properties.

Although several transcription factors have been implicated in this developmental process, the precise mechanisms governing cell fate decisions towards the generation of blood precursors remain largely unknown. The research performed in our group aims at further understanding the transcriptional networks that orchestrate this developmental process. Many transcriptional regulators implicated in haematopoietic specification during embryogenesis are also linked to leukemogenesis events, often as a result of aberrant expression. Through a better understanding of the function of these transcriptional regulators at the onset of haematopoietic specification, we hope to gain insight into their potential role in the initiation and maintenance of haematological malignancies.

The first haematopoietic and endothelial cells of vertebrates emerge extra-embryonically within the yolk sac. Long-standing observations early in the 20th century initially suggested that both lineages originated from a common precursor, the haemangioblast. However, the existence of this progenitor was only demonstrated much later in vitro using the differentiation of mouse embryonic stem cells as a model system and more recently through in vivo studies. The differentiation of embryonic cells is a powerful method to explore the molecular and cellular mechanisms that regulate embryonic haematopoiesis. Using this model system, elegant studies have revealed that the haemangioblast generates blood precursors through the formation of an intermediate specialised endothelium termed haemogenic endothelium. The precise molecular mechanisms that underlie the specification of haemangioblast to haemogenic endothelium and blood precursors still remain poorly understood. It is known that the transcription factor SCL is critical for the generation of haemogenic endothelium cells and that these cells require RUNX1 expression to generate definitive haematopoietic progenitors. Studies in our laboratory over the past few years have been aimed at understanding the role of the transcription factor SOX7 during this early developmental process.

SOX7 is transiently expressed at the onset of haematopoietic development

This transcription factor belongs to the SRY-related HMG-box family and shares similar protein structure with SOX17 and SOX18, two other members of the F subgroup. Early in murine development, *Sox7* transcripts can be detected in the parietal endoderm and in vascular tissues. Compelling evidence supports the idea that SOX7, together with SOX17 and SOX18, plays an important role during vertebrate cardiovascular development. The role of SOX F proteins in haematopoiesis is far less understood. Recent studies have demonstrated that SOX17 regulates the proliferation of neonatal and foetal haematopoietic stem cells, whereas we have recently established the involvement of SOX7 and SOX18 at the earliest stages of blood development. In these previous studies, a genome-wide expression analysis of differentiated embryonic stem cells revealed that *Sox7* was up-regulated at the onset of haemangioblast development. To further characterise the pattern of *Sox7* expression in early haematopoiesis, we studied its detailed kinetics of transcription during haemangioblast differentiation. Upon culture in the presence of VEGF, FLK1$^+$ haemangioblasts give rise to haemogenic endothelium cells which then further mature into blood precursors. The haemangioblast-derived haemogenic endothelium has been characterised as a population of cells expressing endothelial cell surface markers, such as TIE2, PECAM-1 or Ve-Cadherin along with the tyrosine kinase receptor cKIT. Upon differentiation, this population gradually loses its endothelial identity and inversely, gains a haematopoietic immuno-phenotype, with the rapid up-regulation of the alpha-IIb integrin CD41 followed by a slower acquisition of the pan-haematopoietic marker CD45. The careful analysis of SOX7 expression in the context of these cell surface markers revealed that this transcription factor is strictly associated with the expression of endothelial markers and sharply down-regulated during further commitment to haematopoiesis.

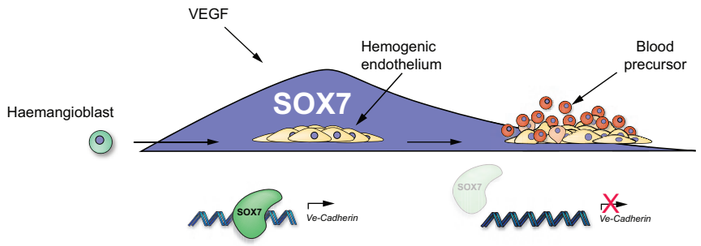


Figure 1
A model for the action of Sox7 during haematopoietic development.

The enforced expression of *Sox7* maintains haemogenic endothelium precursors

To understand the functional role played by SOX7 during this differentiation process, we took advantage of a mouse embryonic stem cell line containing a doxycycline inducible *Sox7* cDNA. This line allowed us to counteract the bona fide transient expression of *Sox7* during haematopoietic development and to assess the impact of maintaining the expression of this transcription factor beyond its normal time frame. Unlike the control culture, FLK1$^+$ haemangioblast cells differentiated in the presence of doxycycline failed to down-regulate the endothelial programme and showed little sign of commitment toward haematopoiesis. The continued expression of *Sox7* appears to inhibit the full differentiation of haemangioblast, maintaining an immature endothelial-like state. Given that *Sox7* is endogenously expressed in the transient haemogenic endothelium population, and that enforcing its expression beyond its normal time frame maintained an endothelial cell population, we performed further experiments demonstrating that the differentiation blockage induced by *Sox7*-enforced expression led to the maintenance of haemogenic endothelium progenitors.

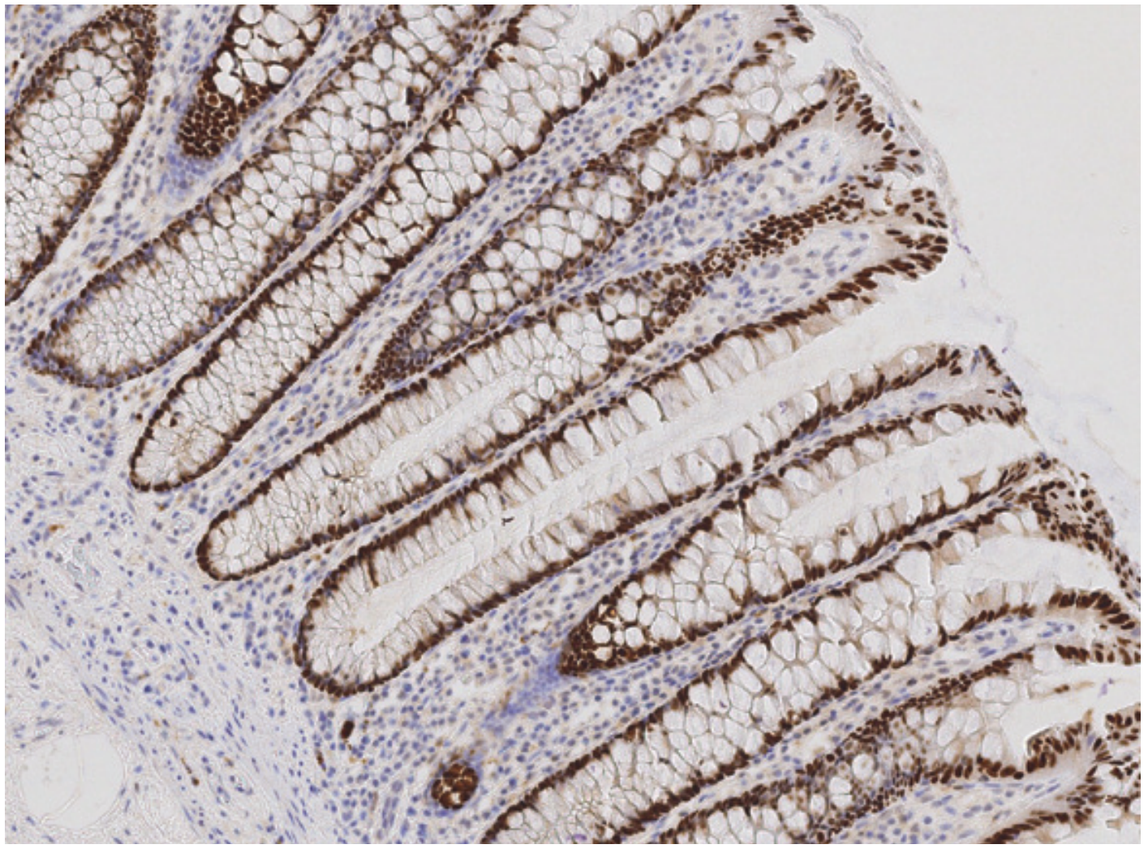
Ve-Cadherin is a transcriptional target of SOX7

The expression of Ve-Cadherin in embryonic haematopoiesis is associated with its expression in the haemogenic endothelium. Understanding the regulation of *Ve-Cadherin* expression is of crucial importance to gain insight into the poorly explored molecular mechanisms that drive haemogenic endothelium specification from the haemangioblast. In order to shed light on the transcriptional activity of SOX7, we focused on the expression of Ve-Cadherin a potential target of SOX7 at the onset of haematopoiesis. The promoter of mouse Ve-Cadherin has been described as a region of 2,500 base pairs, sufficient to drive the transcription of this gene in endothelial cells. In order to define whether SOX7 may bind and activate this promoter, we first identified potential SOX DNA binding motifs in this promoter sequence. To explore whether SOX7 regulates the activation of Ve-Cadherin through these motifs, we generated reporter plasmids containing either full-length or truncated promoter fragments to perform transient transactivation assays. Interestingly, SOX7 induced an eight-fold increase in transcriptional activity regardless of the length of the promoter region used, demonstrating not only that the Ve-Cadherin promoter was activated by SOX7, but also that the most proximal region studied, containing only one SOX binding motif, was sufficient for this effect. We next undertook a series of experimental assays to evaluate the interaction of SOX7 with this motif. Through both in vitro and in vivo assays, we were able to demonstrate that SOX7 binds the most proximal SOX DNA binding motif on the promoter of Ve-Cadherin. Together, these data reveal that SOX7 regulates the expression of Ve-Cadherin by binding to its promoter, hence identifying Ve-cadherin as a transcriptional target of SOX7.

SOX7 is a critical regulator of haemogenic endothelium at the onset of haematopoiesis

In summary, our data reveal that SOX7 is specifically expressed in haemogenic endothelium cells at the onset of haematopoietic development and that SOX7 controls the expression of Ve-Cadherin, a gene critically associated with the endothelial nature of the haemogenic precursors. It is tempting to propose a model in which SOX7 acts upstream of endothelial-related genes in the haemogenic endothelium before the haematopoietic differentiation has been initiated (see Figure 1). Thereafter, the down-regulation of this transcription factor is necessary for the dynamic process of blood precursor emergence from the haemogenic endothelium.

Publications listed on page 75



Normal human colon stained with an antibody to the marker CDX2 (brown). CDX2 is used as a marker in colorectal cancer prognosis. The tissue has been counterstained with haematoxylin to show the nuclei of cells (blue).

Image provided by Darren Roberts from the Immunology Group.

THE UNIVERSITY OF MANCHESTER INSTITUTE OF CANCER SCIENCES

RESEARCH GROUPS

CHILDREN'S CANCER

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Graduate Student
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Administrator
Parisa Mahjoob-Afag

[1]joined in 2012

Our group conducts investigations into the biological mechanisms responsible for the variations in the therapeutic response in children with acute lymphoblastic leukaemia (ALL). We conduct a number of international clinical trials, which provide us with the data and clinical material for hypotheses based laboratory investigations.

Clinical

Childhood ALL is a rare disease with high cure rates. Our interests are in refractory and relapsed disease. Thus most of the clinical trials in which we participate are done as part of international collaborations. In the largest such collaborative study ever performed, we showed that contrary to conventionally held beliefs, some children with ALL who do not respond to initial therapy can be salvaged by allogeneic stem cell transplantation. Moreover as these children can be identified by typical cytogenetic changes, this study offers practical therapeutic strategies (Schrappe *et al.*, 2012). In another international study (EsPhALL), we were involved in designing and conducting a randomised trial of the tyrosine kinase inhibitor (TKI) imatinib in children with Philadelphia positive ALL. This is the first such randomised trial of a TKI in such a rare sub group. The results do not show a significant difference in outcome between those who received and did not receive imatinib (Figure 1). However, the study is perhaps underpowered to establish the difference in survival. Imatinib was well tolerated in the context of intensive therapy, and there appears to be trend in its favour and thus the randomisation was discontinued (Biondi *et al.*, 2012). In the international trial for relapsed patients (ALLR3) we demonstrated a benefit of the drug Mitoxantrone over Idarubicin. This is one of the largest differences ever observed by a single drug intervention in the context of combination chemotherapy and the randomisation was stopped. The trial continues to enrol patients to answer secondary objectives and with over 400 now recruited, is one of the largest of trials of its kind worldwide. Further investigations include the detection of clone specific molecular biomarkers to risk stratify patients for transplantation and evaluation of the new agent Clofarabine. A new international trial for relapsed patients, designed by us and funded by the FP7 program, is due to launch in 2013 (http://www.intreall-fp7.eu/).

Laboratory

Investigating the biological mechanisms of therapeutic failure in collaboration with Dr Krstic-Demonacos (University of Manchester, Faculty of Life Sciences), we have shown that steroid sensitivity in ALL is controlled through a series of feedback loops operating in differential temporal patterns that will, at least in part, determine cellular levels of GR, AP-1, Erg and Bim, ultimately contributing to cell fate (Chen *et al.*, 2012). However, emerging evidence from our clinical trials suggests that recurrence cannot be explained only by intrinsic resistance. To investigate extrinsic mechanisms, Jizhong Liu has created an in vitro organotypic, orthotropic model of the bone marrow microenvironment, developed using mesenchymal cells obtained from bone marrow aspirates (BMSCs). Conditioned media from this system is able to confer chemo-protection in ALL cells by activating prosurvival signalling pathways. Our analyses suggest that BMSCs dynamically regulate mitochondrial redox status and antiapoptotic gene expression, which confer resistance to chemotherapy in leukaemia cells. Targeting this process using drugs that modulate the redox adaptation reverses the chemoresistance leading to cell death. This has potential implications for future therapeutic strategies not only in childhood ALL but in other cancers. These data earned Jizhong a highly rated award for his poster at the AACR meeting in Chicago in April.

Our interest in the interaction between the bone marrow microenvironment and the leukaemic cell has led us to hypothesise a model of two-way communication. We believe microvesicles (MV) produced by the leukaemic cell to be one such mechanism by which the leukaemic cell modulates its environment. Suzanne Johnson has characterised leukaemia cell-derived microvesicles, showing that they have distinct gene expression and protein profiles compared to the parent cell. Genes involved in redox status, histone regulation and lipid metabolism are upregulated in the MV. MVs are enclosed in a lipid bilayer and lipidomic analyses (in collaboration with Professor Goodacre, Manchester Institute of Biotechnology) revealed a unique profile from MVs produced under hypoxic conditions. Electron microscopy has identified a heterogeneous population of MVs including exosomes and larger MVs derived from both cell lines and ALL patient cells which contain organelles including mitochondria, lysosomes, endoplasmic reticulum and polyribosomes (Figure 2). We have also shown that human leukaemia cells are able to produce MVs in vivo which are internalised by mouse stromal cells in the bone marrow. In vitro internalisation of the leukaemic cell MVs by BMSCs appears to effect key signalling pathways involved in metabolism. Thus tumour derived MVs may modulate the host cells to create an environment that favours the survival of leukaemic cells over normal haematopoietic precursors in targeted niches.

Figure 1 EsPhALL UK: PFS Imatinib vs No Imatinib

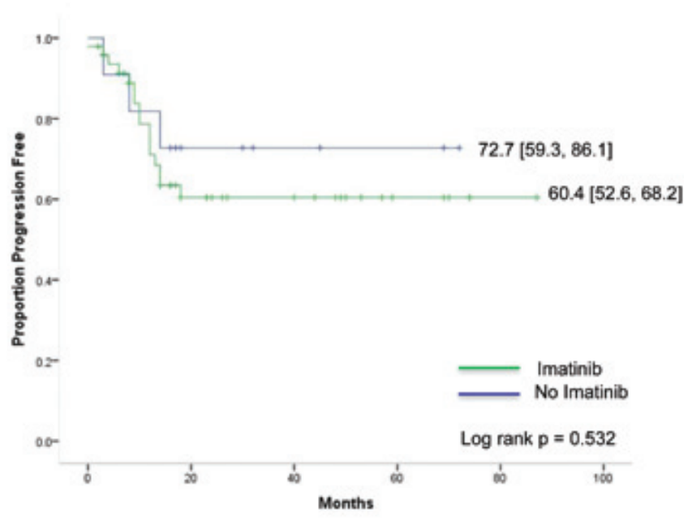

Figure 1
Progression free survival of EsPhALL patients in the UK; Kaplan-Meier analysis of Imatinib versus no Imatinib showed no significant difference in PFS between those patients who received Imatinib and those who did not.

Clare Dempsey and Seema Alexander have created a number of xenograft mouse models to study pathogenesis and understand the effects of therapy. Collaboration with Professor Stern (Paterson Institute) using such a model showed that a subpopulation of leukaemic blast cells expressing 5T4 displayed invasive and chemotactic behaviour, suggesting a model of recurrent disease (Castro *et al.*, 2012). Stephanie Harrison is investigating the role of the adhesion molecule LFA-1, a molecule we have previously identified using a discovery based proteomic approach, in chemo-resistance, adhesion and invasion. She has identified a panel of high and low LFA-1 expressing cell lines and measured their response to the bacterial protein Leukothera (collaboration with Dr Scott Kachlany, New Jersey); which binds LFA-1 and induces cell death.

We are pleased to report that Ashish Masurekar successfully defended his PhD thesis, Steph Harrison made the transition to 2nd year PhD student and Wayne Pinto gained his MRes. We bid farewell to Adiba Hussain, and Mark Holland who moved on to pastures new, and welcome Dr Yujun Di and Tasos Ioannou to our group.

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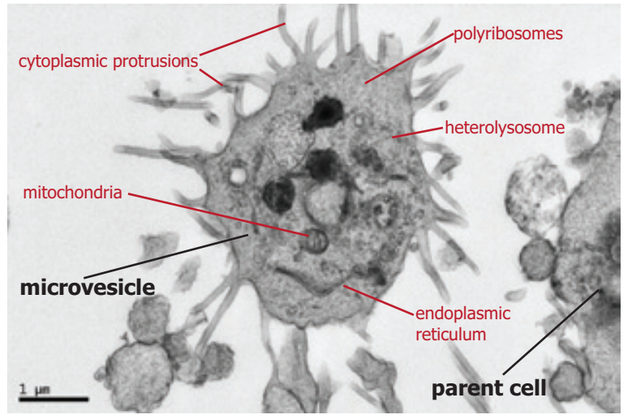
Figure 2
Transmission electron micrograph showing a large microvesicle derived from an acute lymphoblastic leukaemia cell (x 2900 magnification). Image shows intact membrane with cytoplasmic protrusions and intra-vesicle organelles.



MEDICAL ONCOLOGY: CLINICAL AND EXPERIMENTAL IMMUNOTHERAPY

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David Gilham

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Lyndon Ridges-Jones[2]
Elizabeth O'Donovan[1]

Renal Cancer Data Manger
Dionne Lawrence

Project Managers
Nikki Price
Helena Kondryn

[1]joined in 2012
[2]left in 2012
[3]Cellular Therapeutics Unit

The Medical Oncology Clinical and Experimental Immunotherapy Group undertakes translational research to develop immunotherapy for cancer. This is based around clinical expertise with immunotherapy in renal cancer and is developing into work in gastro-intestinal cancer, lymphoma and melanoma. The major focus is on the development of adoptive cellular therapy and a major recent infrastructure development is the recently licensed Good Manufacturing Process (GMP) Cellular Therapy Unit base in the UMIC building on the main Medical School site. This enables us to produce clinical grade cells for trials and has also led to a spinout company. The group lead several EU consortia in the field and much of our research links with other leading EU centres for this type of work.

Clinical/translational research renal cancer

A number of major clinical trial results were released in 2012 with the renal cancer clinical group playing a major role. These included the comparative trials of Sunitinib and Pazopanib assessing both patient preference (PISCES) and efficacy (COMPARZ). These were presented at ASCO and ESMO respectively and demonstrated similar efficacy and a clear patient preference for Pazopanib. The renal team have also established a major data base for audit and research of renal cancer management. The initial research output was presented at ESMO assessing the value of CAIX expression as a biomarker to predict response to high-dose interleukin-2 in addition to our previously published selection criteria (Shablak *et al.*, 2011). In addition, this database has facilitated the setting up of collaborative projects with the Christie pathology department and the Clinical Genetics department at St Mary's Hospital to assess the potential value of further histological and genetic biomarkers.

Cell therapy

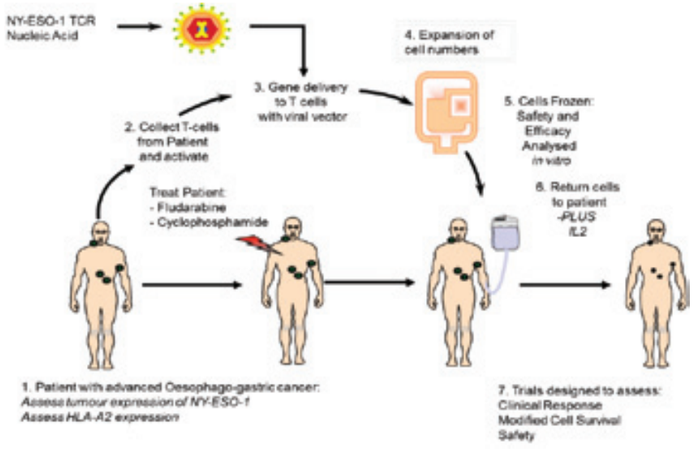
The year has seen a major step forward in the clinical /translational development of cellular therapy. The Cellular Therapy Unit (CTU) has begun making clinical cell products and the first patients have been treated with tumour infiltrating lymphocytes. This along with other immunotherapy research by the group was featured on a BBC Newsnight programme in August 2012. The opening of the CTU has also enabled a phase I trial targeting CD19 in B-cell malignancies to reopen. This trial had previously shown very encouraging responses at the lowest dose and it is hoped that the further cohorts planned will produce further clinical benefit. In addition, the EU has awarded a major clinical trial grant (€ 6M) to target NY-ESO-1 (FP7 ATTACK: Adoptive T-cell Therapy to Achieve Cancer Killing). NY-ESO-1 is a cancer testis antigen which is strongly expressed in a proportion of many common cancers. The use of T-cells to target NY-ESO-1 has been tested in melanoma and in synovial sarcoma with excellent clinical response rates and no significant on-target toxicity. This background has allowed us to set up this project to test two major hypotheses:

1. Is adoptive T-cell therapy active in a common epithelial malignancy where NY-ESO-1 is over-expressed? Dr Thistlethwaite leads this project as joint-chief investigator and the overview of the trial in illustrated in figure 1. This trial is scheduled to start in 2013 (Q2).

2. Does selecting cells with better repopulating ability and optimised production processes (see below – pre-clinical section) produce better cell survival and better clinical responses? This trial will be undertaken in malignant melanoma where high clinical response rates have been seen. The trial is planned as a randomised phase II study and should commence in 2013 (Q3) once the pre-clinical validation of the processes has been achieved.

The ATTACK consortium (Co-ordinator Robert Hawkins) comprises 17 partners with many major clinical centres in Europe and these trials will be the first multi-centre engineered T-cell trials in Europe. As well as the clinical centres there are four companies (three SMEs and one large company). One of the SMEs is Cellular Therapeutics Ltd – a spinout company from the University/this Group. The inclusion of relevant commercial companies accords with the EU focus on developing novel therapies in a sustainable way that should both improve health outcomes and provide employment and economic value.

Figure 1
The ATTACK clinical trial in oesophago-gastric cancer. Patients will be screened for HLA-A2 tissue type and to assess if their tumour expresses NY-ESO-1. Patients who are eligible will undergo leukapheresis to obtain large number of T-cells. After activation, the T-cells are genetically modified using a lentivirus to produce cells engineered to target NY-ESO-1. Patients are treated with pre-preconditioning chemotherapy to deplete normal host T-cells - this results in the in vivo homeostatic expansion of the returned engineered T cells. The engineered T cells are also supported with ectopic cytokines. The trial is designed to assess the RECIST response rate to the treatment and it is anticipated to include up to 28 patients.



Major Histocompatibility Complex proteins. However, it is also clear that the interactions of T cell-tumour proteins outside of the initial CAR-target interaction are important. Indeed, the optimal activity of CAR-T cells is dependent upon interactions including that of the CD2 receptor with its cognate ligands (Cheadle *et al*, 2012).

Importantly, we are seeking to develop new model systems to test the power of gene modified T cells. To this end, our collaborative studies with the Immunology group (Professor Peter Stern) targeting the mouse 5T4 antigen has identified some of the issues that relate to immunological tolerance (Castro *et al.*, 2012). We have generated CAR's specific for the mouse 5T4 protein and mouse T cells engrafted with these CAR's show specific functional activity against mouse 5T4 expressing targets. Our future studies will investigate the potency of these 5T4-specific T cells and investigate what role immune tolerance and expression of the natural antigen has upon the in vivo function, persistence and trafficking of CAR T cells.

It seems self-evident that the long-term persistence of therapeutic T cells in the patient is likely to correlate with potential clinical response. However, many of the methods used to generate CAR T cells results in the polarisation of the T cells towards a differentiated phenotype which tends to result in reduced in vivo persistence. To counter this, we have investigated whether cytokines can modulate gene-modified T cell phenotype. Indeed, the combination of IL-7 and IL-15 during the culture of T cells drives these T cells towards a less differentiated phenotype and consequently, results in an improved engraftment potential of T cells in mouse models. This work has formed the basis of a successful European Union Framework 7 programme ATTACK where we will test the effect of IL-7/IL-15 on T cells engrafted with a NY-ESO-1 T cell receptor in comparison to T cells cultured in standard methods in a randomised phase II Malignant Melanoma clinical trial setting. Our future studies are focusing upon the selection of T cells for specific chemokine receptors to investigate whether the specific selection of T cell subsets prior to genetic modification further improves the in vivo potency of gene-modified T cells.

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MEDICAL ONCOLOGY: TRANSLATIONAL ANTI-ANGIOGENESIS

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Cristina Ferreras

Clinical Fellows
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Kalena Marti Marti (joint with CEP)
Danielle Shaw

Scientific Officer
Graham Rushton

Angiogenesis has been validated as a target in ovarian cancer therapy through clinical trials in which the VEGF inhibitor bevacizumab, in combination with cytotoxic chemotherapy, resulted in improved progression-free survival. The majority of angiogenic cytokines, including VEGF, depend on heparan sulphate (HS) for their activity, thus presenting an opportunity to target cytokine-HS interactions and thereby inhibit cytokine activity. Our organic HS synthesis programme generated inhibitory HS sequences where differentially 6-O-sulphated sequences target specific angiogenic cytokines. In addition to HS structure-function studies, we focus on defining the phenotypes and functions of circulating endothelial progenitor cells (EPCs) isolated from ovarian cancer patients which might lead to identification of new cellular targets for anti-angiogenic therapies.

Significance of 6-*O*-sulphation in HS oligosaccharides

(collaboration with Dr. John Gardiner, Manchester Interdisciplinary Biocentre)

Our previous work showed that a synthetic HS dodecasaccharide, consisting of six repeating disaccharide units that were composed of iduronate 2-*O*-sulphate linked to *N*-sulphated glucosamine (ISNS) was a potent inhibitor of FGF2- and VEGF165-induced endothelial cell responses in vitro (Cole *et al.*, PLoS One, 2010). We also showed that 6-*O*-sulphate levels in endothelial cells play a major role in regulating responses to FGF2 and VEGF (Ferreras *et al.*, J Biol Chem, 2012). To understand the significance of the number and position of 6-*O*-sulphates in HS we generated a synthetic ISNS dodecasaccharide with either one 6-*O*-sulphate moiety at the non-reducing end of the molecule (ISNSmono6S) or a uniformly 6-*O* sulphated molecule (ISNS6S). The inhibitory properties of ISNSmono6S were enhanced leading to a more pronounced inhibition of FGF2- and VEGF165-dependent endothelial cell proliferation, migration, sprouting and tube formation. During in vivo tumour xenograft growth studies, ISNSmono6S dodecasaccharide significantly reduced FGF2-induced microvessel density and the average vessel size. Correspondingly, there was a reduction in phosphorylation levels in FRS2, FGFR1 adaptor molecule, within the tumour vessels (Figure 1). In contrast, a synthetic tri-sulphated dodecasaccharide (ISNS6S) was a poor inhibitor of FGF2- and VEGF165-dependent endothelial cell functions. Significantly, testing the effects on chemokine-induced endothelial cell migration revealed that ISNSmono6S, but not ISNS, potently inhibited Sdf-1α-induced endothelial cell migration, whereas IL-8-induced endothelial cell migration was inhibited by ISNS dodecasaccharide, but not ISNSmono6S variant. Such specificity was also reflected in inhibition of SDF-1α-induced phosphorylation of Erk and IL-8-induced phosphorylation of Stat3, where ISNSmono6S was more potent in inhibiting SDF-1α-induced Erk phosphorylation, while ISNS inhibited IL-8-induced Stat3 phosphorylation. This suggests that the number of 6-*O*-sulphate residues and their position are of major importance in differentially inhibiting specific cytokines.

Figure 1
ISNSmono6S inhibits FGF2 signalling in tumour vasculature. Immunostaining with antibodies recognising endothelial cell marker CD31 and phosphorylated FRS2 of endometrial carcinoma xenografts over-expressing exogenous FGF2 in mice treated with saline, ISNSmono6S or sunitinib.

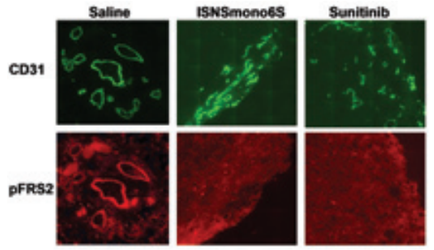
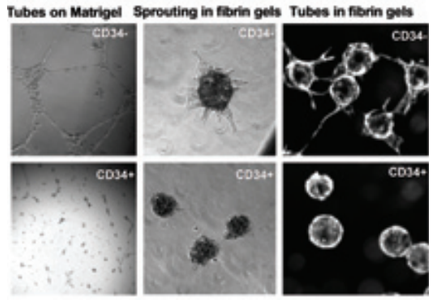


Figure 2
Assessment of endothelial functions in CD34-negative (CD34-) and CD34-positive (CD34+) EPC populations. CD34- EPCs show angiogenic phenotypes when tested for tube formation on Matrigel, sprouting and tube formation within three-dimensional fibrin matrix. CD34+ EPCs show no angiogenic activity in these assays.



Phenotypic and functional evaluation of EPCs from ovarian cancer patients and healthy controls

Through a number of studies it has been shown that EPCs play an important role in promoting physiological and pathological angiogenesis, although their exact role in tumour angiogenesis remains controversial. EPCs are usually defined as cells present in bone marrow, umbilical cord blood or peripheral blood that express endothelial, but not hematopoietic, cell surface markers and show high proliferative potential. However, the definition of EPC phenotype and function still poses a major challenge in the field.

We established an EPC ex vivo expansion procedure where we routinely detect the outgrowth of endothelial cell colonies from the peripheral blood mononuclear cell population of healthy females and ovarian cancer patients. We have identified two distinct EPC populations based on the expression of the progenitor stem cell marker CD34. No differences were found in the expression of endothelial cell surface markers CD31, CD146, CD105 and VEGFR2 within both populations, while the expression of the haematopoietic marker CD45 was undetectable in CD34-negative and CD34-positive cell populations. CD34-negative cells formed an endothelial tube network when plated on Matrigel, were able to sprout and formed endothelial tubes in a three-dimensional fibrin matrix (Figure 2). The CD34-positive cell population showed no sprouting and tube formation either on Matrigel or in a three-dimensional matrix (Figure 2). Evaluation of the chemotactic capacity of EPCs toward VEGF165 showed a better response rate in CD34-negative population when compared to CD34-positive cells. Together these data show the morphological and functional differences between CD34-negative and CD34-positive populations within EPCs.

Clinical studies

(collaboration with Clinical and Experimental Pharmacology, led by Professor Caroline Dive)

Angiogenesis has been validated as a target for the treatment of ovarian cancer in four randomised trials; two in the first line setting and two in recurrent disease. One of the trials carried out in chemo-naïve patients was ICON7, which randomly allocated patients to receive carboplatin and paclitaxel or the same regimen supplemented with the anti-VEGF antibody, bevacizumab. The results showed that in patients with advanced disease (FIGO stage III or IV) there was a statistically significant improvement in progression free survival and revealed early data that were suggestive of an overall survival advantage.

We carried out a translational research project through investigation of the angiogenic cytokine concentrations in the plasma of patients before treatment in ICON7; the aim being to identify the patients who will most benefit from bevacizumab. During the clinical trial Professors Dive and Jayson collaborated with Dr Alison Backen to validate, to the standards of GCLP, a multiplex ELISA system that was capable of measuring the concentration of 16 angiogenic growth factors simultaneously in small volumes of plasma. Our analysis showed that patients with high plasma concentrations of Ang1 and low Tie2 were those who benefitted the most from bevacizumab. Ang1 is implicated in maintaining a quiescent endothelium and potentially, our data suggest that in patients with high plasma concentrations of Ang1 the tumour vasculature is more dependent on VEGF and that is why these patients benefit most from bevacizumab. We will attempt to verify the findings in an independent sample set over the next year.

These results are important not only because bevacizumab can induce toxicity and is expensive but also because new anti-angiogenic agents that target Angiopoietin are in late stage development. Conceivably, these data will allow us to use such tests to select the right anti-angiogenic agents for the right patient thereby reducing cost and toxicity while improving the survival of our patients.

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TARGETED THERAPY

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Grazyna Lipowska-Bhalla

MD Student
Clara Chan

MRes Student
Benjamin Sanderson

BSc Pathology Student
Conor McKenna

This last year has been highly successful for the Targeted Therapy Group and the highlights have included important research findings leading to publication in two key research areas within the programme, namely combining RT with immunotherapy and mechanisms of action of antibody action. Earlier in the year we provided new insights into the role of Reactive Oxygen Species (ROS) in the mechanism of induced tumor cell death with the anti-CD20 antibody (GA101, Obinutuzumab) in (Honeychurch et al 2012) and followed this with a novel approach for the treatment of cancer using radiotherapy in combination with a TOLL-like receptor (TLR)-7 agonist, which was also published in Blood as a plenary paper with associated editorial (Dovedi et al 2012). Tim Illidge was awarded the Faculty of Medical and Human Sciences Researcher of the Year.

Combining radiation therapy with immunotherapy approaches

Effective anti-cancer treatments often result in the induction of large amounts of tumour cell death. RT is an effective cancer treatment that plays an important part in the local control of many tumours and often leads to high response rates in patients. RT induced tumour cell death has the potential to stimulate immune responses against the cancer cells. However, these immune responses are usually too weak to lead to durable anti-tumour responses that might subsequently lead to improvements in patients' outcome. However, combining RT with novel agents that are capable of stimulating the immune system has the potential to generate durable and effective anti-cancer immune responses that are able to eradicate widespread malignant disease and reduce disease recurrence.

By using a synthetic agonist of TOLL-like receptor family (TLR-7) which activates a systemic immune response by mimicking a viral infection our data demonstrate that the anti-tumour efficacy of RT can be enhanced in pre-clinical models of lymphoma. The combination of TLR-7 and RT but not single-agent treatment resulted in long-term clearance of tumour and enhanced survival (Figure 1). Combination therapy led to an increase in the frequency of $CD8^+$ cytotoxic T-cells present in the circulation. Our investigations revealed that the efficacy of this combination therapy was dependent upon the activity of these $CD8^+$ cytotoxic T-cells as their depletion completely abrogated the therapeutic response. Moreover, the combination of RT and systemic TLR7 therapy led to the induction of long-term immunological memory, which was able to protect against disease recurrence. These data revealed that combination therapy with radiation and a TLR7 agonist was able to generate tumour-specific immunological memory. These findings demonstrate the potential for novel therapeutic combination approaches involving RT and immunotherapy for the treatment of cancer. We are currently expanding this study to include additional syngeneic models of solid tumours. In addition, we are also evaluating a series of novel small molecule TLR7 agonists with the aim to test these combinations in the clinic. Further work is ongoing to elucidate the role of immune effector cells such as DC, helper-T cell, B cell, NK cell and macrophage in the generation of protective anti-tumour immunity post combination treatment with TLR7 and radiotherapy.

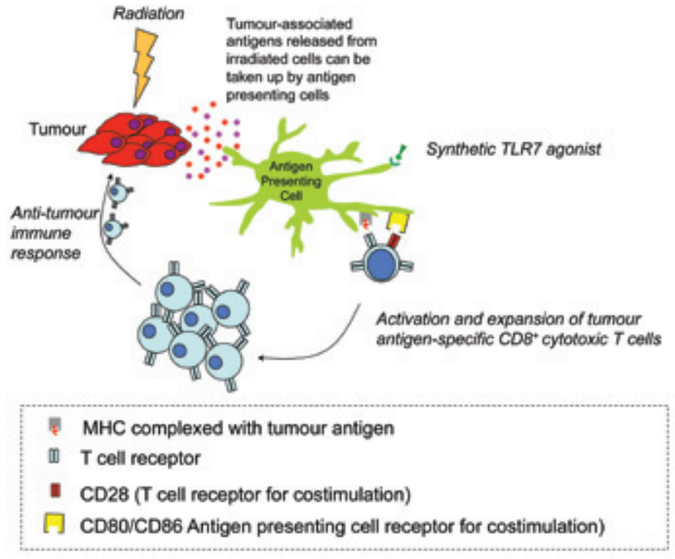


Figure 1
Treatment with radiation therapy (RT) in combination with a synthetic TOLL-like receptor (TLR)-7 agonist leads to the activation of antigen presenting cells (APCs). Following activation APCs present tumour associated antigens and the co-stimulatory factors required to bring about the activation of cytotoxic T cells. These activated T cells are then able to selectively kill tumour cells.

Host immune recognition of treatment induced tumour cell death

We have also investigated in vivo the host immune response to dying tumour cells. Whilst apoptosis is generally considered non-immunogenic, recent evidence suggests that some anti-cancer therapies that induce apoptosis can elicit anti-tumour immune responses. In collaboration with Clinical Experimental Pharmacology, led by Professor Caroline Dive, we have developed a number of doxycycline (Dox)-dependent caspase-3 "death switch" syngeneic murine tumour models. In these models a doxycycline inducible, constitutively active caspase-3 ('death switch') was constructed in murine tumour cell lines to explore the impact of the host immune response to rapid, synchronous and substantial tumour cell apoptosis. In vitro, activation of the death switch triggers up to 80% apoptotic cell death that is accompanied by release of 'danger signal' molecules such as HMGB1 and HSP90, 12-24 hours post-induction. In vivo, death switch induction provoked rapid, pronounced tumour xenograft regression in immune-competent and immune-deficient mice but sustained tumour eradication was observed only in immune-competent mice. Moreover, mice which were tumour free after death switch induction in their tumours were protected (< 60%) from rechallenge by the same tumour cells. These data suggest that sustained tumour eradication after substantial tumour apoptosis requires an anti-tumour host immune response which prevents tumour relapse. In many patients, cancer therapies produce encouraging initial responses that are only short lived. These results provide new insights that may have important implications for further development of strategies that result in long term tumour clearance after initially effective anti-cancer treatment. We are currently investigating these models further to assess immune responses to cell death in vivo in established tumours.

Novel Mechanisms of antibody induced cell death

Monoclonal antibodies (mAbs) have revolutionised the treatment of B-cell malignancies. Although Fc-dependent mechanisms of mAb-mediated tumor clearance have been extensively studied, the ability of mAbs to directly evoke programmed cell death (PCD) in the target cell and the underlying mechanisms involved remain under-investigated. We recently demonstrated that certain mAbs (type II anti-CD20 and anti-HLA DR mAbs) potently evoked PCD through an actin-dependent, lysosome-mediated process (Ivanov *et al* J Clin Investigation 2009, Alduaij *et al* Blood 2011). More recently we have demonstrated that the induction of PCD by these mAbs, including the type II anti-CD20 mAb GA101 (obinutuzumab), directly correlates with their ability to produce reactive oxygen species (ROS) in human B-lymphoma cell lines and primary B-cell chronic lymphocytic leukemia cells. ROS scavengers abrogated mAb-induced PCD indicating that ROS are required for the execution of cell death. ROS were generated downstream of mAb-induced actin cytoskeletal reorganisation and lysosome membrane permeabilisation. ROS production was independent of mitochondria and unaffected by BCL-2 overexpression. Instead, ROS generation was mediated by NADPH oxidase. These findings provide further insights into a previously unrecognised role for NADPH oxidase-derived ROS in mediating nonapoptotic PCD evoked by mAbs in B-cell malignancies. This newly characterised cell death pathway may potentially be exploited to eliminate malignant cells which are refractory to conventional chemotherapy and immunotherapy. These observations are important for the understanding of cell death induced immune responses, which suggest that the generation of a successful host immune response towards tumour cell death is dependent on several factors, including the amount of cell death induced, the immunogenicity of the cell death and the tumour micro-environment.

Publications listed on page 77

TRANSLATIONAL RADIOBIOLOGY

Group Leader
Catharine M.L. West

Postdoctoral Fellows
John Hall
Amanda Williamson
Janet Taylor[1]

Clinical Fellows
Jonathan Bernstein
Navin Mani

Scientific Officers
Joely Irlam-Jones
Helen Valentine

Graduate Student
Guy Betts

Masters Students
Kenneth Oguejiofor
Junaid Iqbal Wahid

Scientific Administrator
Rebecca Elliott

[1]joint with Applied Computational Biology & Bioinformatics

The Translational Radiobiology Group explores approaches for predicting how a cancer patient is likely to respond to radiotherapy with a goal of developing biomarkers for the future individualisation of treatment. The group studies tumour hypoxia, tumour radiosensitivity and normal tissue radiosensitivity.

Can hypoxia biomarkers predict benefit from hypoxia-modifying therapy?

In head and neck and bladder cancer tumour hypoxia is associated with a poor prognosis. The addition of hypoxia-modifying agents to radiotherapy improves outcome and hypoxic status can predict treatment benefit, but there is no universal measure of hypoxia in the clinic. We are investigating whether a hypoxia-associated 26-gene signature reflects the hypoxic status of a tumour and predicts benefit from hypoxia-modifying treatment. The 26-gene signature was derived from three independent head and neck microarray datasets and showed prognostic significance in multiple cancers (head and neck, lung, breast). Multiplex markers such as gene signatures better reflect the complex cellular response to hypoxia. Work carried out by Guy Betts showed that a multiplex marker approach can account for intra-tumour heterogeneity of hypoxia better than use of single markers such as CA9 or pimonidazole (Betts *et al* 2012; Eur J Cancer).

Over the past year Amanda Williamson, Navin Mani and Joely Irlam-Jones showed that the 26-gene signature predicts benefit from hypoxia-modifying therapy in head and neck but not bladder cancer. Samples were available from 157 T2-T4 laryngeal cancer and 185 T1-T4a bladder cancer patients enrolled on the ARCON and BCON phase III randomised trials of radiotherapy alone or with carbogen and nicotinamide (CON) respectively. Customised TaqMan Low Density Arrays (TLDAs) were used to assess expression of the 26-genes using quantitative real-time PCR. The median normalised expression of the 26 genes was used to derive a hypoxia score (TLDA-HS). Patients were classified as TLDA-HS low (≤median) or TLDA-HS high (>median). The primary outcome measures were regional control (ARCON) and overall survival (BCON). Laryngeal tumours classified as hypoxic (TLDA-HS high) showed greater benefit from ARCON than non-hypoxic tumours (TLDA-HS low). Five-year regional control was 81% (accelerated radiotherapy alone) versus 100% (CON) for TLDA-HS high (log rank P=0.009). There was no significant improvement in five-year regional control for ARCON patients with TLDA-HS low. TLDA-HS did not predict benefit from CON in bladder cancer. Gene by gene analysis of expression of the 26-gene signature showed a similar profile in both cancer types (Spearman ρ=0.73, p<0.0001; Figure 1A) but differential expression was much greater in laryngeal cancer (Figure 1B). The median fold increase was 57.2 (range 5.7-8.3 x 103; laryngeal) and 43.3 (range 6.2-1.3 x 103; bladder). For TLDA-HS fold increase was 429.6 and 66.5 for laryngeal and bladder cancer respectively. The 26-gene hypoxia signature predicts benefit from hypoxia-modifying treatment in laryngeal cancer but not bladder cancer. In laryngeal cancer inter-tumour variation in TLDA-HS is 6.5-fold greater than in bladder cancer. The large dynamic range may permit more distinct categorisation of patients with clinically relevant tumour hypoxia. The predictive ability of the TLDA-HS now needs testing prospectively in a clinical trial of hypoxia modifying therapy in head and neck cancer.

HPV genotype does not influence tumour radiosensitivity

A long sought goal in radiotherapy related research is to derive not only a reliable approach for assessing tumour hypoxia but also an assay that measures tumour intrinsic radiosensitivity. As radioresistance limits the number of cancer patients cured, there is a need to understand the molecular mechanisms underlying radioresistance in order to develop methods to identify patients with radioresistant tumours and to develop new drugs targeting radioresistance to combine with radiotherapy. Radiosensitivity is a broad term applied to individuals, tissues and cells – it is genetically determined. The main mechanism of action of radiation is via induction of unrepaired double strand DNA breaks. Defects in DNA damage response pathways can have a large impact on radiosensitivity. However, the molecular mechanisms underlying differences in radiosensitivity between different types of tumours and tumours of the same histological type are not well understood. The group is interested in deriving gene signatures that reflect radiosensitivity in cervix and head and neck cancers. Human papillomavirus (HPV) are associated with the aetiology of both cancer types and work by John Hall and Jan Taylor over the past year explored whether HPV genotype influenced tumour radiosensitivity.

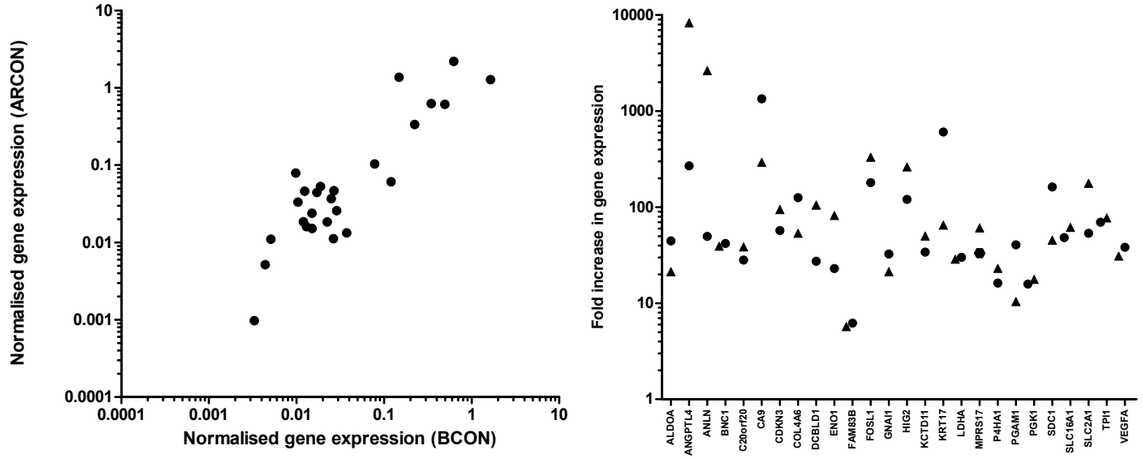


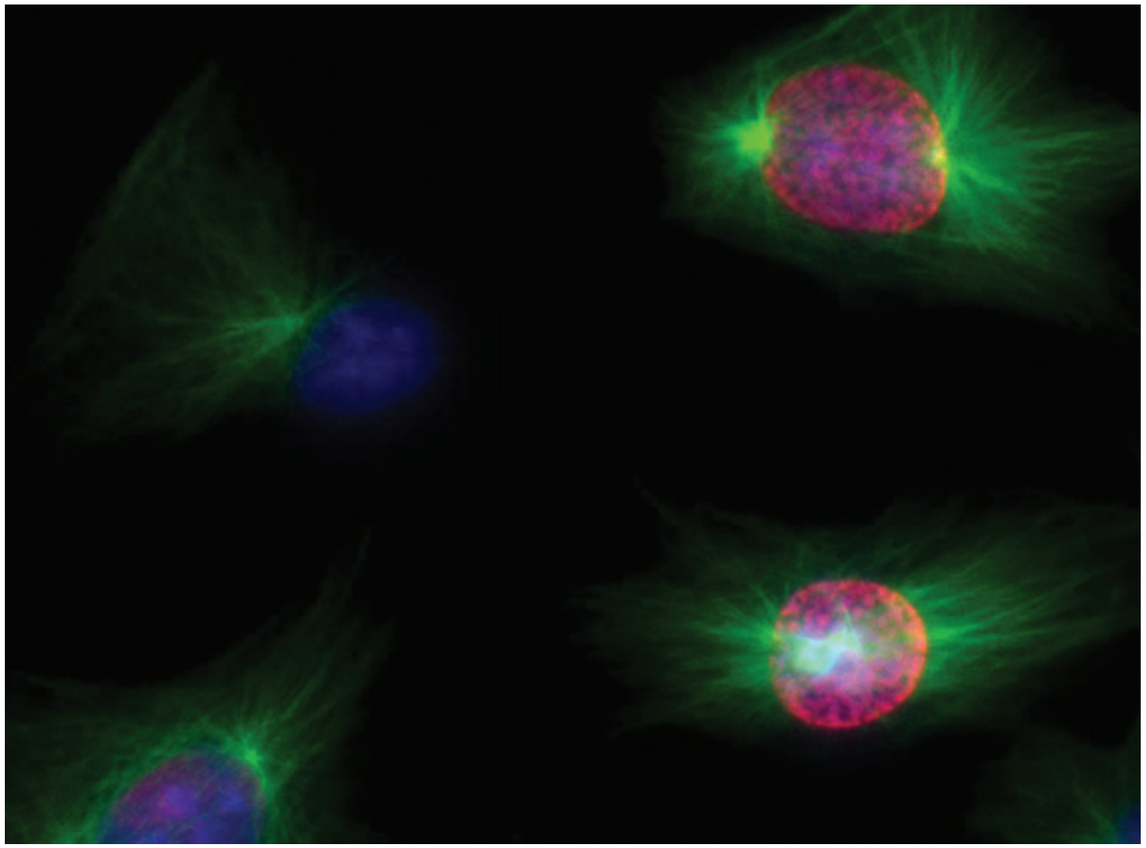
Figure 1
Gene by gene comparison of the 26-gene signature in laryngeal and bladder cancer. Left: Median expression of each gene is plotted for 157 ARCON laryngeal and 185 BCON bladder cancer patients. Right: Fold increase in gene expression between lowest five and highest five patient samples (■ =bladder; ▲=larynx).

Most cervical carcinoma are HPV positive with HPVα9 (primarily HPV16) and HPVα7 (primarily HPV18) genotypes accounting for >80%. As patients with α7 tumours tend to have poorer prognosis, we investigated the relationship between HPV genotype with outcome following radiotherapy and intrinsic radiosensitivity. HPV inno-LiPA25 was used to genotype cervix tumour biopsies (n=197). Papillocheck and qRT-PCR was used to genotype cervix cancer cell lines (n=16). Local progression-free survival following radiotherapy alone was assessed using log-rank and Cox proportionate hazard analyses. Intrinsic radiosensitivity was measured as surviving fraction at 2 Gy (SF2) using clonogenic assays. Of the 197 tumours, 106 (53.8%) were positive for HPV16, 28 (14.2%) for HPV18, 8 (4.8%) for multiple genotypes, 9 (4.6%) for HPV45, 23 (11.7%) for other HPV genotypes and 22 (11.2%) were negative. In 148 patients with outcome data, those with HPVα9-positive tumours had better local control compared with α7 patients in univariate (p<0.004) and multivariate (HR 1.54, 95% CI 1.11-1.76, p=0.021) analyses. There was no difference in the median SF2 of α9 and α7 cervical tumours (n=63). In the cell lines, there was also no statistically significant difference in SF2 between the α9 and α7 positive cells.

Normal tissue radiosensitivity

The group is also interested in developing biomarkers that predict a cancer patient's risk of toxicity following radiotherapy. The group co-ordinates the national RAPPER (Radiogenomics: Assessment of Polymorphisms to Predict the Effects of Radiotherapy) study (Rebecca Elliott, Helen Valentine), which involves a long-running collaboration with researchers at Cambridge University. Blood samples are collected from patients enrolled in late-phase clinical trials in patients undergoing potentially curative radiotherapy. Total accrual at the end of November 2012 was 4,891 with enrolment running 9% ahead of target. The excellent recruitment to the study reflects the commitment of the UK radiotherapy community with samples collected in 48 centres. Analysis of a genome wide association study in the first 1,853 patients was completed in 2012. The work was helped by the Radiogenomics Consortium, which the group helped to establish in 2009. Samples from the Radiogenomics Consortium were used in a rapid replication of the top single nucleotide polymorphisms (SNPs) identified. The work has shown there are many SNPs associated with radiotherapy toxicity and that larger cohorts must be studied to identify them with certainty.

Publications listed on page 77



Retinal Pigment Epithelial cells (RPE1) were arrested at the G2/M transition using the CDK1 inhibitor RO-3306, then released for 20 minutes to allow the cells to go into mitosis. Cells were fixed and stained with DAPI (blue), tubulin antibody (green), and phospho-histone H3 antibody to stain the cells which have successfully entered mitosis (red).

Image provided by Helen Whalley from the Cell Signalling Group.

PATERSON INSTITUTE

RESEARCH SERVICES, PUBLICATIONS AND ADMINISTRATION

RESEARCH SERVICES

www.paterson.man.ac.uk/research/

Head of Research Services
Stuart Pepper

The remit of the Research Services is to provide technology platforms that will support the research programmes of the Institute. During the past year, there have been a number of significant changes as well as notable achievements in these facilities. The head of our Flow Cytometry service, Morgan Blaylock, moved on to a new position having spent the last five years building up our in house FACS service to a very high standard. Morgan's contribution to the Institute will be greatly missed as he brought a great deal of expertise to his role.

Head of Research Services

Stuart Pepper

A common theme this year has been to look at the remit of each service and see how different technology areas are best supported. One result of this process has been the merging of Advanced Imaging and FACS into a single unit. This change will give a more robust team structure to manage technology platforms that support a broad variety of cell imaging applications. The need for close cooperation between different technical areas has also been recognised by the appointment of a post to link between Histology and Advanced Imaging to provide a seamless user experience for quantitative analysis of histological specimens.

The theme of collaboration has been evident in other facilities too; the Molecular Biology Core Facility has worked closely with the Clinical and Experimental Pharmacology (CEP) and Applied Computational Biology and Bioinformatics (ACBB) Groups to develop a workflow that allows robust expression profiling of single cells, while the Mass Spectrometry team have been involved in a number of collaborations which have led to several publications. Other major initiatives this year have been undertaken in the Biological Resources Unit (BRU) and Laboratory Services. Lab Services have had a new autoclave and glass washer installed as well as a laboratory refurbishment that will allow a new solution provision service to be developed. The BRU has been busy relocating transgenic breeding work from the Institute to our new facility in the University of Manchester's Incubator building. An ambitious program of re-derivation of mouse lines is currently underway.

Significant funding from the UK Research Partnership Investment Fund, that was awarded towards the end of 2012, is set to dramatically expand the range of technology platforms across the Research Services over the next couple of years. As the year comes to an end we are already beginning to plan how to use this funding to maximum effect to support our research programmes.

Advanced Imaging and Flow Cytometry Facilities

Steve Bagley, Jeff Barry, Mike Hughes, Abi Johnson[1], Kang Zeng[1]

[1]joined in 2012

During 2012, the management of the facility that visualises and analyses cells in high detail, and the facility that both sorts and analyses thousands of cells quickly, have been merged in order to provide a seamless array of tools for the researcher. Within the facility there have been a range of new developments and tools have been introduced which will provide faster methods of translating analogue systems (the cell) to digital data.

To help answer the questions that our researchers pose, the flow cytometry section of the facility comprises a range of flow cytometry sorters (BD Influx, BD Aria II and Jazz) and analysers (LSR Foretessa, Caliburs for 4 and 3 colour, and a FACS array). In the coming year an additional sorter and analyser will be installed to address equipment demands. Within imaging there are five large microscope systems (Deltavision, time-lapse, low light imaging, spinning disk confocal and macro-confocal), two scanners for digitising histology slides and two high content screening systems. Alongside the equipment are IT solutions for image processing and analysis (Imaris, Huygens and Definiens). A third histology scanner will be added in 2013 and additional high content screening systems and a super resolution microscope for molecular imaging will also be introduced in response to the demands for translational and molecular imaging.

In the Flow Cytometry laboratory a member of staff has been appointed to help calibrate equipment and to assist researchers with sorting samples quickly and efficiently. Usually sorting is used to find sub-populations of cells but there is also a requirement to sort cells singularly so that variation in gene expression and other parameters can be enumerated. Development over this year is ongoing to allow single cell sorts for molecular biology methods, which will be increasingly in demand over the coming year and will be achieved in conjunction with the Molecular Biology Core Facility. With the appointment of a new support post in the imaging facility, the main focus during 2013 is towards automated imaging so that numerical analysis can be achieved at a faster rate.

The Institute has been using automated systems to scan primary tissue for five years as this process removes the possibility of user-induced error and expedites analysis. This process employs pyramidal data formats, which is common with satellite and mapping data so the tissue can be visualised at a range of magnifications. Over the last year software has been introduced for the mathematical modelling of multiple labels in primary tissue and tissue microarrays. In conjunction with the Histology Facility, procedures are being put in place to allow histological preparations to be standardised for computerised analysis rather than relying on human inspection. This form of translational imaging and mathematical modelling is to be expanded in 2013 in order to allow more data to be processed at an increased turnover.

To advance development within the Drug Discovery Group, multi-well plates are now taken through high content screening so that responses to potential drugs and combinational drugs can be numerated. Multi-well plates are imaged and analysed in a semi-automatic manner to mathematically describe the effect of potential drug candidates.

New methods are being developed for the imaging and assessment of biomarkers in a cell population to model the heterogeneity of gene expression. Systems are being developed for scanning populations of primary cells and then numerically describing the data; in the coming year these techniques will be extended by using imaging flow cytometry, three dimensional culture and high resolution imaging.

Systems have been put in place during 2012 to allow as much of the equipment as possible to be run in a standardised, calibrated manner. As the amount of work that requires good clinical practice (GCP) standardisation increases, the facility has responded by implementing daily and weekly checks as well as incorporating a paper trail on all of the equipment. Training for researchers has been increased so that investigations can be designed incorporating the new techniques that are available and with a view to statistically analysing the results.

The data load for the facilities grew again this year to 17TB per annum, and the amount of raw data that was then processed for analysis also increased dramatically. A new member of staff will be appointed early in 2013 as a joint histology/imaging post; the role will be helping to develop numerical analysis of histological data and to fine tune the digitisation process.

Development projects over the coming year include: the merging of data from histology with other sources; investigating novel histology digitisation methods; sorting of single cells for clonal sequencing; development of protocols for imaging flow cytometry; the introduction of robotics and improved confocal optics for high content screening and the development of procedures for the modelling of variations in cell populations and photon based super-resolution microscopy by gated Stimulated Emission Depletion in order to visualise events at a molecular level.

Biological Mass Spectrometry Facility

Duncan Smith, Yvonne Connolly, John Griffiths

The key role of the facility is to enhance the research output of the Institute by facilitating access to Proteomic workflows for all groups onsite. The facility spans areas of activity from routine service provision, project design and data interpretation through to the bespoke collaborative development of novel workflows designed to answer previously intractable biological questions.

We recently acted as a beta test site for a new nLC MS (nano-liquid chromatography mass spectrometry) interface project named 'Kevlar'. This project has involved optimising the interface between ultra-high performance nano separations and electrospray mass spectrometry. As a direct result of these optimisations, we have improved the sensitivity of our Orbitrap based workflows by eight-fold. The beta test period involved the facility acting in an expert consulting and directing role. The conclusion of this period resulted in the gift of a full production unit of ThermoFisher Scientific's 'EASYSpray' apparatus allowing the Institute to benefit from this technology on a permanent basis.

During 2012, members of the facility were authors of seven peer reviewed publications including main authorship on three papers directly from our research and development pipeline. The first describes the development and utilisation of enhanced two dimensional peptide separation technologies in complex proteome analysis (Griffiths et al). The second describes a novel approach to the LCMSMS analysis of SUMOylation (Chicoree et al). Another study, (also Chicoree et al) describes the chemical derivitisation of Ubiquitin remnant isopeptides to facilitate the generation of diagnostic ions enabling their screening in advanced LCMS analysis. These three workflows are now directly accessible by PICR research groups.

Biological Resources Unit

Biological Resources

The animal facility has been fully occupied throughout 2012 both for Transgenic Production and Experimental Services with a total of 15 Project Licences in place with primary and secondary availability. The renewal of the Transgenic Service Licence over the summer has ensured production of progeny for the next five years for the scientific programmes.

Transgenic Services

The transfer of the Paterson Institute transgenic lines to the additional site at the University of Manchester's Incubator Building began at the end of the summer with the requirement to re-derive approximately 100 lines via embryo transfer; to date we have achieved 28 viable productions and this will continue throughout 2013. During this period the breeding and production at the Paterson Institute facility has been maintained to ensure that the research goals of the scientific groups are not disrupted.

The arrival of Professor Richard Marais, as both head of the Molecular Oncology Group as well as Director of the Institute, required an extensive re-derivation programme of the group's transgenic lines from The Institute of Cancer Research. During this period we transferred 20 live lines into our quarantine area and maintained minimal breeding programmes due to space restrictions whilst transferring clean embryos into the facility's transgenic area. These programmes are now expanded and to date we have 31 lines actively producing progeny for scientific use.

This year has also seen a steady demand for embryonic stem cell (ES Cell) microinjections with no less than 20 clones processed. Assistance has also been given to the Stem Cell Biology Group for the re-derivation of embryonic stem cell lines via blastocyst immuno-surgery for the production of numerous mouse embryonic fibroblast lines. Cryopreservation has continued throughout 2012 with approximately 4,200 embryos being archived. Sperm cryopreservation has also complimented the banking of strains during this period.

Experimental Services

The experimental area has been supported by four experienced licensed technicians who have delivered a varied range of non-surgical and surgical procedures for 11 research groups. The requirement for orthotopic models has increased this year; refinements have been made for the delivery of cells via intra-femoral injection and skills adapted and increased for the melanoma models.

As always, we have ensured that the highest quality of care has been provided, and that Home Office legislation has been adhered to, by performing daily health and welfare checks along with extensive monitoring for animals under procedure.

The experimental area of the animal facility will be required to expand in the foreseeable future as the transgenic area is decanted to the Incubator facility; the planning for this will begin in 2013.

The UK implementation of Directive 2010/63EU will take place on the 1st January, 2013, which will lead to some local changes for the management of the Certificate of Designation which will become the Licence 2C for the establishment. The requirement for a Training and Competency Officer will ensure that all Personal Licence Holders are assigned the correct category of authorisation and that in house processes are streamlined. During 2013 we will be working towards the required retrospective review for all animals under procedure to ensure that we best capture the required information for statistical return.

Histology

Garry Ashton, Caron Abbey, Michelle Greenhalgh (MCRC, Tissue Biobank) David Millard (Histology /Tissue Biobank), Deepti Wilks (Haematological Malignancy Biobank)

As the Histology Facility continues to be developed, the range of services offered continues to grow. As a result the unit has seen exceptional demand this year from both existing groups and new groups, resulting in all services offered being used extensively. The continued professional development and retention of staff has ensured that we continue to offer a comprehensive and flexible service. We specialise in the histological preparation and analysis of a wide range of samples including mouse model tissues, whole mouse embryo preparations, cell preparations and human biopsies. The samples are either frozen or embedded in paraffin. Several special stains have been used including Pearl's and Turnbull's Blue together with the Quincke's reaction for ferrous/ferric iron, Massons Trichrome for collagen, Masons Fontana, PAS and Neutral Red. In addition the use of special stains and the subsequent downstream extraction of nucleic acids from these tissues have also been evaluated.

The Stem Cell Biology Group has recently found that the first blood cells are generated from specific types of endothelial cells with haematopoietic potential and that the transcription factors Gfi1 and Gfi1b are critical for the development of these cells. Using serial frozen sections of developing embryos, together with immunohistochemistry (IHC), the group is trying to determine the spatial and temporal expression of these two genes, and investigate their potential co-expression, at both different sites of emergence of blood cells and in the context of other markers of endothelial and blood cells.

As the expansion of tissue biomarkers continues to gather pace, the construction of tissue microarrays (TMAs) has also seen a large increase with high throughput, and accurate TMA construction of tumour-specific and custom arrays giving true representation. Even more challenging has been the continued development of a truly representative prostate

core TMA. A new evaluation process has been introduced allowing the more accurate reporting of TMAs and a second platform has recently been introduced to cope with demand.

The Leica Bondmax automated IHC platform, together with the open Biogenix i6000, continues to offer a high throughput, routine, troubleshooting and antibody validation service. Demand on all these services has seen a sharp rise. Many antibodies have now been validated using the BondMax. In addition dual IHC and in situ hybridisation have also been performed on the platform. With increasing demand it is anticipated that a second platform will be purchased early in 2013. The antibody validation service together with a high throughput image analysis service will continue to play a pivotal role in tissue biomarker research.

In addition, we have continued to focus on the optimisation of pre capture variables and methods of cell identification for laser capture microdissection. Immunofluorescence and chromogenic IHC have both been used to identify specific cell immunophenotypes. The use of holding buffers during capture and sample acquisition have been evaluated. Results have been encouraging, allowing the extraction of good quality RNA and DNA. In addition, we have also successfully extracted good quality nucleic acids from both macrodissected tissue and whole samples using morphological analysis as a guide to disease status.

The unit continues to process formalin-fixed paraffin- embedded (FFPE) and frozen samples for the MCRC Biobank. To date, samples from over 3600 patients have been collected, with the largest number of samples from lung and prostate cancer. There has also been a significant increase in collections of colorectal and breast cancer samples as well as melanoma. Blood, bone marrow and plasma (at various disease status time points) from over 250 haematological malignancy patients has also been collected. To date, over 50 research projects have been approved. Samples have also been released for method validation studies. The number and types of samples processed for the Cancer Research UK Stratified Medicine Programme has also increased. Genetic results are now routinely being fed back to clinicians for these cancer disease types. Extensive quality control measures and the results from the Stratified Medicine Programme demonstrate the high quality of all the samples processed by the unit.

A technique that we developed that allows both the extraction of multiple cellular components from the same piece of tissue, in addition to allowing the morphology of the sample to be reported, has been used on several projects. In addition studies looking at the possible use of molecular-friendly fixation and processing, together with improvements in tissue stabilisation at time of sample acquisition, are ongoing.

Laboratory Services

Mark Craven, Andy Burns, Tony Dawson, Corinne Hand, John Higgins, Frances Hockin, Amy Moloney, Christine Whitehurst,

The Laboratory Services area has undergone some major improvements during 2012, and our extensive refurbishment plan will continue into 2013. At the start of the year the capabilities of the service were enhanced by the addition of a new glass washer and the replacement of an old autoclave with an updated model. This has allowed the team to maintain the provision of sterile glassware and solutions at times of peak demand.

This year there has also been a shift in the team operation to enhance the robustness of the service provided. The old system of having an individual lab aide allocated to each research group is being replaced by a centralised solution provision service. To facilitate development of this service a lab space has been completely refurbished to provide a clean environment for making solutions and media, and a cold room is being developed to allow bulk storage of media.

The team also provides a centralised Legionella monitoring service covering every tap in the building.

Laboratory Services also supports the Institute in other ways including monitoring and restocking First Aid supplies, maintaining the supply of clean lab coats and managing the two Institute X-ray film processors. Taken together the changes made to Laboratory Services during 2012 have allowed a small team to operate very efficiently, and support the Institute research programs across a range of services. In 2013 the refurbishment programme will be finished and the team will be able to further extend the range of media and solutions provided to the Institute.

Molecular Biology Core Facility and Cancer Research UK Microarray Service

Stuart Pepper, Chris Clark, Yvonne Hey, Rheanna Makorie[2], Gill Newton, Julie Watson[2], John Weightman[1], Jodie Whittaker

[1]joined in 2012, [2]left in 2012

The Molecular Biology Core Facility provides a range of services to support the research groups on site. These include diagnostic services, such as cell line authentication and mycoplasma screening, and research services for expression profiling and DNA sequence analysis.

Over the last year there has been an increase in the use of Next Generation Sequencing (NGS) to support a wide variety of projects. In the last year 19 NGS projects have been completed including sequencing of total RNA, PolyA RNA, microRNA, human exomes, Chromatin Immunoprecipitation (ChIP) samples and yeast genomes. During this we year we have also been part of a collaboration with the ACBB and CEP Groups to develop a workflow for sequencing RNA from single cells. This protocol was initially tested on expression arrays and then modified to be compatible with NGS. This workflow is now available to groups on site and will be of great interest for projects studying circulating tumour cells or cancer heterogeneity.

Alongside the demand for NGS we have also seen a continued need for microarray based expression analysis. The Cancer Research UK microarray service, based in MBCF, has processed around 500 arrays this year and we have seen 50 new projects either completed or initiated. With the advent of NGS for quantitative expression profiling it has been predicted that microarray expression profiling will be superseded; however, whilst we have seen some drop in demand for this service we are still seeing more new projects then many other facilities.

For expression profiling of individual genes, rather than entire transcriptomes, the facility has two ABI7900 machines with a choice of 96 or 384 well formats. These systems, in conjunction with our Probe Library, continue to be heavily used and provide a very cost effective route for small experiments on small numbers of samples.

The need for cell line authentication has become widely accepted in the research community. We have had a service up and running for three years and have seen good uptake by research groups. All cell lines in use in the building can now be regularly authenticated by Short Tandem Repeat (STR) profiling, which is the gold standard method for checking cell line identity. Combined with regular mycoplasma screening this gives researchers the necessary confidence in the cell lines they are working with.

The regular plasmid DNA preparation and Sanger sequencing services have continued to function well. Whilst demand for minipreps has dropped over the last year we have seen a continued high demand for DNA sequencing. As more validation work from NGS projects feeds through to the Sanger sequence service it is likely that our 3130xl sequence instrument will no longer have sufficient throughput. This machine has given reliable service in the facility for over a decade but looking ahead to next year we may need to expand our capacity for this service.

RESEARCH PUBLICATIONS

Crispin Miller (page 14)
Applied Computational Biology and Bioinformatics Group

Refereed Research Papers

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Carcinogenesis Group

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Cell Cycle Group

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Cell Regulation Group

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Clinical and Experimental Pharmacology

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DNA Damage Response Group

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Drug Discovery Group

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Immunology Group

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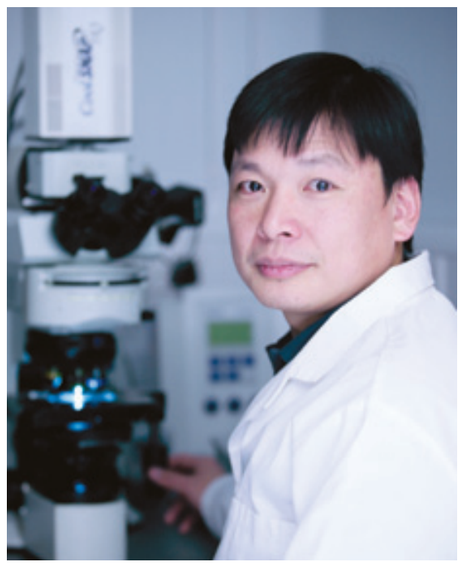

Nullin Divecha (page 36)

Inositide Laboratory

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John Brognard (page 42)
Signalling Networks in Cancer Group

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Georges Lacaud (page 44)
Stem Cell Biology Group

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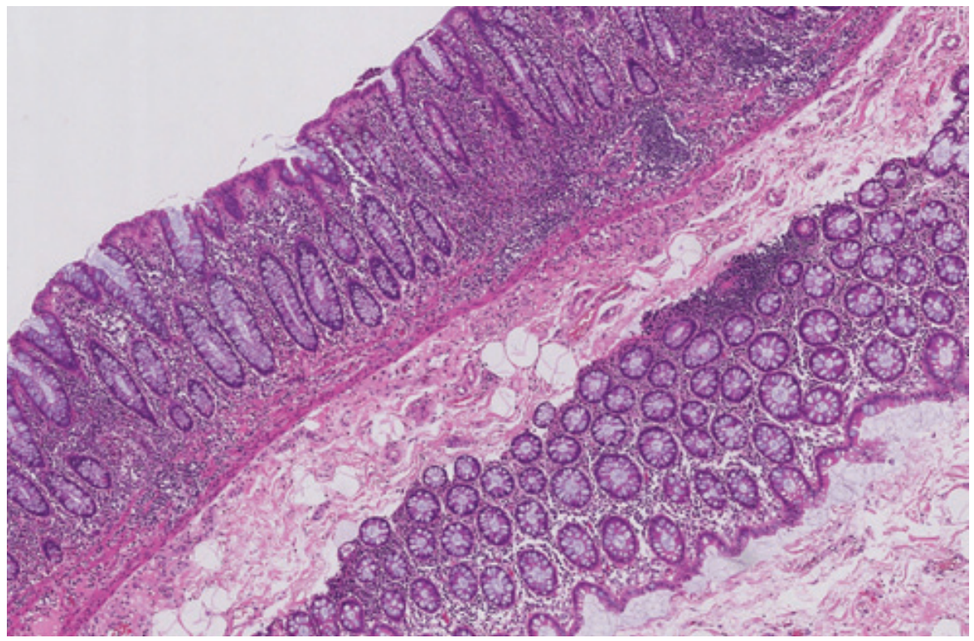

Valerie Kouskoff (page 46)
Stem Cell Haematopoiesis Group

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Vaskar Saha (page 50)
Children's Cancer Group

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Robert Hawkins (page 52)
Medical Oncology: Clinical and Experimental Immunotherapy Group

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Gordon Jayson (page 54)

Medical Oncology: Translational
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Tim Illidge (page 56)

Targeted Therapy Group

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Catharine West (page 58)

Translational Radiobiology Group

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Active Patents

WO Application No: PCT/EP2010/070583
Applicant: CR Technology Ltd. Hypoxia tumour markers.

Additional Publications

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PICR THESES

Elvan Boke

William Harris

Timurs Maculins

Lilly Sommer

Chong Tan

Ahmet Acar
Stromal-Tumour Interactions Group
The role of Notch Signalling in Human Breast Carcinoma-associated Fibroblasts

Julian Blaser
Leukaemia Biology Group/ Inositide Laboratory
The utilisation of shRNA screens to investigate the role of phosphoinositide modulator genes in ALL

Elvan Boke
Cell Division
An interplay between PP1 and PP2A phosphatases coordinates mitotic progression in fission yeast

William Harris
Leukaemia Biology Group
Regulation of Self Renewal in Human MLL-AF9 Acute Myeloid Leukaemia Stem Cells

Sarah Hughes
Clinical and Experimental Pharmacology
A pre-clinical and clinical evaluation of the specific endothelin-A antagonist zibotentan in prostate cancer

Timurs Maculins
Cell Cycle
Regulation of the replisome by the ubiquitin ligase SCFDia2

Avinash Patel
Cell Division Group
Interrogation of fission yeast polo kinase with quantative phosphoproteomics and chemical genetics

Andrzej Rutkowksi
Immunology Group
Investigation of a novel locus encoding a putative paralogue of 5T4 oncofoetal glycoprotein

Lilly Sommer
Inositide Laboratory
Nuclear phosphoinositides - Novel interactors and the regulation of the transcriptional coactivator TAF3

Chong Tan
Cell Signalling Group
A novel regulation of Tiam1 protein stability by ubiquitylation and its role in hepatocyte growth factor-induced cell scattering

EXTERNAL SEMINAR SPEAKERS 2012

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. We had another successful year for scientific interaction with an excellent set of internationally renowned speakers visiting the Institute. In its fourth year, The Breakthrough Breast Cancer Research Unit seminar series continues to produce an outstanding range of speakers. The postdoctoral researchers at the Institute also give weekly seminars which are very well attended and help to integrate the entire cancer research efforts of the Institute.

Dario Alessi
MRC Protein Phosphorylation Unit, University of Dundee

Peter Andrews
The University of Sheffield

Buzz Baum
MRC Laboratory for Molecular Cell Biology, University College London

Olivier Bernard
Institut Gustave Roussy, France

Christoph Borner
Institute of Molecular Medicine and Cell Research, University of Freiburg, Germany

Bob Brown
Imperial College London

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London Research Institute

Aled Clayton
Cardiff University School of Medicine

Ruud Delwel
Erasmus Medical Center, The Netherlands

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Institute of Microbiology, Oslo University Hospital, Norway

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Ulrike Gruenberg
Department of Biochemistry, University of Oxford

Kamil Kranc
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Massimo Lopes
Institute of Molecular Cancer Research, University of Zurich, Switzerland

Laura Machesky
The Beatson Institute for Cancer Research

Thomas Milne
The Weatherall Institute of Molecular Medicine, University of Oxford

Jacques Neefjes
The Netherlands Cancer Institute, Amsterdam

David Neuhaus
MRC Laboratory of Molecular Biology, Cambridge

Alexandra Newton
University of California at San Diego, USA

Mark O'Connor
AstraZeneca

Peter Parker
London Research Institute

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German Cancer Research Centre (DKFZ), Heidelberg

Erik Sahai
London Research Institute

JJ Schuringa
University of Groningen, The Netherlands

Charles Swanton
London Research Institute

Nic Tapon
London Research Institute

Ali Tavassoli
University of Southampton

Helle Ulrich
London Research Institute

Christopher Ward
The University of Manchester

Heidi Welch
The Babraham Institute

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LSU Health Science Center New Orleans, USA

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University of Texas at Austin, USA

Elad Katz
Breakthrough Breast Cancer Research Unit,
University of Edinburgh

Richard Pestell
Director of the Kimmel Cancer Centre at Jefferson, USA

Matthew Smalley
European Cancer Stem Cell Research Institute, Cardiff University

Marc Tischkowitz
University of Cambridge

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University of Cambridge

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Education Manager

Crispin Miller
Postgraduate Tutor
until September

Ian Waddell
Postgraduate Tutor
from October

A well-supported graduate programme is of fundamental importance to a research institute such as the Paterson, both to train the researchers of tomorrow, and for the valuable contribution made by our students to the laboratories that they are working in. In 2012, we welcomed another eight graduate students from around the world to join our PhD programme, working in fields as diverse as yeast genetics, stem cell biology and clinical research. During the course of the year, PhDs were awarded to nine graduate students and one clinical fellow.

The Paterson graduate programme

We aim for each student to receive high quality training in scientific research through an intellectually demanding but achievable research programme. Each project is peer-reviewed in advance and monitored throughout the course of their studies, via a mixture of seminars, written reports, and progress and planning meetings. These are designed not only to provide formal points at which progress (of both the student and the project) can be monitored, but also to help develop the presentation skills that are so fundamental to the majority of careers in science and elsewhere. Graduate training is monitored by an Education Committee, which features Group Leaders, senior clinicians and scientists, and student representatives (see below). Following the directive from the University of Manchester following a QAA (Quality Assurance Agency) audit, students registering from September 2011 onwards have also been appointed a second co-supervisor who is able to provide additional advice and consultation on academic and non-academic matters. Each student is also assigned an advisor (similar to a personal tutor on an undergraduate programme) whose role is to provide impartial support and advice. Further support is also available individually from the Education Committee Chair Postgraduate Tutor, Postgraduate Manager, or collectively as the Education Committee Administration Group.

The Paterson Institute runs an external seminar series featuring talks from many of the key players in cancer research, and students are expected to attend all of these external seminars. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding work from the leaders in different disciplines, which will give them a broad understanding of many aspects of cancer research and basic biology. In addition, we hold a series of weekly postdoctoral research seminars that the students attend, while students themselves are asked to give talks at key points during their PhD studies. They also have opportunities to present their work at lab meetings within the Institute.

The annual Paterson Institute Colloquium, held in September, is an excellent opportunity for our new intake of students to meet other established PhD students, as well as other members of the Institute, including Group Leaders, Postdoctoral Fellows, and Scientific Officers. This forum provides up to date scientific presentations by Group Leaders and second year PhD students together with poster presentations by Postdoctoral Fellows, Scientific Officers and other PhD students.

PhD studentships

All our CR-UK core funded studentships are four years in duration, and consist of an approved research project in one of our research groups. Some students have joint supervisors in different groups, fostering collaborations and exposing the students to different disciplines. Recruitment is highly competitive, with hundreds of applications competing for approximately 4-8 places each year. Interviews are typically conducted over a two-day period in January.

All our students benefit from access to advanced state-of-the-art facilities including advanced imaging, biological mass spectrometry, microarrays, flow cytometry, histology and next generation sequencing. Our research groups offer PhD studentships and projects covering the entire breadth of research within the Institute.

Fellowships in Clinical Pharmacology Research

In order to help train the next generation of clinical pharmacologists with expertise in oncology, the Paterson Institute, in collaboration with the MCRC and AstraZeneca, established a fellowship scheme in Clinical Pharmacology Research in 2007. The fellowships are open to applicants who have obtained, or are close to obtaining, their Completed Certificate of Specialist training (CCST) in Medical Oncology.

Each clinical Pharmacology Research Fellow undertakes a three-year PhD project, which provides training in biomarker discovery, method development/validation, and in clinical trial methodology. During tenure, at the Christie NHS Foundation Trust/Paterson Institute, the post holders receive clinical supervision from Malcolm Ranson, and laboratory- based training from Caroline Dive in CEP (in collaboration with MCRC colleagues); at AstraZeneca they receive training in clinical trials management, regulatory interaction, translational research through project management and attendance at investigator meetings. Clinical training includes one research clinic per week, training in clinical trial design and methodology, ICH-GCP, EU Directives and research governance. Biomarker method development and application take place on both sites in all projects, with mutual benefit as each Fellow brings newly acquired knowledge to each site. Regular meetings take place between the Fellows, their supervisors, as well as other staff members involved in the project, ensuring true collaboration and a 'joined-up' approach.

Education Committee 2012

Our goal is for every student to have a project that is both achievable and intellectually demanding. Projects and students are monitored by the Education Committee which makes sure that the proposed plan of research is suitable, and that progress follows an appropriate timeline throughout the course of the studentship. Various assessments throughout the PhD programme, including regular talks, progress meetings and written reports are an essential key in ensuring successful completion of the PhD programme.

Valerie Kouskoff (Chair, Education Committee)
Anne Armstrong[1]
Fiona Blackhall
Richard Cowan
Julie Edwards
Dave Gilham
Ian Hampson
Sacha Howell[1]
Karim Labib
Was Mansoor[1]
Angeliki Malliri
Crispin Miller (Postgraduate Tutor until September)
Donald Ogilvie
Vaskar Saha[2]
Tim Somervaille
Ian Waddell (Postgraduate Tutor from October)
Catharine West
Caroline Wilkinson
Richard Marais[1] (Ex-Officio Member)

Student Representatives

Emily Holmes[2]
Hadir Marei
Danish Memon

[1] Joined in 2012
[2] Stepped down in 2012

OPERATIONS

Margaret Lowe
Head of Finance

Stuart Pepper
Head of Research Services

Rachel Powell
Head of Human Resources

Caroline Wilkinson
Scientific Operations Manager

The Operations Department provides the necessary infrastructure and services to facilitate the running of the Institute. Finance and purchasing, as well as Estates and Logistics, fall under the leadership of Margaret Lowe, while Stuart Pepper oversees IT as well as Health and Safety; Rachel Powell is head of HR and Caroline Wilkinson is responsible for all aspects of Scientific Administration. This year we said goodbye to Amy Weatheritt but welcomed Siana Peters to the Administration team. Gillian Campbell was recruited to the Scientific Administration team, in the newly created post of Grants Advisor, to help the Institute's scientists to secure additional external funding which will support the full breadth of research that we would like to undertake.

Administration Department

Siana Peters (EA to the Director), Ruth Perkins, Steven Morgan

Siana Peters joined the Institute in early 2012 to provide personal assistance to Richard Marais in his post as Director of The Paterson Institute as well as overseeing the management of the administration team. The department has assisted with the organisation of several events over the course of the year, including the Paterson Colloquium and quinquennial reviews, as well as running the main Institute reception and coordinating catering requirements. Administrative support is also provided for the external seminar series. The 2012 series has been a great success and the 2013 programme is already in place. This will aim to provide a varied programme of national and international speakers, serving to foster collaboration and encourage positive interaction within the wider scientific community.

Details can be found at:
www.paterson.man.ac.uk/Seminars/.

Estates

Steve Alcock, Graham Hooley, Tony Woollam

A number of small projects have been carried out in order to improve certain areas and ensure that the building remains fit for purpose. Improvements to the Laboratory Services area are ongoing which will allow for the expansion of these facilities in order to incorporate the new media preparation service. Other improvements included the conversion of an old storage area into a tissue culture facility and the refurbishment of laboratory and office space to accommodate the new Director and his group.

The Estates team endeavour to identify sustainable solutions when implementing new schemes together to help reduce the Institute's carbon footprint whenever possible.
The team has been pro-active throughout the year, attending to many legislative requirements including Legionella best practices and fire alarm testing. A continual objective is to acquire new skills and refresh existing ones and with this in mind, team members have attended a number of relevant courses to keep their knowledge up to date with current working practices and changing legislation.

Finance & Purchasing

Margaret Lowe, David Jenkins, Denise Owen, Muhammad Raja, Debbie Trunkfield

The Finance Department is responsible for providing a comprehensive purchasing, travel and finance service to the Research Groups and Service Units within the Paterson Institute. The procurement team continues to work with the research groups to identify savings in consumable spending.

In May, the University introduced a new Travel Management System, Egencia, to cover all corporate travel needs. This enables the university team to capture all the necessary data to allow them to have up-to-date management information and identify trends for the Central Procurement Office to negotiate route/volume deals with airlines and hotels. Egencia has a price match promise on all hotels and flights. Our procurement team has worked closely with staff to ensure their needs are met and all bookings are authorised immediately.

In addition to the everyday purchasing and finance procedures, we have continued to support the research groups by providing effective and efficient professional advice when costing new research proposals and administering existing grants. The Institute has been successful in securing several new external grant awards that will be activated in 2013.

Health & Safety

Colin Gleeson

A new intranet based genetic modification (GM) risk management system was developed, tested and then introduced throughout the Institute in 2012. This comprises of risk assessment forms for assessing GM work and an integrated document management system. The new system should enable efficient risk management of GM work and facilitate our compliance with the Genetically Modified Organisms (Contained Use) Regulations.

A number of pro-active initiatives were undertaken throughout the year to help safeguard health and safety. These included the annual self–assessment biosafety survey, the formal laboratory inspection programme, a review of UV personal protective equipment and a well-being back care programme, which included sessions in Alexander technique and Pilates. Some remedial actions were generated and completed successfully. The back care programme received highly positive feedback from the participants.

Following analysis of the performance of the health and safety management systems, additional Key Performance Indicators (KPIs) were introduced which will provide better information about the performance of the management system. The KPIs selected include pro-active and reactive elements of the management system.

A number of compliance surveys were undertaken to ensure that the Institute meets its legal duties for biosafety-associated legislation; this included schedule five of the Anti-Terrorist Crime and Security Act, Misuse of Drugs legislation, the Human Tissue Act and the Chemical Weapons Convention.

The Paterson Institute was visited by two enforcing authorities in 2012. In April the Greater Manchester Fire and Rescue Service carried out a fire safety audit. Encouragingly, no specific recommendations were made in relation to fire safety legislation. In July, the Environment Agency carried out a radiation audit which found the Institute to be compliant and did not lead to any remedial actions.

Training has been provided for new starters in the form of induction training, and for laboratory workers who are working with biological agents and hazardous chemicals. For this purpose, a new training record form was developed and is being utilised by staff.

Finally a review of respiratory protective equipment is being undertaken, in light of updated guidance from the Health and Safety Executive. This will continue into 2013.

Human Resources

Rachel Powell, Laura Jones, Julie Jarratt

Over the past year the Human Resources Department has continued to successfully provide a high quality professional and pro-active HR service to the Institute. This includes providing advice and guidance to managers and staff on all employment-related matters such as recruitment, policy guidance, legislation and best practice.

The last twelve months has seen the successful appointments of 51 individuals who will endeavour to complement and enhance the work of the Institute. Throughout the year we always look for ways to improve our processes and the service that we provide to staff. This year, for example, we have improved our new starter process. We ensure that all new employees attend an HR induction on their first day. It is important that an individual is given the best start and that their time at the Institute is an

informed and enjoyable experience from the beginning.

We have continued our joint partnership working with the unions which has resulted in the agreement of several new policies, including the Staff Training and Development Policy, and the revision of existing ones such as the Mentoring Framework and the Flexible Working Policy. Over the next year the main focus for the HR department will be the successful implementation of the online probationary process system and a review of the current contribution review process.

Information Technology

Malik Pervez, Brian Poole, Steve Royle, Zhi Cheng Wang, Matthew Young

The IT department provides a balance of quick response user-facing services and strategic developments to ensure that the IT infrastructure remains fit for purpose as the Institute develops. During 2012, much of the strategic development has been to enhance the support for mobile computing, along with upgrades to both the hardware and software that comprises the core IT infrastructure of the building.

The most significant IT challenge was the ever increasing demand for storage capacity. In order to address this need a new storage system has been procured and installed. The new unified storage solution is highly resilient and scalable and will allow highly cost effective expansion of storage in the future.

During 2012, we also improved the IT Business continuity strategy to reflect the new storage solution and ensure that we have the required resilience if the need arises. We also re-evaluated and subsequently optimised the back-end systems to improve performance, stability and security. As the need for mobile computing continues to increase, we developed a strategy for mobile devices and during the year we procured in excess of 50 mobile devices in the form of tablets and smart phones to give service users as much freedom and flexibility with their off-site computing as possible. A further programme of work was developed to set up and support staff based at a satellite office on the main University campus.

Plans to upgrade the high performance cluster (HPC) have been developed and will be implemented in the first quarter of 2013. The changes will provide the computing power that is necessary to analyse the large data sets being generated by the latest technological platforms.

Logistics

Maurice Cowell, Sedia Fofana, Antony Griffin, Phil Jackson, Andrew Lloyd, Jonathan Lloyd

The Logistics facility plays a vital role in supporting the research carried out at the Institute. This includes the receipting, checking, booking in and distribution of goods ordered by staff as well as the collection and removal of waste. The team also provides assistance with moving equipment and supplies and therefore helps facilitate internal rearrangements and the arrival of new groups. A number of larger liquid nitrogen vessels are now in operation, increasing the usage of nitrogen and its transportation to the labs. This is performed three times a week with dry ice deliveries taking place twice a week. Gas cylinders are also monitored and replaced as necessary. Savings have been made following an audit of gas usage which allowed us to reduce cylinder rental charges.

Researchers can order central stores stock items via the intranet which are then distributed by the Logistics team who also ensure that adequate stock levels are maintained at all times. Included in this system are the enzymes and media stored in the Institute freezers (Sigma, Invitrogen, Roche, Promega, New England Biolabs, Fisher kits and Qiagen). This year we have had a Bio-Rad fridge/freezer installed which operates on a password and scanning system. The department works closely with all the research groups and helps out where necessary, for example by tracing and confirming delivery of goods with suppliers. The team also manages the moving of heavy equipment or furniture, and the reconfiguration of meeting rooms for numerous events.

Scientific Operations

Caroline Wilkinson, Gillian Campbell

Scientific administration is overseen by the Scientific Operations Manager, Caroline Wilkinson, who provides support to the Director in order to facilitate the day-to-day running of the Institute. The Scientific Operations Manager provides reports for, and liaises with, a number of sources, including Cancer Research UK and the University of Manchester, as well as editing publications such as the Annual Scientific Report and Institute Newsletter and writing updates for the intranet and external website. Talks and tours are also provided for a packed programme of fundraiser events throughout the year.

Further responsibilities include organising Quinquennial and mid-term reviews, liaising with library staff at the University of Manchester to secure access to appropriate electronic journals, maintaining detailed databases of the Institute's publications, arranging a weekly seminar series presented by our post-doctoral research fellows and organising the Institute colloquium. For the second year running, the Colloquium was held at Lancaster University with over 100 Institute scientists enjoying two days of scientific talks and poster sessions. All grant submissions submitted by our researchers are screened for the appropriate ethical approvals as well as the ability of the Institute to accommodate the proposed programme of work. This year, the new post of Grants Advisor was created to facilitate the procurement of external funding by Group Leaders through identifying relevant opportunities, assisting with grant preparation and overseeing an internal grant review process. Gillian Campbell was recruited to this post in May 2012 and also participates in other aspects of scientific administration.

Cancer Research Technology

Martyn Bottomley

Cancer Research Technology (CRT) is a specialist oncology-focused development and commercialisation company wholly owned by Cancer Research UK. CRT aims to maximise patient benefit from publicly funded research worldwide by advancing research discoveries into development with pharmaceutical and biotechnology parties.

At CRT we bridge the gap between cutting edge academic research and industrial development of cancer therapeutics and diagnostics. We achieve this by working closely with prestigious international research institutes, such as the Paterson Institute, and with funding bodies to develop, protect and commercialise oncology related discoveries. Core activities of business development and drug discovery are supported by specialists, integrated in the business with expertise in patents, legal, finance and marketing. Our exclusive focus in oncology provides an unrivalled depth of knowledge and experience in cancer-specific translational development and commercialisation. By arrangement with The University of Manchester, CRT owns and is responsible for the development and commercialisation of intellectual property arising from Cancer Research UK funded research at The University of Manchester (including the Paterson Institute).

Our relationship with the Paterson Institute reflects the specific requirements of the scientist, the Institute itself, Cancer Research UK and the individual project. To effectively facilitate these requirements and interactions, Martyn Bottomley, a CRT Business Manager, is based on-site dedicated to working closely with the staff at the Institute and also The University of Manchester. Martyn offers access to oncology focused expertise in technology evaluations, patent applications and management, funding for development, commercialisation, drug discovery, market intelligence, and project management. He also works closely with UMIP, The University of Manchester technology transfer organisation. CRT continues to work very closely with the Drug Discovery Laboratories based at the PICR to facilitate the development of small molecules drug therapies to satisfy the unmet clinical needs of cancer patients. CRT is currently actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the PICR that continue to attract commercial partners (ranging from enabling technologies through to drugs in late stage clinical trials). We look forward to building on our successes and continuing to work closely with the PICR to advance discoveries that will allow us to beat cancer in the years ahead.

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Cancer Research UK's Research Engagement Manager

James Dunphy

The Paterson Institute has had a fantastic year of supporting fundraisers, thanking them and joining them in a range of activities.

Representatives from the Institute have been involved in over 50 events, reaching approximately 25,000 supporters. The Institute has welcomed record numbers of the public through its doors, with over 500 people visiting a variety of laboratory tours, open days, open evenings and other public events. This also included an event to launch the new Cancer Research UK brand to local staff, researchers and fundraising groups.

The Institute has enthusiastically entered teams into a wide variety of fundraising events and challenges, raising thousands of pounds for charity. This includes; running, 24 hour and 40 mile walking, mountain climbing, cake-baking, hairdressing and moustache-growing.

A team of 13 chemists and biologists from the Drug Discovery Group participated in the Great Manchester Run. They formed part of the 1400-strong team who took part, raising an estimated £180,000 for Cancer Research UK. Kate Smith explained their reasons for taking part:

"We all felt we should do something to raise money for CR-UK as we know only too well how vital fundraising is to the charity. It was also our way of getting out and thanking all the fundraisers who work so hard for us and allow our team to carry out such fantastic work here in Manchester. The total raised as a team was £3200 and we thoroughly enjoyed taking our lab coats out of the lab and onto the streets of Manchester!"

The Paterson Institute also entered a team in the local Relay for Life event. The relay is a 24 hour event where at least one member of the team has to walk around the rugby pitch at any time. During the day the team engage with supporters by showcasing local research in an interactive way. This year was the fourth year a team has been entered and they raised over £2300 for CR-UK.

Shameem Fawdar (a Postdoctoral Fellow in the Signalling Networks in Cancer Group) demonstrates gel loading to Celia Russell, one of the grand-daughters of Drs Ralston and Edith Paterson. Watching the demonstration is Dr Elspeth Russell, the daughter of the Patersons along with one of her grand-daughters, Phoebe.

The Drug Discovery Chemists get ready to run.

The Relay for Life team made up of some of the Institute's Post-doctoral Fellows and PhD students.

One of the most important events of the year is the annual open day. The open day provides the Institute with the opportunity to give supporters and the general public a research update along with demonstrations of the science that is undertaken, whilst thanking them for their endeavours. This year was the most successful open day yet, with over 120 visitors enjoying a presentation from Professor Richard Marais about the Institute and his research into melanoma followed by a tour of several labs. Some very special guests were also invited and they took the time to write in after their visit:

"As the daughter of Ralston and Edith Paterson (the original Directors of the Institute), I particularly enjoyed the Open Day as did my daughter Celia and grand-daughter, Phoebe who came along with me. We found the visits to the research laboratories both interesting and informative. My parents would have been very proud of the enormous strides made in research at the Institute. Elspeth Russell (nee Paterson)."

The Paterson was once again a designated 'pit-stop', used to support CR-UK fundraisers who were walking 26 miles for the Shine event. This year 15 volunteers from the Institute worked through the night, providing hot drinks, food and encouragement to the 2000 participants who passed by. Head of Research Services, Stuart Pepper, was in charge on the night:

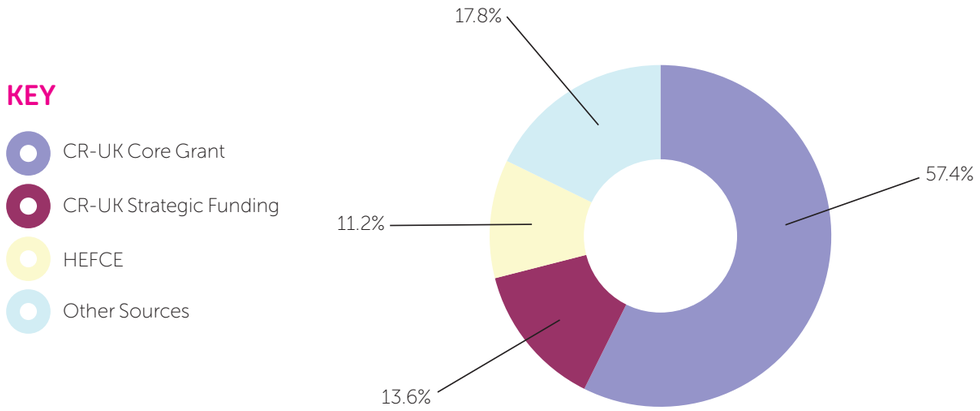
"Helping these committed fundraisers on their way can be both uplifting and quite emotional as people often want to share a few words about their experiences ".

It is hoped that the 3000 people who took part in the 13 mile and 26 mile walks will raise over £500,000 for the charity.

ACKNOWLEDGEMENT FOR FUNDING OF THE PATERSON INSTITUTE

The total funding of the Paterson Institute for 2012 was £18.7m. The major source of this funding was awarded by Cancer Research UK (CR-UK) via a core grant of £10.7m plus additional strategic funding of £2.5m. This funding enables the various scientific groups and service units within the Institute to carry out their research.

PATERSON INSTITUTE FUNDING 2012



The infrastructure of the Paterson Institute is funded by HEFCE generated income at a cost of £2.9m.

The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- Astra Zeneca
- Roche
- European Commission
- BBSRC
- Leukaemia & Lymphoma Research Fund
- Novartis
- Qiagen
- Chugai
- Abbott Laboratories
- Cambridge University
- GlaxoSmithKline
- Christie Hospital NHS Foundation Trust
- Association for International Cancer Research
- Medical Research Council
- Kay Kendal Leukaemia Fund
- Wellcome Trust
- Parsortix

We are immensely grateful to all our sponsors.

CAREER OPPORTUNITIES AT THE PATERSON INSTITUTE

The Paterson Institute is located alongside The Christie NHS Foundation Trust, and has a strong programme of basic and translational research. There are very close links with clinical and translational research groups throughout the Christie Hospital site.

The Manchester Cancer Research Centre (MCRC) was created nearly six years ago by its partners The University of Manchester, The Christie Hospital NHS Foundation Trust and Cancer Research UK. This is an extremely exciting development which is enhancing all aspects of cancer research, education and treatment. The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular services, a microarray platform, proteomics, flow cytometry, histology, the production of knock-in/knock-out animal models, real-time PCR, next generation sequencing and advanced imaging. Details of all groups and facilities are given throughout this report, and can guide interested parties to the appropriate contacts.

Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitable qualified graduates to apply to join either the PhD or MD programmes. Graduates with a first or 2.1 honours degree in a biological science can apply each year to train for a four-year PhD in one of our research laboratories. The University of Manchester offers a wide range of training for new and existing students which provides opportunities to acquire skills that will complement the research programme and help achieve personal and career development goals. At the Paterson, we also ensure that postgraduate students are provided with high quality, relevant and appropriate training alongside development opportunities. The Institute also has a well-developed process for ensuring suitable pastoral care and mentoring for all students.
Postdoctoral applicants of high calibre are regularly sought. Although post docs will be encouraged to apply for their own fellowships, funded positions are available for outstanding candidates. Interested applicants should contact the Group Leaders directly, with details of their area of interest and recent experience.

In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding for personnel, recurrent expenditure and equipment. Junior Group Leaders are appointed for an initial six-year period with a review between five and six years for consideration for promotion to Senior Group Leader, with Senior Group Leaders appointed to non-time limited positions.

Specific vacancies can be found on our web pages (*http://www.paterson.man.ac.uk/jobs*), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

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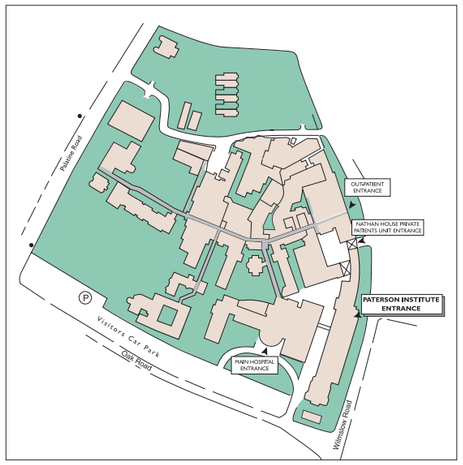
Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).
Registered address: Angel Building, 407 St John Street, London, EC1V 4AD.

Tel 44(0) 20 1234 5678
www.cruk.org



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