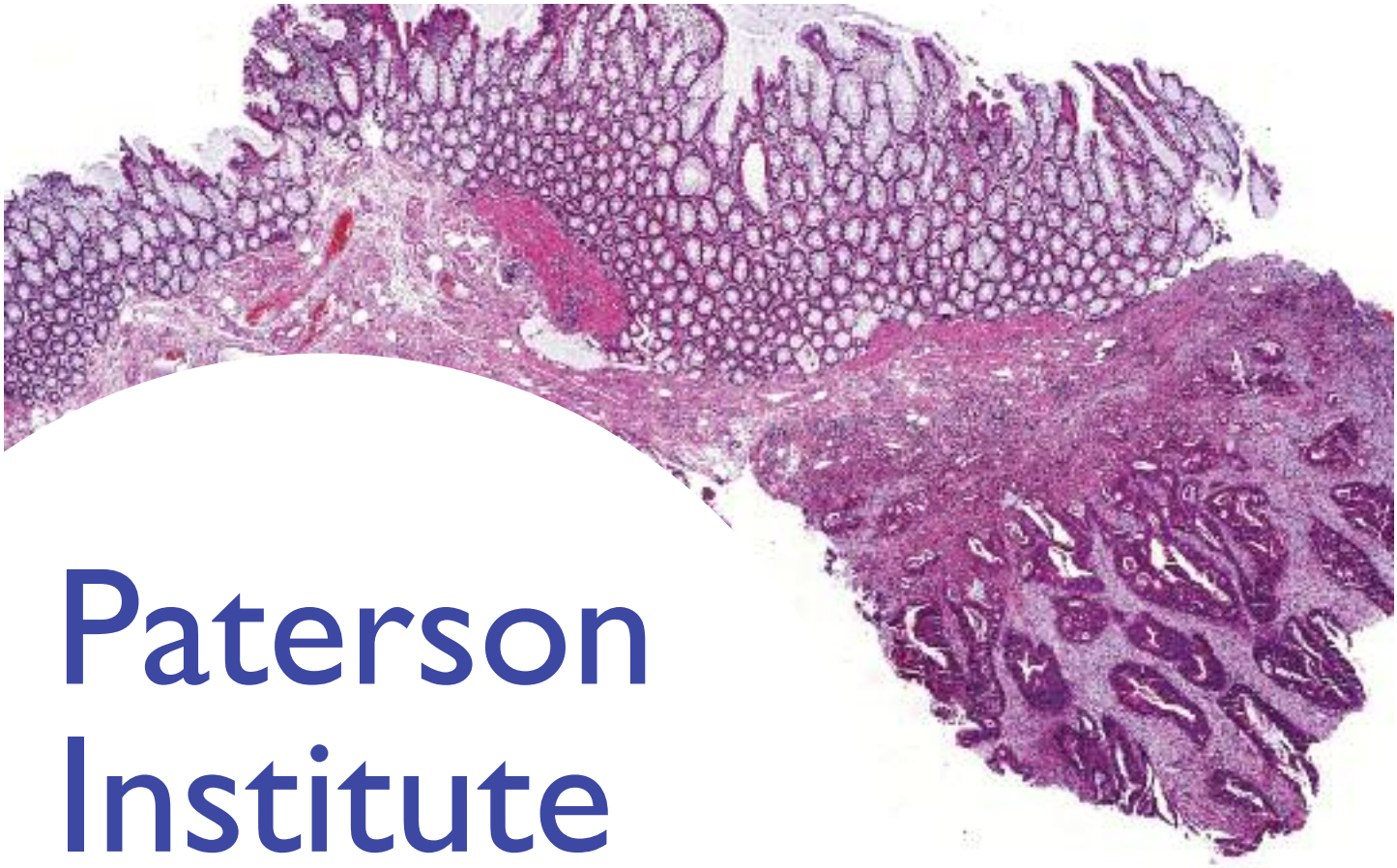

Paterson Institute for Cancer Research

Scientific Report 2011

Together we will beat cancer

CANCER RESEARCH UK

Cover images

Top
A histological section of a colorectal tumour, showing both the tumour itself on the right and the more organised normal tissue in the centre and on the left. The section has been stained with haematoxylin and eosin to show the various structures within the tissue. The sample is approximately 5mm in length. Image provided by Darren Roberts from the Immunology Group.

Bottom
This is a cytospin preparation of bone marrow harvested following treatment of mice with 10mg/kg oxaliplatin & stained with May-Grunwald-Giemsa stain. Evidence of dying cells can be seen in this sample, as well as the usual bone marrow cells - macrophages, red blood cell precursors, megakaryocytes & neutrophils. This experiment forms part of a project that is looking to correlate the expression of a potential toxicity biomarker in the blood with toxicity in bone marrow. Image provided by Cassandra Hodgkinson from the Clinical and Experimental Pharmacology Group.

Scientific Report 2011

Paterson Institute for Cancer Research

Paterson
Institute for Cancer Research

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK

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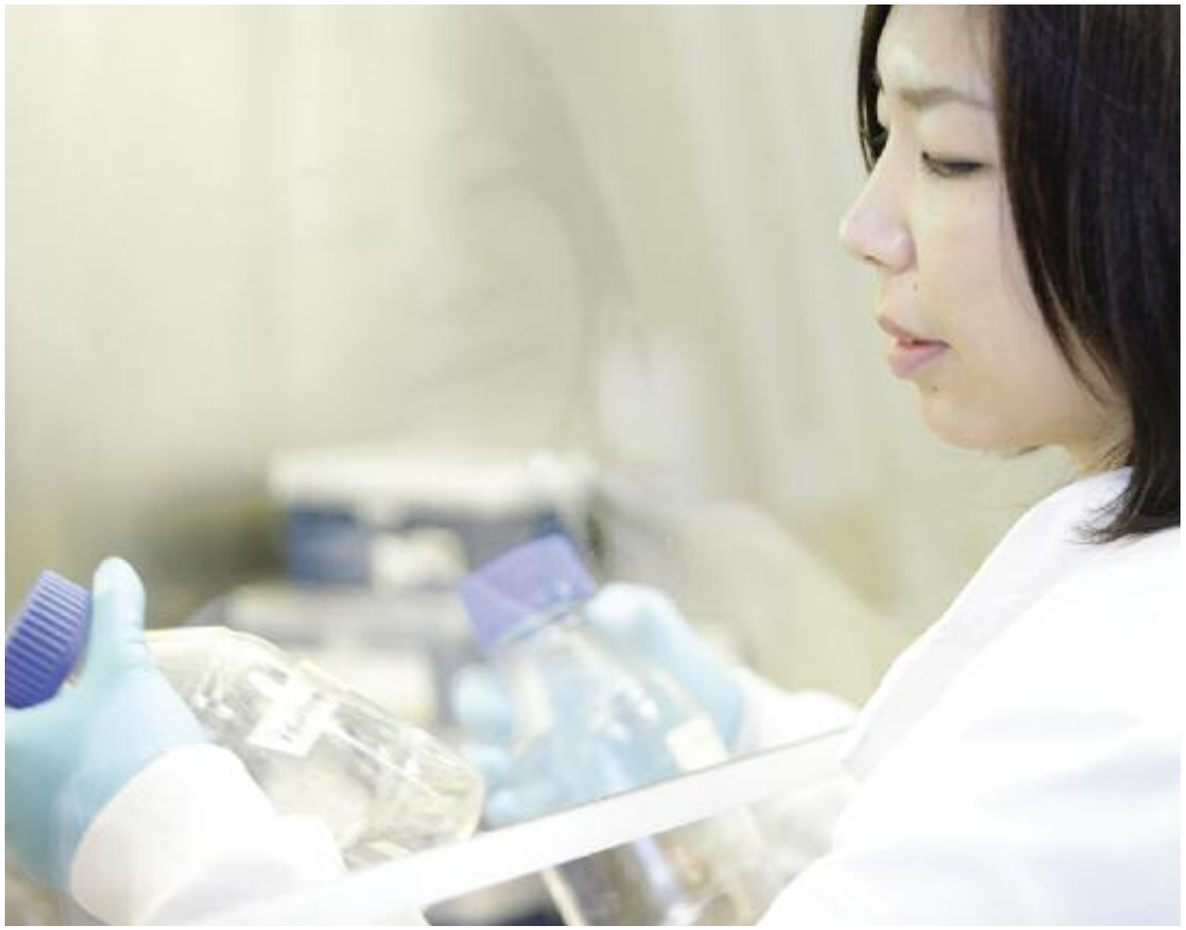

Professor Caroline Dive
(Senior Group Leader)

Professor Iain Hagan
(Senior Group Leader)

Pippa McNichol
(Director of Operations)

Senior Management Team's Introduction

Welcome to the 2011 Paterson Institute Annual Scientific Report. There have been significant changes at the Institute over the past year, most notably with Nic Jones stepping down as Director to take on the prestigious and challenging role of Chief Scientist at Cancer Research UK. We take this opportunity to reflect on Nic's achievements during his tenure as Director, to celebrate our success stories of 2011 and to look forward to an exciting new era in the development of the Institute.

Nic Jones joined the Institute in 1999 and, following a scientific review, instigated a major re-organisation of its research programmes and operations. He spent twelve years leading the Paterson during which time it has undergone enormous development. The number of research groups has expanded, a CR-UK funded drug discovery programme has been introduced, and a research services strategy established to support the increasingly complex technology that underpins world class cancer research. These achievements have led to higher quality research and greater international recognition of the Institute.

One of Nic's strongest legacies has been the development of strategic partnerships which serve to facilitate a fully integrated approach to cancer research in Manchester. Most significantly, in 2006, the Paterson became an Institute of the University which was accompanied by the creation of the Manchester Cancer Research Centre. This federation brings together the basic and translational cancer research carried out at the University of Manchester with the clinical research of the Medical School and The Christie NHS Foundation Trust. This helps to foster collaborations between basic scientists and clinicians that are essential if we are to develop more effective ways of diagnosing and treating cancer. The development of the MCRC, along with the activities of the Paterson Institute during Nic's tenure, played a large part in the University of Manchester being judged the leading university in the UK for cancer research in the 2008 Research Assessment Exercise. Fortunately, Nic will retain strong links with Manchester, by continuing to run his research group here and remaining as Director of the Manchester Cancer Research Centre.

At the start of 2011, we formed a Senior Management Team to oversee the Paterson Institute in the interim period between Nic's departure and the appointment of a new Director. We have been helped enormously in this task by the example set by Nic's strong leadership and the Institute infrastructure put in place during his tenure as Director. Some new appointments also eased the operational burden significantly, Stuart Pepper as Head of Research Services and Caroline Wilkinson as Scientific Operations Manager. These positions were created upon the departure of Jenny Varley, the Assistant Director of Research, who retired in March after ten years in this post, preceded by nine years as a Paterson Institute Group Leader. One of Jenny's key responsibilities was to develop and oversee the research services, the excellent standard of which serves as one of her strongest legacies. Pippa McNichol also moved on from the Institute at the end of the year, after nine years as Director of Operations. Her professionalism, energy and enthusiasm made a big impact upon the Institute, and along with Jenny, she will be greatly missed.

The search for a new Director began in early 2011 and was completed in September with the recruitment of Professor Richard Marais who

A Cancer Research UK fundraiser takes a closer look at our work during the Institute's open day

takes up the Directorship in early 2012. We are delighted by his appointment as it will initiate an exciting new phase in the development of the Paterson Institute. Richard's research focuses on signalling through the RAS/RAF pathway and the role that this plays in the development and progression of melanoma, the most deadly form of skin cancer. His work was pivotal in determining how mutated BRAF drives the formation of this disease and he is now translating his findings via the development of therapeutic agents that are undergoing early clinical trials. His wide span of interests from basic science to transgenic models of disease, coupled with his active engagement in both drug discovery and clinical trials, perfectly mirrors the range of research activities being undertaken at the Institute.

Despite changes to its leadership, the Institute has continued to thrive, with our scientists enjoying a great deal of success in 2011. First of all, the DNA Damage Response Group, led by Ivan Ahel, in collaboration with Professor David Leys' group at the University of Manchester, published their ground-breaking work describing the crystal structure of a poly (ADP-ribose) glycohydrolase in the highly prestigious journal, Nature. This valuable structural insight will help direct mechanistic studies which should further the ultimate goal of developing PARG inhibitors for use in future cancer therapies. Ivan's continuing success was rewarded with his acceptance onto the EMBO Young Investigator Programme.

The Cancer Research UK Translational Cancer Research Prize for 2011 was awarded to the leaders of the Clinical Experimental Pharmacology team, Caroline Dive and Malcolm Ranson along with Fiona Blackhall, the lung cancer translational research lead within CEP and Andrew Hughes from AstraZeneca. The judging panel highlighted the outstanding contributions made by the team to biomarker-enhanced early clinical trials as well as the significant impact made by their work on circulating tumour cells and circulating micrometastases. The award, consisting of a research grant of £25,000, was presented at the NCRI conference in Liverpool where there was also success for Ahmet Acar from the Stromal-Tumour Interaction Group, led by Dr Akira Orimo, who was runner up in the best student poster competition. His work describes how notch signalling mediates myofibroblast differentiation of carcinoma-associated fibroblasts. Caroline Dive and Malcolm Ranson led a successful application to renew the funding of the Manchester Experimental Cancer Medicine Centre which was one of three that will receive the maximum level of funding (£500,000 per annum for five years). The renewal will allow the centre to develop its experimental cancer medicine portfolio in areas such as radiotherapy, proteomics, metabolomics and imaging and continue its early phase clinical trial programme.

Waleed Alduaij, who recently completed his PhD thesis in the Targeted Therapy Group, was awarded the Royal Society of Medicine Oncology Section - Sylvia Lawler Prize. Matthew Krebs, from the Clinical and Experimental Pharmacology Group, won the Institute's "Dexter Young Investigator" award. His PhD studies have culminated in three first author publications, including a report describing the presence and prognostic significance of circulating tumour cells in non-small-cell lung cancer which was published in the Journal of Clinical Oncology. The previous winner of this award, Danny Bitton (ACBB Group), published two first author papers this year in collaboration with the Cell Division Group. One of these studies, which showed that fission yeast meiosis is regulated by a novel mechanism using antisense regulatory transcripts, was made possible by the addition of next generation sequencing capability to our research services. We look forward to migrating the expertise and knowledge gained from these model organism studies to human biology and ultimately to clinical samples. These new and exciting technological platforms will keep us in a strong position to contribute to CR-UK's Stratified Medicine Programme. The international profile of the Institute was boosted this year when Iain Hagan teamed up with Jon Pines from the Gurdon Institute to organise the UK-Japan Cell Cycle workshop in a surprisingly sunny Lake District in April. Further enhancement came from the hosting of the European Bioconductor Developers' Workshop by the ACBB Group which attracted participants from all over the world.

Our Drug Discovery Group has expanded after a successful period of recruitment. This includes a new Head of Biology, Dr Ian Waddell, who joined us after eighteen years as Director of Discovery Medicine at AstraZeneca. He will oversee a team of ten bio-scientists working with a portfolio of early phase drug targets. Some of these have arisen as a result of collaborations with Paterson Institute Group Leaders illustrating the benefit of having drug discovery capacity embedded within the Institute.

Ged Brady was appointed as Deputy Group Leader of the Clinical and Experimental Pharmacology (CEP) group where he has expanded the CEP Biomarker Portfolio to include a Nucleic Acids Biomarkers team. Stephen Walker has also joined CEP and is playing a critical role in the management of the increasing number of clinical studies underway in this group and will also assist in the development of circulating tumour cell and blood based biomarker assays.

In an increasingly challenging economic climate, it is more vital than ever that we engage with our fundraisers at every opportunity, not only to communicate our progress, but also to convey our gratitude for their support. Events which have enabled us to do this in 2011 included our Institute open day which saw ninety local supporters attend lab demonstrations. Forty local sixth form students got a taste of life in the lab when they took part in practical scientific sessions as part of the Institute's annual schools' day. Our scientists also volunteered at local race for life and relay for life events as well as providing support by manning an official pit stop at the Institute during the Manchester Shine walk which aims to raise £2m for CR-UK. The Institute also welcomed a variety of visitors onto our monthly lab tours including CR-UK shop volunteers, local fundraising groups, two local MPs, a MEP and a premiership footballer! It is always humbling to meet our supporters and we are immensely grateful to all who give their time and money so generously to help fund our research.

The coming year promises to be particularly eventful, not only due to the arrival of our new Director and his research group, but also as a result of further Group Leader recruitment, which had been put on hold pending the new Director's arrival. Five Senior Group Leaders will undergo Quinquennial Reviews which will allow us to take stock of past achievements and prioritise our future research activities. There will also be reviews of all our Research Services which will direct us towards the necessary changes that will enable us to meet the evolving needs of the Institute. It promises to be an exciting time and we look forward to the continuing strengthening and development of the Institute under Richard Marais' leadership allowing the Paterson to play its part in realising the goals of Cancer Research UK.

Senior Management Team: Professor Caroline Dive (Senior Group Leader), Pippa McNichol (Director of Operations), Professor Iain Hagan (Senior Group Leader)

Research Highlights

In this section we are highlighting some research publications from 2011 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.

Bitton, D.A., Grallert, A., Scutt, P.J., Yates, T., Li, Y., Bradford, J.R., Hey, Y., Pepper, S.D., Hagan, I.M. and Miller, C.J.
Programmed fluctuations in sense/antisense transcript ratios drive sexual differentiation in S. pombe.
Mol Syst Biol, 2011, 7: 559.

It has recently been shown that the majority of the human genome is transcribed into non-coding RNAs that are never translated into functional proteins. This revelation has led to considerable interest in the potential role of these novel transcripts. The fission yeast *Schizosaccharomyces pombe* is an important model organism for studying these mechanisms, since many of the key pathways involved in RNA interference (RNAi) are conserved in humans. In collaboration with the Cell Division group, the Applied Computational Biology and Bioinformatics (ACBB) group has been using *S. pombe* to study these basic processes. Bitton, Grallert et al., performed strand specific deep sequencing of total RNA over a timecourse of sexual differentiation. These data revealed a set of 'Antisense Regulatory Transcripts', or ARTs, that on further manipulation were found to be responsible for regulating the protein levels of some of the key coordinators of sexual differentiation. A significant number of ARTs arise from adjacent and overlapping pairs of protein coding genes transcribing towards each other from opposite strands of the genome. As well as demonstrating a critical role for these transcripts in controlling meiosis, a key finding of the paper, therefore, is that neighbouring genes can regulate each other; a critical factor when considering the effects of knockout or knockdown studies, since perturbations to one locus may lead to unintended disruptions to another.

Mehrotra, P.V., Ahel, D., Ryan, D.P., Weston, R., Wiechens, N., Kraehenbuehl, R., Owen-Hughes, T., and Ahel, I.
DNA repair factor APLF is a histone chaperone.
Mol Cell, 2011, 41: 46-55.

There are many pathways and signalling strategies that control genome stability in humans. Many of these pathways are regulated by poly(ADP-ribosyl)ation. Poly(ADP-ribosyl)ation is a post-translational protein modification synthesised by the poly(ADP-ribose) polymerase (PARP) family of enzymes, using the vital cellular cofactor NAD as a substrate. The recent development of potent, cell-permeable PARP inhibitors has provided powerful tools to study the pathways regulated by poly(ADP-ribose), as well as providing a promising class of novel drugs in the treatment of cancer. Poly(ADP-ribosyl)ation plays a major role in DNA repair, where it regulates chromatin relaxation as one of the critical events in the repair process. However, the molecular mechanism by which poly(ADP-ribose) modulates chromatin remains poorly understood. Here we identified the poly(ADP-ribose)-regulated protein, APLF (Aprataxin and PNK-Like Factor), as a protein with the remarkable ability to bind histones – major structural components of chromatin. We demonstrate that APLF possesses histone chaperone activity in vitro, which we suggest is important in the regulation of chromatin structure in response to DNA damage and also for efficient DNA repair.

Slade, D., Dunstan, M.S., Barkauskaite, E., Weston, R., Lafite, P., Dixon, N., Ahel, M., Leys, D. and Ahel, I.
The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase.
Nature, 2011, 477: 616-620.

Poly(ADP-ribosyl)ation is a post-translational protein modification that serves to regulate many cellular pathways important for genome stability including DNA repair, mitosis and apoptosis. The Poly(ADP-ribose) chemical signal is synthesised by the PARP family of enzymes using cofactor NAD as a substrate. PARP inhibitors have been showing great promise in clinical trials for patients with breast, ovarian and prostate cancers caused by mutations in genes called BRCA1 and BRCA2. The main protein that removes poly(ADP-ribose) signals after the specific pathway has been completed is called poly(ADP-ribose) glycohydrolase (PARG). Scientists believe that, similar to PARP inhibitors, drugs designed to block the action of PARG could be effective in treating cancer, but the progress in developing permeable, small-molecule PARG inhibitors has been limited due to the lack of mechanistic and structural data for PARG. In this work we present the first insight into the three-dimensional structure of a PARG enzyme. This structure, in combination with functional analysis, provides a vital understanding of how PARG works and will facilitate the development of small cell-permeable PARG inhibitors.

Ferreras, C., Lancrin, C., Lie, A. L. M., Kouskoff, V. and Lacaud, G.
Identification and characterization of a novel transcriptional target of RUNX1/AML1 at the onset of haematopoietic development.
Blood, 2011, 118: 594-7.

The gene *AML1/RUNX1* (*Acute Myeloid Leukaemia 1*) encodes a transcription factor that regulates the expression of haematopoietic genes and is critical for haematopoietic development. Alteration in the activity of AML1/RUNX1 by either mutation or translocation is the most frequent initiating event leading to leukaemia. To better understand the consequences of alteration of the activity of the protein AML1/RUNX1, it is important to define the downstream transcriptional targets of AML1/RUNX1.

In this study, we compared gene expression between AML1/RUNX1$^{+/-}$ and AML1/RUNX1$^{-/-}$ cells at a stage where blood development is halted in the absence of AML1/RUNX1 activity. Among several other genes differentially expressed, we selected AI467606 for further studies. AI467606 encodes a protein with no known function and with no homology to other proteins. We validated the difference in AI467606 expression and demonstrated by chromatin immunoprecipitation and promoter assay the direct regulation of AI467606 expression by AML1/RUNX1. We further established that AI467606 is specifically expressed in the haematopoietic system from its establishment early in ontogeny to its maintenance throughout adult life. Taken together our findings indicate that AI467606 is a novel transcriptional target of RUNX1/AML1 widely expressed within the haematopoietic system. We are currently evaluating to what extent AI467606 expression is altered in leukaemia cells and are further investigating the function of this intriguing novel protein in haematopoiesis.

Harrison, L. R., Micha, D, Brandenburg, M., Simpson, K. L., Morrow, C. J., Denneny, O., Hodgkinson, C., Yunus, Z., Dempsey, C. Roberts, D., Blackhall, F., Makin, G. and Dive, C.
Hypoxic human cancer cells are sensitized to BH-3 mimetic-induced apoptosis via downregulation of the Bcl-2 protein Mcl-1.
J Clin Invest, 2011, 121(3): 1075-87.

The majority of solid tumours contain regions of hypoxia, due to insufficient tumour vasculature. Tumour cells which reside in the hypoxic region are resistant to the majority of conventional chemotherapeutic agents and radiotherapy. They also have an increased invasive capacity and are enriched for stem cell markers. Therefore, novel agents which preferentially kill hypoxic cells are of great therapeutic interest. The BH3 mimetic ABT-737 induces apoptotic cell death by interrupting the protein-protein interactions between pro- and anti-apoptotic Bcl-2 family proteins. Harrison et al investigated the effect of ABT-737 on normoxic and hypoxic small cell lung cancer and colorectal cancer cells. This work demonstrated that hypoxic cells were more sensitive to ABT-737 induced apoptosis than their normoxic counterparts and that this was due to down-regulation in hypoxia of the ABT-737 resistance biomarker Mcl-1, a Bcl-2 family protein for which ABT-737 has only poor affinity. ABT-737 sensitisation was independent of Hif-1α (the main regulator of hypoxic response) and Mcl-1 down-regulation was caused by reduced translation of the Mcl-1 gene. These data suggest that combining ABT-737 with other therapeutic agents such as radiotherapy that preferentially target normoxic regions of tumours may prove clinically beneficial and this is being investigated in further studies.

Krebs, M. G., Sloane, R., Priest, L., Lancashire, L., Hou, J. M., Greystoke, A., Ward, T.H., Ferraldeschi, R., Hughes, A., Clack, G., Ranson, M., Dive, C. and Blackhall. F.H.
Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Non-Small-Cell Lung Cancer.
J Clin Oncol, 2011, 29(12): 1556-63.

Lung cancer is the leading cause of cancer-related death worldwide with Non-Small Cell Lung Cancer (NSCLC) accounting for 85% of cases. There are few predictive biomarkers to help guide therapeutic choices. Circulating tumour cells (CTCs) are an appealing biomarker - as the cells responsible for metastasis, their molecular characterisation will lead to increased understanding of the biology of metastasis and will help develop novel treatment strategies. This study explored the presence and prognostic value of CTCs in patients with advanced stage NSCLC using the FDA approved CellSearch™ system. CTCs (≥2 CTCs/7.5ml blood) were detected in 32% of patients with advanced NSCLC and the presence of 5 or more CTCs in 7.5ml blood was strongly associated with poor prognosis, compared to those patients with less than 5 CTCs. In multivariate analysis, CTC number was the strongest independent prognostic factor amongst all known standard prognostic variables. Furthermore, a pharmacodynamic effect was demonstrated where a reduction in CTC number after a single cycle of standard-of-care chemotherapy predicted for better outcomes than in those patients whose CTC number increased. This study has formed the basis for ongoing studies in lung cancer focused on CTC characterisation with a view to identification of new treatment targets and to developing CTCs as predictive biomarkers.

Brognard, J., Zhang, Y. W., Puto, L. A. and Hunter., T.
Cancer-associated loss-of-function mutations implicate DAPK3 as a tumor-suppressing kinase.
Cancer Res, 2011, 71:3152-61.

There has been a recent flood of cancer kinome sequence data, but the functional consequences of the reported protein kinase mutations have been inferred largely through statistical approaches. Our study, published in Cancer Research, represents a critical first step in assessing the functional relevance of putative driver mutations experimentally. The study illustrates how bioinformatic approaches can be utilized to mine large cancer genomic datasets to accurately identify important functional mutations. We were able to demonstrate that mutations in DAPK3 are loss-of-function mutations that are essential for maintaining the tumorigenic phenotypes of lung cancer cells. We demonstrated the importance of DAPK3 mutations by reconstituting lung cancer cells that harboured a loss-of-function mutation in DAPK3, with the wild type functional kinase, which resulted in the lung cancer cells being more responsive to apoptotic stimuli and chemotherapeutics. As we progress into the age of personalized medicine it will be essential to determine causal mutations in cancer patients and to use this knowledge to administer drug cocktails tailored to an individual tumour that target aberrantly activated or inactivated pathways ultimately leading to the specific killing of cancer cells.

Tamm, T*., Grallert, A*., Grossman, E. P., Alvarez-Tabares, I., Stevens, F. E. and Hagan, I. M.
Brr6 drives the Schizosaccharomyces pombe spindle pole body nuclear envelope insertion/extrusion cycle.
J Cell Biol, 2011, 195: 467-84.

Yeast biology has contributed much to our understanding of the basic biology of a cell. While the function of the majority of the genes encoded within the genome is understood or can be derived from comparisons between homologues in different systems, functional interrogation via genetic screens can still prove informative. We identified the fission yeast Brr6 molecule in a screen for mutants that were unable to form a mitotic spindle. Significantly Brr6 is required for an aspect of mitosis that is unique to organisms that undergo a closed mitosis in which the nuclear envelope does not break down: polar fenestration. Polar fenestration is the localised disruption of envelope integrity to enable the spindle pole to nucleate microtubules that contact the cell cortex at the same time as nucleating the microtubules of the mitotic spindle. As the nuclear envelope breaks down during every division in human cells they do not require a member of this highly conserved protein family. The highly structured nature of the conserved domain in Brr6 and its apparent role in modifying the lipid composition of the nuclear envelope makes it an ideal target for the development of novel anti-eukaryotic microbial therapies.

Alduaij, W., Ivanov, A. Honeychurch, J., Cheadle, E., Potluri, S., Lim, S. H., Shimada, K., Chan, C. H., Tutt, A., Beers, S. A., Glennie, M. J., Cragg, M. S. and Illidge, T. M.
Novel type II anti-CD20 monoclonal antibody (GA101) evokes homotypic adhesion and actin-dependent, lysosome-mediated cell death in B-cell malignancies.
Blood, 2011, 117: 4519-29.

The anti-CD20 monoclonal antibody (mAb) rituximab has substantially improved the clinical outcome of patients with a wide range of B-cell malignancies. However, many patients relapse or fail to respond to rituximab, and thus there is intense investigation into the development of novel anti-CD20 mAbs with improved therapeutic efficacy. The Targeted Therapy Group, led by Tim Illidge, has previously shown that certain antibodies including anti-HLA-DR and type II anti-CD20 mAbs efficiently induce programmed cell death (PCD) through a process involving lysosomsal death (Ivanov et al., J Clin Investigation, 2009). Now, for this first time, they have demonstrated that the humanized, glycol-engineered anti-CD20 mAb GA101, which is currently undergoing extensive clinical testing, can trigger non-apoptotic PCD in a range of B-lymphoma cell lines and primary B-cell malignancies. This research demonstrates that GA101-induced cell death is dependent on actin reorganization, can be abrogated by inhibitors of actin polymerization and is independent of BCL-2 over-expression and caspase activation. GA101-induced PCD is executed by lysosomes which disperse their contents into the cytoplasm and surrounding environment. Taken together, these findings reveal that GA101 is able to potently elicit actin-dependent, lysosomal cell death, which may potentially lead to improved clearance of B-cell malignancies in vivo.

Holland, M., Castro, F.V., Alexander, S., Smith, D., Liu, J., Walker, M., Bitton, D., Mulryan, K., Ashton, G., Blaylock, M., Bagley, S., Connolly, Y., Bridgeman, J., Miller, C., Krishnan, S., Dempsey, C., Masurekar, A., Stern, P., Whetton, A. and Saha, V.
RAC2, AEP, and ICAM1 expression are associated with CNS disease in a mouse model of pre-B childhood acute lymphoblastic leukemia.
Blood, 2011, 118: 638-49.

Though <2% of children with acute lymphoblastic leukaemia have central nervous system (CNS) disease at diagnosis, this rises to over 40% at recurrence. The pathogenesis of this process has remained an enigma, with the dogma being that leukaemic cells enter the CNS via burst capillaries. As conventional therapy does not cross the blood-brain barrier, these cells survive giving rise to recurrence. We developed a murine model of CNS disease, which demonstrates that CNS disease occurs via haematogenous dissemination and cellular diapedesis of lymphoblasts across blood-brain and blood- cerebrospinal fluid barriers. These findings replicate autopsy findings in children dying due to CNS leukaemia. Using a semi-quantitative proteomic discovery approach and functional assays, we showed that transgressing cells are motile, invasive, express active RAC2 and have an organised actin cytoskeleton. CNS infiltrating cells from both cell lines and primary material expressed CD70, ICAM1 and LFA-1. This suggests that subclones expressing key adhesion/integrin molecules activate cytoskeletal reorganisation promoting diapedesis on contact with stromal cells. In the future, it may be possible to target these interactions as an adjunctive to chemotherapy and prevent extramedullary recurrences.

Di, Y., Holmes, E. J., Butt, A., Dawson, K., Mironov, A., Kotiadis, V. N. Gourlay, C. W., Jones, N. and Wilkinson, C. R.
H_2O_2 stress-specific regulation of S. pombe MAPK Sty1 by mitochondrial protein phosphatase Ptc4.
EMBO J, 2011, 31(3): 563-75.

Cells need to respond in an appropriate and timely manner to a broad spectrum of stress conditions. A key feature of stress responses is the mobilisation of appropriate defence and repair mechanisms. In fission yeast, many of these responses are orchestrated through the Sty1 MAP kinase. Like its mammalian counterpart, p38, Sty1 is phosphorylated and activated by a variety of stress stimuli. Similar to other MAPK pathways, Sty1 signalling is highly regulated to control the magnitude and duration of signalling. This is achieved through co-ordination of positive and negative acting signals. Positive signals include stress conditions that result in the phosphorylation of Sty1 whereas negative signals involve phosphatases that inactivate Sty1 through dephosphorylation. We found that Ptc4, a PP2C-family phosphatase, regulates both the magnitude and duration of Sty1 activation in response to H_2O_2 but not other stresses.

Surprisingly, Ptc4 localizes to the mitochondria and is targeted there by an N-terminal mitochondrial targeting sequence (MTS) which is cleaved upon import. A fraction of Sty1 also localizes to the mitochondria suggesting that Ptc4 attenuates the activity of a mitochondrial pool of this MAPK and moreover, that Sty1 may phosphorylate and thereby regulate targets in this organelle. Cleavage of the Ptc4 MTS is greatly reduced specifically upon H_2O_2, resulting in the full length form of the phosphatase; this displays a stronger interaction with Sty1 thus suggesting a novel mechanism by which the negative regulation of MAPK signalling is controlled and providing an explanation for the oxidative stress-specific nature of the regulation of Sty1 by Ptc4.

Research groups
Paterson Institute for Cancer Research

Applied Computational Biology and Bioinformatics Group

http://www.paterson.man.ac.uk/bioinformatics

Group Leader
Crispin Miller

Postdoctoral Fellows
Danny Bitton
John Hall (joint with Translational Radiobiology Group)
Hui Sun Leong
Yaoyong Li

Software Architect
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Bioinformatics Programmer
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Bioinformatician
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Paul Scutt

Graduate Students
Danish Memon
Sharmin Naaz (joint with Stem Cell and Haematopoiesis Group)
Andrzej Rutkowski (joint with Immunology Group)
María José Villalobos Quesada (joint with Cell Division Group)

Systems Administrator
Zhi Cheng Wang (joint with IT department)

The advent of high density tiling microarrays and deep sequencing has revealed that the majority of the human genome is transcribed, even though less than 2% of it encodes protein sequences. A major focus of the Applied Computational Biology and Bioinformatics (ACBB) Group is to develop a better understanding of the roles played by these novel non-coding RNA molecules, and to determine how their behaviour is altered in tumours. The group is highly inter-disciplinary and features a mixture of computer scientists, mathematicians and biologists. It collaborates widely with other groups in the Paterson Institute and the Manchester Cancer Research Centre.

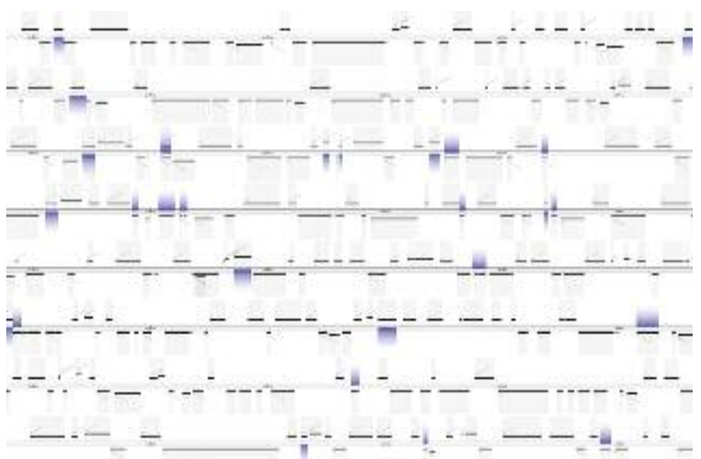
Identification of novel protein coding genes

One possibility raised by the widespread transcription of non-coding RNAs is that some of them encode for previously undiscovered proteins. Using the fission yeast *Schizosaccharomyces pombe* as a model organism, Danny Bitton (at the time, a graduate student within the group), was able to use protein mass spectrometry to test this hypothesis (Bitton *et al.* 2011, *Genetics*). He did this by searching the output of a mass spectrometer against a database derived from the set of all the known and predicted protein sequences in the genome, generated by translating it in all six possible forward and reverse reading frames. This differs from a typical mass spectrometry experiment, which searches only against a database of known proteins, and allows it to be used for protein discovery as well as identification. One of the challenges of such an approach is that the candidate database contains a large number of potential sequences that will never be expressed, substantially increasing the possibility of spurious false positive matches. This requires the careful application of statistics and the incorporation of annotation data from other sources, in order to ensure that the error rate is minimised whilst allowing the maximum scope for real matches to be identified. Using these approaches, we were able to add an additional 0.8% to the protein coding complement of the fission yeast *Schizosaccharomyces pombe*, and to revise the structure of many other protein coding genes. *S. pombe* is an important model organism that shares many key pathways with human cells (see below). In collaboration with the Cell Division Group, we were then able to demonstrate that these predictions did indeed result in the expression of novel proteins, that some of these proteins were essential for cell viability, and that the deletion of others resulted in strong phenotypes including slow-growth and delayed-division. These novel genes (*new1-new25* and *tam1-tam14*; transcripts altered in meiosis) have been incorporated in PomBase, the fission yeast reference genome database (http://www.pombase.org). CENPW/Cug2, the human homologue of one of these genes, *new1*, is a putative oncogene found upregulated in many human cancers (McAinsh & Meraldi 2011 *Semin Cell Dev Biol*; Chun *et al.* 2011 *J Biol Chem*).

ARTs – Antisense Regulatory Transcripts

RNA interference (RNAi) is the process by which double strand RNA molecules are used to regulate gene expression. Many of the key components of the RNAi pathways found in human cells are also present in fission yeast, making it an important model system for studying non-coding RNAs. In collaboration with the Cell Division group, we have been studying the role of non-coding RNAs in regulating meiosis. Strand-specific deep sequencing of total RNA taken from *S. pombe* cells over a time course of meiosis was used to investigate how the transcript profile of the cells changed as they underwent sexual differentiation (Bitton et al. 2011, Mol Syst Biol, 7:559). Analysis of these data identified hundreds of novel transcripts, and revealed that a significant number of these were differentially expressed, suggesting that they are under regulatory control and may therefore be functional. A subset of these molecules originated from the opposite strand to a known protein coding gene, resulting in an antisense transcript. We hypothesised that these transcripts may serve to regulate the amount of protein expressed by their corresponding protein coding gene. We were able to test this hypothesis for four key regulators of meiosis (Spk1, Dis1, Spo4, Spo6), each of which was found to have an associated antisense transcript. In every case, over-expression of the antisense transcript from an integrated ectopic locus resulted in the same phenotype as a deletion of the corresponding protein coding gene. For one locus, *dis1*$^{+}$, the availability of an antibody made it possible to measure protein expression. As predicted, over-expression of the *dis1* antisense transcript resulted in a reduction in Dis1 protein levels. We refer to these transcripts as Antisense Regulatory Transcripts, or 'ARTs'. Further analysis demonstrated that for each of the four loci tested, the action of these ARTs was dependent on core components of the RNAi machinery.

Figure 1
A small region of the S. pombe genome showing sites of ART (Antisense Regulatory Transcript) activity (blue) as identified in Bitton et al., (2011, Mol Syst Biol, 7:559). Each grey line represents a fragment of chromosome, with known genes represented as grey boxes (highlighted by black lines) appearing above or below, according to whether they are expressed on the forward or reverse strand.



Antisense expression can arise from a number of sources including the expression of independent non-coding RNA transcripts, the transcription of protein coding genes that overlap at their 3' ends, and a phenomenon known as 'bi-directional transcription' in which the polymerase appears to transcribe away from its promoter in the 'wrong' direction. All these phenomena were observed in our data, including antisense arising from overlapping protein coding genes transcribing towards each other on opposite strands of the genome. This led to the conclusion that adjacent genes can regulate each other, and has profound implications for gene deletion experiments, since care must be taken to ensure that the phenotype observed when knocking out one gene is not the result of an unexpected interaction with one of its neighbours.

Software engineering

Bioconductor is an international collaboration to write open source software for the analysis of biological data. With the advent of high throughput technologies including deep sequencing, proteomics and microarrays, computing is becoming increasingly ubiquitous within the biosciences, and the software tools generated by projects such as Bioconductor are fundamental to much of the research conducted in an institute such as the Paterson. Some of the software packages developed by the ACBB group are downloaded thousands of times each month. In December, we hosted the European Bioconductor Developers' Workshop, a meeting designed to help foster the exchange of technical expertise, to keep contributors up to speed with the latest developments in Bioconductor, and to coordinate related efforts. This year, the meeting featured speakers from the Paterson Institute, the Fred Hutchinson Cancer Research Center, Harvard University, Johns Hopkins University, Cambridge University, the CR-UK Cambridge Research Institute, IPB Halle and The University of Zurich.

Building gene expression classifiers from clinical data

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 extract meaningful transcription data from FFPE material. In collaboration with the Translational Radiobiology Group we have been developing bioinformatics approaches to support the analysis of microarray data generated from FFPE tissue. Recently, we were able to show not only that it is possible to use FFPE RNA to build a meaningful gene expression based classifier capable of separating squamous cell carcinoma (SCC) and adenocarcinoma (AC), but that transcripts from this classifier could be used to correctly segregate an independent cohort of samples using a different technology, QuantiGene (Hall, Leong *et al.* 2011 BJC).

Publications listed on page 66

Carcinogenesis Group

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Jo Kelly (Until September)
Sarah Pinder (From July)
Vitaly Sukhinin (Until August)
Emma Williams (From July)

The group's work focuses on the mechanism of action of a group of chemical compounds called alkylating agents. Agents of this type display a wide range of biological effects in living organisms all of which are attributed to the introduction of various types of DNA damage. The ability of these agents to kill cells is exploited in their use as antitumour agents in the treatment of certain types of cancer. Although a number of different lesions can be generated in DNA, one of these, O^6-alkylguanine, is considered to be the most important. We are trying to establish precisely how cells respond to this damage and the impact that this has on the biological effects of these agents.

Background

The Cancer Research UK drug Temozolomide, which is used in the treatment of glioma and melanoma, kills cells by chemical modification of DNA, in particular at the O^6-position of guanine. Such lesions can be repaired by the damage reversal protein O^6-methylguanine-DNA methyltransferase (MGMT), which simply removes the methyl group from O^6-methylguanine and restores the DNA to its pre-damaged state and hence protects against Temozolomide toxicity. To combat this, in collaboration with Prof Brian McMurry and the late Dr Stanley McElhinney (and their group at the Chemistry Department, Trinity College, Dublin) we developed the potent MGMT inactivating drug, Lomeguatrib. However, in clinical trials, Lomeguatrib did not enhance the therapeutic effect of Temozolomide and we are now investigating the possible basis of this by using fission yeast to characterise alternative repair pathways.

Clinical studies

The dose-limiting toxicity of Temozolomide occurs in bone marrow cells. To assess if this could be predicted using cells in the circulation (peripheral blood mononuclear cells: PBMCs) we quantified MGMT levels in ninety-three melanoma patients treated with temozolomide or dacarbazine in four clinical trials. A model of the interaction between MGMT expression and haematological toxicity was constructed. Nadir white-cell and platelet counts were related to, and hence could be predicted from, pretreatment MGMT. Leucopenia and thrombocytopenia were more prevalent amongst patients with low pretreatment PBMC MGMT, according to the highest grades of toxicity experienced and/or the dose intensity patients could sustain. It is reasonable to conclude that the determination of MGMT in PBMC may be used to identify patients at greatest risk of toxicity or who are suitable for dose intensification.

Alkyltransferase-like proteins

We have previously described some of our studies of a novel family of proteins, the Alkyltransferase-like (ATL) proteins that we so named because they resemble the alkyltransferase family, but with the major difference that the cysteine residue which accepts the alkyl group is replaced, usually by tryptophan. Genes encoding these proteins are present in prokaryotes, archaea and eukaryotes, but it is intriguing that some organisms seem to need both functions. Thus budding yeast expresses only an alkyltransferase and *S.pombe* only has an ATL (Atl1), whereas *E.coli* expresses both alkyltransferase genes (in fact two: *ada* and *ogt*) and an ATL gene (eATL). The evolutionary basis of these differences are open to speculation.

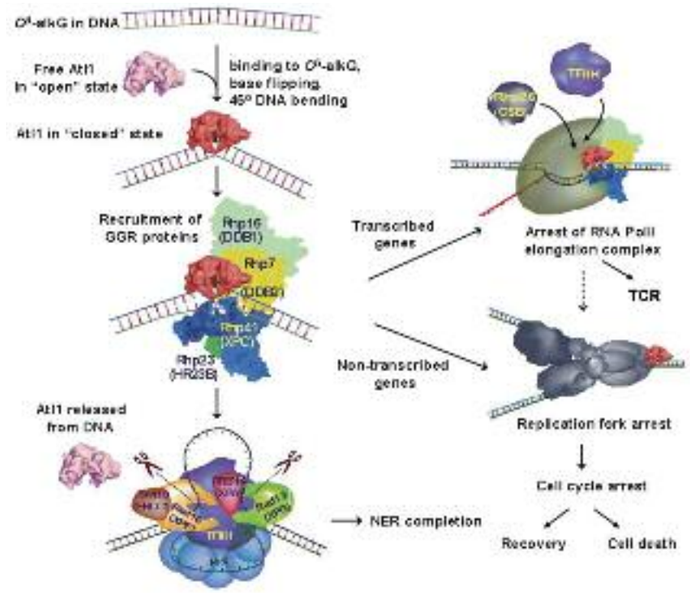

Figure 1
Current model for the processing of O6-alkylguanines in S.pombe DNA by Atl1. GGR; global genome repair, NER; nucleotide excision repair, TCT; transcription-coupled repair. See text for other abbreviations and explanation.

One of the original observations we made was that both eATL and Atl1 were able to rapidly bind to methylated DNA and inhibit the action of MGMT on its normal substrate, O^6-methylguanine. One of the many questions this raised was to what extent these proteins would bind to other O^6-alkylguanines in DNA. We have been able to pursue this through a collaboration with Dr David Williams in the Chemistry Department of Sheffield University who has synthesized a large number of short oligonucleotides containing a wide range of O^6-alkylguanines. We have been examining the interaction of Atl1 and eATL with these oligos by a variety of methods. More recently, in collaboration with Andy Povey at the University of Manchester, we have also investigated them as substrates for MGMT. John Tainer and his group (Scripp's research Institute, La Jolla), have obtained additional crystal structures with Atl1 and these are telling us more about the molecular interactions that take place. The crystal structures add further support to our previous suggestion that Atl1 binds to DNA, "flips" out the O^6-alkylguanine from the base stack using an arginine "finger", rotates the phosphodiester bond by means of a tyrosine residue and accommodates the base in the binding pocket, stabilized by various ionic interactions with amino acid residues in the vicinity. In the case of MGMT, this allows alkyl group transfer to the cysteine residue and rapid dissociation of the MGMT, but ATL, unable to transfer the alkyl group, remains bound. However, it is worth noting that this juxtapositioning is not the only prerequisite for alkyl transfer. We previously showed that mutation of the tryptophan in Atl1 to cysteine, as in MGMT did not confer alkyltransferase activity.

The subsequent steps in Atl1-mediated processing of the lesion have not been completely elucidated but current evidence indicates that the bound Atl1 is a substrate for and/or recruits components of the nucleotide excision repair system which ultimately results in the elimination of the lesion from DNA. Figure 1 shows our current thinking of the factors involved. It is clear that in some cases, repair is probably mediated by the downstream components of the global genome repair (GGR) pathway involving the interaction of the *S.pombe* equivalents (Rhp7 and Rhp14 respectively) of the human DNA damage response (DDR) factors DDB1 and DDB2. This is probably followed by the binding of the *S.pombe* equivalents (Rhp41 and Rhp23 respectively) of the human proteins Xeroderma Pigmentosum complementation factor C (XPC) and homologous recombination factor 23b (hHR23b). After this, transcription factor TFIIH is recruited and two helicase proteins therein unwind the DNA, allowing the region containing the lesion to be removed by dual endonuclease activities. This is followed by copying of the complementary strand to fill in the gap and rejoining of the break by DNA ligase. In other cases, however, the Atl1-Rhp7/14/23/41 complex might be more stable. If such a complex is present in the transcribed regions of transcribed genes, transcription-coupled repair (TCR) factors seem likely to be involved in processing the lesions (see Figure 1). In non-transcribed genes, and in cases where TCR is impaired, the stable complex probably stalls DNA replication forks. Our current concept is that such structures may be extremely difficult to resolve and may account for the lethality of the agents that generate such lesions. Further studies will hopefully confirm or refute our hypotheses.

CHEMORES

We are a member of a European Union Framework 6 programme-supported Consortium that is investigating alternative mechanisms of chemotherapy resistance. Our focus is on aspects of DNA repair in a number of human melanoma cell lines, some of which we are generating by repair gene transfection. We are using biochemical assays of functional activity of DNA repair proteins along with highly sensitive methods of quantifying DNA lesions generated by treatment with Temozolomide in attempts to identify pathways, perhaps resembling the Atl1 pathway in *S.pombe*, that may provide resistance.

Publications listed on page 66

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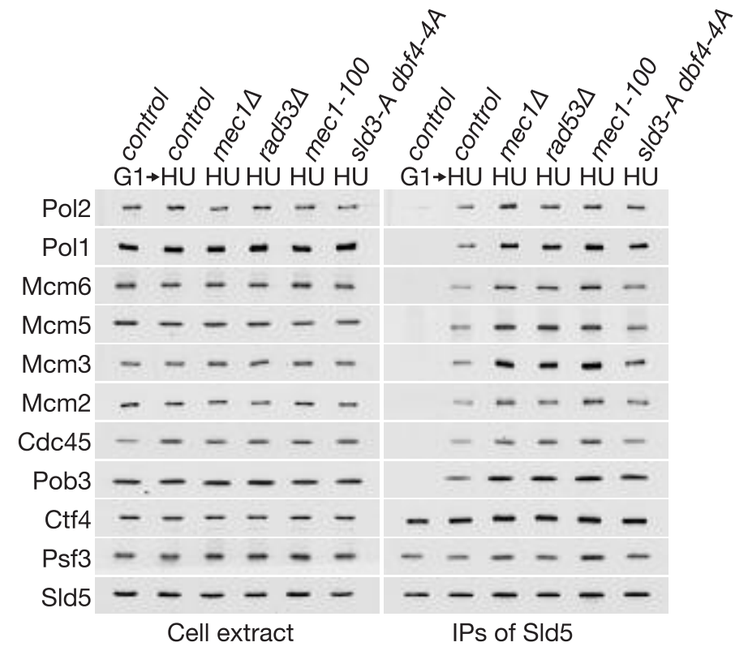
Our group studies the mechanisms and regulation of chromosome replication and cytokinesis. Eukaryotic cells have evolved complex regulatory pathways to survive replication stress and preserve genome integrity, and this regulation is likely to be of particular importance in many human tumours that have inherent defects in chromosome replication. In response to replication stress, cells activate a signalling pathway called the S-phase checkpoint, which involves activation of a series of checkpoint kinases. Previous work indicated that a key role of the checkpoint kinases was the preservation of replisome stability at defective DNA replication forks. To test this model directly, we isolated replisome material from extracts of budding yeast cells suffering replication stress. Our data indicate that replisome stability is actually independent of S-phase checkpoint kinases, which instead might regulate replisome function in ways that help preserve genome integrity at defective replication forks.

For most of the cell cycle, eukaryotic chromosomes exist in a highly stable form as double strand DNA that is compacted into chromatin. Nevertheless, duplication of the genome during the S-phase of the cell cycle involves unwinding of the DNA duplex by a DNA helicase, to produce the single strand DNA template upon which DNA polymerases can act. At each DNA replication fork the replicating DNA has a unique structure that exposes single strand DNA as well as unprotected DNA ends on the leading and lagging strands, greatly increasing the possibility that nucleases or recombination factors might gain access and generate DNA damage or chromosomal rearrangements. This risk is much higher when problems in DNA synthesis increase the amount of unprotected ssDNA at replication forks, and eukaryotic cells have evolved sophisticated regulatory pathways to detect problems in DNA replication and preserve the functional integrity of defective DNA replication forks.

In human cells, the ATR kinase (ATR = Ataxia and rad related) is recruited to defective DNA replication forks and leads to activation of the downstream kinase Chk1, which is thought to mediate many of the roles of the checkpoint response. ATR also seems to have additional functions not shared with Chk1, such as phosphorylation of Histone H2AX that helps recruit other checkpoint proteins and repair factors. The budding yeast orthologue of ATR is known as Mec1 and leads to activation of the Rad53 kinase in response to replication defects. Rad53 protects cells from replication stress in many ways, such as by inhibiting mitosis, preventing the activation of late origins of replication, and stimulating the activity of ribonucleotide reductase (Labib, K. and De Piccoli, G., 2011, *Phil. Trans. R. Soc. B,* **366**, 3554-3561). Previous studies indicated that both Mec1 and Rad53 were required for an additional and apparently critical aspect of the checkpoint response, namely the stabilization of the replisome at defective DNA replication forks, so that DNA synthesis is able to resume subsequently when the source of replication stress is removed or repaired. This conclusion came from a variety of chromatin immunoprecipitation experiments that monitored the association of replisome components with DNA around origins of replication following replication stress. Whereas replisome factors were clearly still associated with DNA at replication forks in the presence of checkpoint kinases, this association appeared to be lost in cells lacking Rad53 or Mec1.

Figure 1
Stability of the replisome at defective DNA replication forks is independent of S-phase checkpoint kinases. Cells were synchronised in G1-phase and then released for 90 minutes into S-phase in the presence of 0.2M hydroxyurea (HU), to induce replication stress. Replisome material was isolated by immunoprecipitation of the Sld5 subunit of the GINS complex.



To study how the checkpoint response might regulate the replisome following replication stress, we developed a new approach based on the direct isolation of replisome material from extracts of budding yeast cells. Surprisingly, we found that replisome stability is actually independent of checkpoint kinases, and in fact more replisome material can be isolated from cells lacking Mec1 or Rad53, due to the activation of both early and late origins when cells lacking checkpoint kinases suffer replication stress (Figure 1). The amount of replisome material recovered from *mec1Δ* or *rad53Δ* cells is very similar to the amount that can isolated from other mutants such as *mec1-100* or *sld3-A dbf4-4A*, which cannot restrain the activation of late origins following replication stress but do preserve the functional integrity of defective replication forks (De Piccoli *et al*, submitted).

The resumption of DNA synthesis at defective DNA replication forks is highly defective in the absence of Mec1 or Rad53, and the same is true in human cells lacking ATR. Although replisome stability is independent of the checkpoint response, it now seems very likely that the checkpoint kinases will regulate replisome function, as part of a highly complex and multi-faceted response that preserves genome integrity in response to replication stress. Previous large-scale screens for targets of checkpoint kinases identified several replication factors, and we have identified a series of new targets by analyzing replisome material isolated from cells suffering replication stress, and looking for gel-shifts that represent phosphorylations (Giacomo de Piccoli and Karim Labib, unpublished data). The nature of this regulation will be explored in the future, but one attractive idea could be that checkpoint kinases reduce the rate of fork progression in response to replication stress, as this might reduce the risk of mutations that could result from DNA synthesis on a damaged template, and might also reduce the exposure of unprotected ssDNA at defective forks. The underlying mechanisms remain to be determined, but our unpublished data indicate that the replicative DNA helicase is a direct target of multiple checkpoint kinases. Future work will focus on mapping novel phosphorylation sites amongst replisome components, and exploring whether such modifications do indeed regulate fork progression, or else act in other ways, for example by recruiting other factors to defective DNA replication forks.

Publications listed on page 67

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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. While the major focus of the laboratory asks how cells take the decision to divide, we also use genetic screens to identify proteins that are required for cell division and have recently developed collaborations 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 (see account by Crispin Miller in the ACBB Group section of this report).

Mitotic Commitment

Commitment to mitosis is regulated by the activity of a protein kinase called MPF. MPF is composed of a catalytic sub-unit encoded by the *cdc2*$^{+}$ gene and a regulatory sub-unit called Cyclin B. Prior to mitosis MPF is inhibited via phosphorylation by the protein kinase Wee1 on a residue (tyrosine 15) that lies within the ATP binding pocket of p34^{cdc2}. This phosphate is removed by the protein phosphatase encoded Cdc25. The balance of activity between Cdc25 and Wee1 determines when MPF will be activated to drive mitotic commitment. Once a critical threshold level of MPF is reached a positive feedback loop is promoted that boosts Cdc25 activity and suppress Wee1 activity, thereby driving full-scale commitment to mitosis. Fully activated MPF then activates a number of highly conserved kinases that are named after the founder members of each group Polo, Aurora and NIMA (Figure 1).

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, mutational enhancement of Cdc25 activity suppresses loss of Cut12 function. Consistently, combining conditional loss of function mutations within *cdc25* and *cut12* in the same strain generates synthetic lethality.

Cut12 and polo in mitotic commitment

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 because there can be no requirement for a phosphatase to remove a phosphate from Cdc2 if the kinase that puts this phosphate there is absent. Thus, MPF activation and mitotic commitment will occur in the absence of Cdc25 when Wee1 is inhibited. A cue as to how this inhibition may arise in *cut12.s11* mutants came from the key role played by Polo kinase in the MPF positive feedback loop in higher eukaryotes. We therefore considered the possibility that Cut12 suppresses ablation of Cdc25 because it inappropriately prompts Polo to shut down Wee1. This line of reasoning uncovered 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. Furthermore, while polo normally associates with the SPB for 30 minutes prior to mitosis, this association occurs 30 minutes earlier in *cut12.s11* cells. We are now exploiting a range of approaches including the mass spectrometric mapping of phosphorylation sites in both targeted and global approaches to identify the means by which a structural component of the spindle pole can exert such a strong influence upon the mitotic commitment switch.

Figure 1

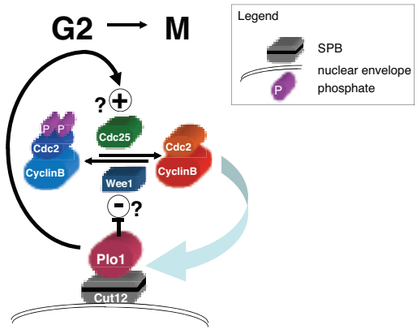
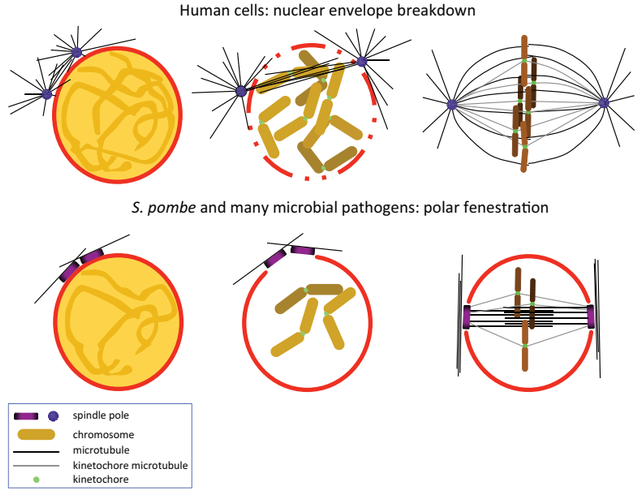


Figure 2



Brr6 – a new target for the fight against eukaryotic microbial infections?

A major challenge in the fight against eukaryotic microbial infections is to identify "druggable" targets or pathways within the microbe that are sufficiently distinct from those of the host that they can be targeted to kill the microbe while leaving the host unscathed. A screen for essential molecules that are required for division of fission yeast conducted in the lab may well have identified a suitable target, Brr6. The basis for this optimism lies in one fundamental difference between the nuclear divisions of many microbes and humans.

When our cells divide we break down the nuclear envelope that separates the nucleoplasm from the cytoplasm. This dispersal enables the centrosome to nucleate two sets of microtubules; those that form the bipolar spindle and the astral microtubules that anchor the spindle at the cortex to ensure that genome partitioning is perpendicular to the plane of division. For a variety of reasons many microbes maintain an intact nuclear envelope throughout division in a "closed mitosis". Because microtubules cannot penetrate the nuclear envelope, this preservation of envelope integrity throughout division presents a major challenge if astral microtubules are required to anchor the spindle to the cortex. To meet this challenge many microbes undergo "polar fenestration" in which a localised breakdown of the nuclear envelope at the poles can enable microtubules to span the nuclear envelope. In some microbes, including yeasts, the polar fenestra is used as a site into which the SPB is inserted to become an integral part of the nuclear envelope. Nucleation from the inner surface of this integrated SPB then generates the microtubules that form the spindle, while the outer face nucleates the astral microtubules that anchor the spindle to the cortex (Figure 2).

We found that Brr6 is an essential molecule that is recruited to the SPB in order to promote SPB insertion into the envelope during mitotic commitment. A domain within the Brr6 amino acid sequence that is bordered by two membrane spanning domains is highly structured and shows striking conservation in a large number of microbes (including malaria and pathogenic fungi). Importantly it is only microbes that undergo polar fenestration that have a molecule that harbours this domain. Given the prevalence of opportunistic fungal infections, the Brr6 pathway offers an attractive set of targets for the development of novel therapeutics.

Publications listed on page 67

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MAP kinase pathways are central in the cellular response to exogenous and endogenous stimuli, and are involved in a multitude of biological activities ranging from cell proliferation, differentiation, and cell death. However, distinct MAP kinase activities regulate different and diverse cellular programmes. Generally, growth factor mediated activation of MAP kinases of the ERK family is commonly associated with cell survival and growth promotion, while stress stimulus activated MAP kinase p38 can induce cell cycle arrest and apoptosis and is therefore generally regarded as growth suppressive. In contrast, the JNK family of MAP kinases has been associated with both growth-promoting as well as suppressing activities. Not surprisingly, deregulated MAP kinase signalling is a critical feature in tumour development.

MAP kinases exert their activities, to a great extent, through changes in transcriptional programmes. Critical targets of their activities include members of the AP-1 complex, a dimeric transcription factor consisting of Jun, Fos, and ATF family DNA binding proteins. Depending on the cellular context the composition of the dimeric complexes determines the regulation of growth, survival, or apoptosis. For example, JNK phosphorylates c-Jun, leading to the dimerisation with c-Fos and activation of AP-1 target genes. JNK, and p38, also phosphorylate ATF2 which leads to the activation of ATF2 homodimers, or ATF2-c-Jun heterodimers and specific transcription activation. Thus, depending on the external stimulus, the AP-1 dependent transcriptional programme contributes to cell proliferation (e.g. through cyclin gene expression) or cell death (e.g. through BH3 domain gene expression).

ATF2 in development and tumorigenesis

To explain ATF2-dependent activities in mammalian systems we undertook mouse knockout studies. We showed that ATF2 (in overlapping functions with its closest homologue ATF7) is essential in the development of embryonic liver and heart (Breitwieser *et al.* 2007). Here, one critical function for ATF2/7 was shown to be in limiting the activities of upstream acting p38 kinase in a negative acting feedback loop. This is achieved by the ATF2 dependent activation of specific MAP kinase phosphatase genes of the DUSP (dual specificity phosphatases) family. Hence in the absence of functional ATF2/7 in embryonic liver cells, p38 MAP kinase was abnormally upregulated, at levels which were shown to be apoptosis inducing.

We also discovered a critical role for ATF2 in the developing central nervous system (Ackermann *et al.*, 2011, PLoS One, 2011, 6(4):e19090).

Deletion of ATF2 caused the loss of specific motor neurons of the hindbrain, which resulted in failure to coordinate breathing activities in postnatal stages and led to invariable death. Analysis in the embryonic brain showed that in the absence of ATF2, specific motor neurons of the hindbrain developed normally but gradually underwent processes reminiscent of neurodegeneration. This observation was correlated with increased levels of phosphorylated (activated) JNK and p38 in these neurons. Laser capture dissection of embryonic motor neurons allowed us to isolate knockout motor neurons and to profile gene expression from recovered RNA. We found that, as observed in the developing liver, specific DUSP gene expression was impaired in these neurons, which could explain the increased levels of activated JNK and p38, and concomitant apoptosis. Thus, in development ATF2 is critical in limiting apoptosis-inducing activities of MAP kinases.

In tumorigenesis, ATF2 can exert pro-tumorigenic but also anti-tumorigenic activities depending on the tumour type. Therefore one focus of our current research has been to decipher these context-dependent activities. In an ongoing project exploiting a mouse model of hepatocellular carcinoma, we found that ATF2 and ATF7 exert novel tumour suppressing activities. Here, double mutant hepatoblasts transformed with oncogenic H-Ras show a significantly stronger tendency to develop into HCC after orthotopic transplantation into recipient livers compared to ATF2 active controls. We also find 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 the upstream kinase JNK and could explain some of the tumour suppressive functions that have been reported for JNK.

Stress Responses in Fission Yeast

The Cell Regulation Group also uses fission yeast as a convenient model to gain insights into the nature and regulation of stress responses. As these processes are conserved in eukaryotes, it is anticipated that these insights will be relevant to mammalian cells. In Schizosaccharomyces pombe, the Sty1 MAP kinase plays a key role in mediating a general stress response. Like its mammalian counterpart, p38, Sty1 is phosphorylated and activated by a variety of stress stimuli and inactivation of the kinase results in pleiotropic stress sensitivity. For any MAP kinase, both the amplitude and duration of signalling is carefully controlled. These parameters can greatly influence the cellular response to the input signal and their control is orchestrated through positive- and negative-acting signals, as well as through the spatial distribution of the pathway's components. The negative signals are mediated through phosphatases which remove the activating phosphate groups. Two classes of phosphatase act upon Sty1: the Pyp and the Ptc phosphatases which dephosphorylate P-tyr and P-thr residues respectively. Ptc4 is a PP2C family phosphatase and we have found that it regulates both the magnitude and duration of Sty1 activation in response to hydroperoxides, but not to other stress conditions. Ptc4 localises exclusively to the mitochondria and is targeted there by an N-terminal mitochondrial targeting sequence (MTS) which is cleaved upon import. As well as controlling MAPK activation, our data indicates that Ptc4 is also a critical regulator of various mitochondrial functions including oxidative phosphorylation. Using immuno-electron microscopy as well as sub-cellular and sub-mitochondrial fractionation techniques, we found that a fraction of Sty1 also localises within the mitochondria, and to the same membrane fraction as Ptc4, suggesting that Ptc4 attenuates the activity of a mitochondrial pool of this MAPK. Cleavage of the Ptc4 MTS is abrogated specifically upon H_2O_2, resulting in the precursor form of the phosphatase; this displays a stronger interaction with Sty1 thus suggesting a novel mechanism by which the negative regulation of MAPK signalling is controlled and explaining the oxidative stress-specific nature of the regulation of Sty1 by Ptc4. We have identified the mitochondrial protease responsible for cleaving Ptc4 and we are currently investigating the mechanism by which oxidative stress inhibits this process. We hypothesize that the fraction of Sty1 localised to the mitochondria contributes to the regulation of this organelle through phosphorylation of targets therein. Indeed, localising a stress-signalling MAPK module to a compartment responsible for the majority of intracellular ROS production could promote a timely response to oxidative damage. One possible function is that Sty1 regulates respiration as deletion of *sty1* results in reduced oxygen consumption although further work will be required to determine if this is a result of directly regulating mitochondrial components through phosphorylation. We are currently trying to identify mitochondrial targets of Sty1 as well as determining the mechanism by which oxidative stress affects the cleavage of Ptc4.

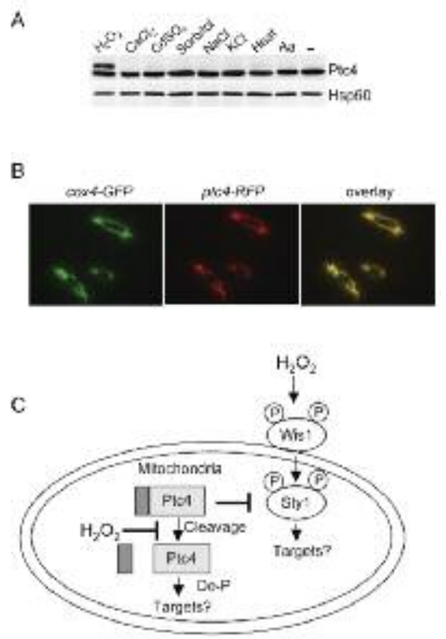

Figure 1
A. Western blot of fission yeast whole cell extract illustrating that Ptc4 exists as an extra isoform upon oxidative stress induced by hydrogen peroxide but not upon other forms of stress.

B. Fluorescence microscopy indicates that Ptc4 localizes to the mitochondria as indicated by co-localization with Cox4, a known protein of the inner mitochondrial membrane.

C. Model to explain how mitochondrial Sty1 is regulated by Ptc4 specifically upon oxidative stress.

Publications listed on page 67

Cell Signalling Group

http://www.paterson.man.ac.uk/cellsignalling

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Tumour initiation and progression result from inappropriate activation of intracellular signalling cascades. Rho-like GTPases are molecular switches in signalling pathways that regulate cytoskeletal and junctional organisation, as well as gene transcription. In this way, Rho proteins influence cell morphology, adhesion, motility, as well as cell cycle progression and cell survival.

Rho proteins are transforming *in vitro* and are essential for Ras-mediated *in vitro* transformation. Moreover, data has emerged to directly implicate Rho proteins in tumour initiation and progression *in vivo*. Our group investigates the mechanisms by which certain regulators of the Rho protein 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 including notably actin cytoskeletal reorganisation. 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 localisation and modulation of Rac1 protein levels. More recently, regulation by post-translational modification has emerged as a significant means of regulating Rac activity. The first such modification identified was C-terminal prenylation to facilitate membrane association but more recently phosphorylation as well as modification with ubiquitin and ubiquitin-like proteins have been described.

Post-translational modifications of the GTPase 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 under such conditions. This revealed the small ubiquitin-like modifier (SUMO) E3-ligase, PIAS3, as a novel Rac interacting protein. We found that PIAS3 interacts better with the GTP-bound form of Rac. We then showed that PIAS3 is required for increased Rac activation and optimal cell migration in response to Hepatocyte Growth Factor (HGF) signalling. Subsequently we demonstrated that Rac1 can be conjugated to SUMO-1 in response to hepatocyte growth factor treatment and that SUMOylation is enhanced by PIAS3. We also showed 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. The finding that a Ras superfamily member can be SUMOylated provides an insight into the regulation of these critical mediators of cell behaviour. Moreover, our data revealed a role for SUMO in the regulation of 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 the HACE1 and Rac1 interaction is enhanced by HGF signalling. Furthermore we showed 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. A non-ubiquitylatable Rac1 rescues the migration defect of Rac1-null cells to a greater extent than wild-type Rac1. These findings identified HACE1 as an antagonist of cell migration through its ability to degrade active Rac1 (manuscript submitted).

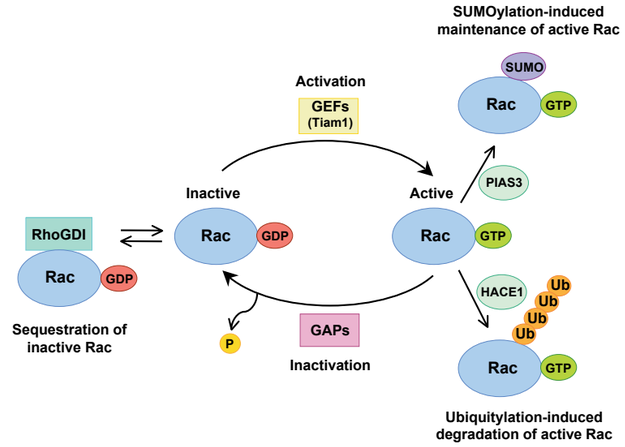

Figure 1
Multiple mechanisms exist to regulate Rac activity. The Rac GTPase cycles between inactive GDP-bound and active GTP-bound states. Rac activation is facilitated by the action of GEFs (such as Tiam1), which promote GDP dissociation from Rac and allow GTP to bind instead. Through the association 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).

Tiam1-Rac signalling regulates bipolar spindle assembly, chromosome congression and mitotic progression dependent on phosphorylation of Tiam1 by Cyclin B/CDK1

Tiam1 (for T-lymphoma invasion and metastasis protein) is a GEF that selectively activates Rac. Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by chemical carcinogens and the few resulting tumours grow very slowly (Malliri *et al*., Nature 2002; 417: 867). To better understand the role of Tiam1 in promoting tumour growth we have examined its role in the cell cycle. During this cycle, centrosomes separate and co-ordinate bipolar spindle formation required for subsequent chromosome segregation during mitosis. Centrosome separation occurs via distinct prophase and prometaphase pathways. Kinesin-5 (Eg5), a microtubule (MT) motor, pushes centrosomes apart during bipolar spindle assembly and its suppression results in monopolar spindles and mitotic arrest. Forces that antagonise Eg5 in prophase are unknown. We identified a new force generating mechanism mediated by Tiam1, dependent on its ability to activate Rac. We revealed that Tiam1 and Rac localize to centrosomes during prophase and prometaphase, and Tiam1, acting through Rac, ordinarily retards centrosome separation. Importantly, both Tiam1-depleted cells in culture and Rac1-deficient epithelial cells *in vivo* escape the mitotic arrest induced by Eg5 suppression. Moreover, Tiam1-depleted cells transit more slowly through prometaphase and display increased chromosome congression errors. Significantly, Eg5 suppression in Tiam1-depleted cells rectifies not only their increased centrosome separation but also their chromosome congression errors and mitotic delay. These findings identified Tiam1-Rac signalling as the first antagonist of centrosome separation during prophase, demonstrated its requirement in balancing Eg5-induced forces during bipolar spindle assembly *in vitro* and *in vivo*, and showed that proper centrosome separation in prophase facilitates subsequent chromosome congression (Woodcock *et al*. Curr Biol. 2010; 20:669). Subsequent to this study, we have found that Tiam is phosphorylated by Cyclin B/CDK1 in mitosis. This phosphorylation, while not required for Tiam1 localisation to centrosomes, appears essential for its role in regulating centrosome separation. Currently we are investigating the mechanism by which phosphorylation of Tiam1 influences its role at centrosomes through attempting to identify interacting partners whose interaction with Tiam1 at centrosomes is dependent on phosphorylation.

Tiam1 antagonizes malignant progression

Despite its role in promoting tumour survival and growth, Tiam1 appears to suppress malignant progression since the few skin tumours arising in Tiam1-deficient mice progressed more frequently to malignancy than those in wild-type mice (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 the accompanying induction of 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 the 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 direct phosphorylation of Tiam1 by Src is required for the initial stages of Src-induced EMT. We showed that Src phosphorylates Tiam1 on tyrosine 384 (Y384). This occurs 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. Significantly, we demonstrated that in human lung, colon, and head and neck cancers phosphorylation of Y384 of Tiam1 positively correlated with Src activity, while total levels of Tiam1 were inversely correlated with Src activity, consistent with the above-mentioned post-translational regulatory mechanism operating in malignancies (Woodcock et al. Mol Cell. 2009; 33: 639).

Publications listed on page 67

Clinical and Experimental Pharmacology Group

http://www.paterson.man.ac.uk/cep

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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.

Two senior appointments this year strengthened CEP; Ged Brady joined us to develop nucleic acid based biomarkers and Stephen Walker will oversee the CEP Biomarker portfolio. We also welcomed back Alastair Greystoke and Emma Dean as NIHR Clinical lecturers developing their independent translational research programmes within CEP. We continue our focus on circulating tumour cells (CTCs), designing and implementing novel proof of mechanism CTC assays and we initiated an exciting collaboration to develop a new CTC technology. Joint working with the MCRC Drug Discovery group is underway such that drugs and biomarkers are developed in parallel. The highlight of 2011 was the award to the CEP based team of the CR-UK Translational Research Prize.

Expansion of the Biomarker Portfolio – The Nucleic Acids Biomarkers Team

The CEP Biomarker Portfolio has expanded to include a Nucleic Acids Biomarkers (NAB) team led by CEP Deputy Ged Brady. NAB projects have been initiated to examine miRNA, methylation patterns present in circulating free DNA (cfDNA), RNA profiling and mutation detection in CTCs (Figure 1). 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 lung cancer patients' blood and tumour samples are underway within CEP and in collaboration with the Cancer Genome Project group at the Wellcome Trust Sanger Institute. New biomarker collaborations are being initiated with companies developing novel CTC/blood biomarker technology platforms.

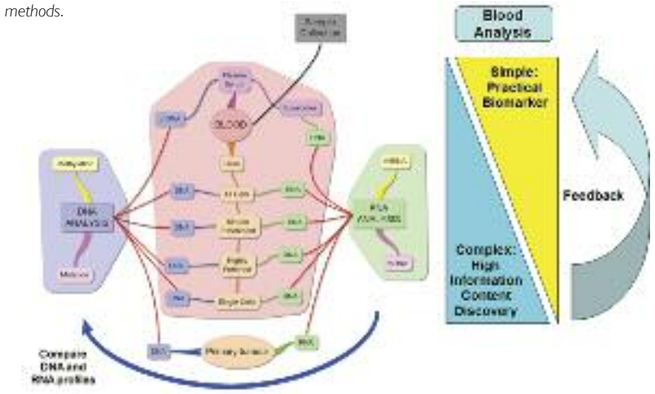
Determining the Apoptotic Potential of Circulating Tumour Cells

An exciting collaboration has begun combining CEP's expertise in isolating and analysing CTCs and the expertise of Professor Tony Letai's group at the Dana Farber Cancer Institute in Boston in the regulation of cancer cell apoptosis. The Letai group have developed a technology, BH3 profiling, which allows the propensity of cancer cells to undergo apoptosis to be determined, either *in vitro* or *ex vivo*. The BH3 profile is predictive of response to various novel and conventional anti-cancer agents however the protocol requires large numbers of cells and is currently limited to lymphomas or tumours which can be readily biopsied. The aim of this collaboration is to develop BH3 profiling for single cell analysis by microscopy and to apply it to CTCs isolated from patients with lung cancer. Dr Radek Polanski received a CR-UK Travel award to visit the Letai laboratory to progress these studies following the visit to PICR of Jeremy Ryan from the Letai Lab. SCLC (small cell lung cancer) is initially chemo-sensitive but it rapidly relapses as a chemo-resistant disease and the molecular mechanism(s) driving this are unclear. By BH3 profiling SCLC patients' CTCs at presentation and relapse, in combination with NGS approaches in the NAB team, we hope to uncover new treatment strategies to overcome drug resistance.

Adding Value to the Drug Discovery Pipeline at PICR – joint working with the Drug Discovery Group (DDG)

As the drug discovery programme initiated in 2009 by Donald Ogilvie in the PICR DDG takes shape, CEP are working closely with DDG colleagues to develop biomarkers early during the drug discovery process, assist in the validation of drug targets and facilitate in-house *in vivo* drug metabolism and pharmacokinetic analyses. The two groups have also worked collaboratively to develop analytical methods for cell based assays that are helping to drive the DDG portfolio. DMPK (Drug Metabolism and Pharmacokinetics) studies on compounds produced by the DDG are ongoing utilising CEP's *in vivo* preclinical pharmacology expertise and mass spectroscopy facilities. With respect to target validation, CEP are investigating whether an enzyme involved in the repair of etoposide-induced damage is a potential drug target in combination with etoposide in SCLC. A joint strategic plan has been constructed to begin biomarker discovery/validation as drug discovery proceeds from hit identification to lead identification, making sure predictive and pharmacodynamic biomarkers aligned to DDG leads are not only useful in preclinical development but are also 'fit for purpose' for deployment in early clinical trial scenarios.

Figure 1
The Figure presents an overview of CEP's blood based Nucleic Acids Biomarkers (NAB) strategy where the aim is to couple a single simplified clinical blood collection protocol with DNA and RNA analysis of both circulating tumour cells (CTCs) as well as the examination of tumour derived nucleic acids present in plasma and serum. The NAB focus will be to carry out a biomarker discovery phase through exhaustive fractionation and profiling of blood samples using CTC fractionation and next generation sequencing followed by an evaluation of potential markers in the clinic using simpler more streamlined methods.



Preclinical models of induced tumour cell death as tools for characterisation of imaging biomarkers

Several collaborations on non invasive imaging of cell death have been carried out this year. We developed and validated a colorectal cancer cell line engineered to express a robust doxycycline (dox) inducible constitutively active mutant of effector caspase 3 (the death switch) that is used in tumour xenograft models. The model allows synchronous and regulated levels of apoptosis to be induced *in vivo* where changes in imaging tissue and blood biomarkers can be monitored and correlated, with the horizon of improved interpretation of multi modality biomarker data in the clinic. In collaboration with Dr Kaye Williams and Dr Chris Cawthorne at the Wolfson Molecular Imaging Centre (WMIC), pilot studies have been performed to study the uptake of [18F]ML-10, a PET imaging probe designed to be specifically taken up by apoptotic cells to address the specificity and sensitivity of this novel tracer. An imaging collaboration with GE Healthcare was initiated using this death switch model investigating the biodistribution of three 99Tc-labelled compounds as specific imaging probes targeted to apoptotic cells. In collaboration with Professor Tim Illidge, a murine cell line containing the death switch has been generated and grown *in vivo* and the impact of induction of tumour cell apoptosis on the immune system is being examined. In collaboration with the University of Manchester Biomedical Imaging Department and Professor Geoff Parker, our CR-UK/AZ clinical pharmacology fellow Danielle Shaw has undertaken two pilot studies using the death switch model to investigate the effects of tumour cell death on the extracellular and extravascular space using Diffusion Weighted MRI.

Portfolio management of clinical biomarker studies

To ensure effective management of the significant number of biomarker studies in CEP, we have initiated the development of a portfolio management system. A key first step has been the recruitment of Stephen Walker as portfolio leader who is responsible for 70 provisional or ongiong clinical biomarker studies in CEP. Biomarkers are being deployed on a range of early clinical trials sponsored by the Manchester Cancer Research Centre (MCRC) and ECMC partner organisations (CR-UK, Christie Clinical Trials Unit (CTU), Manchester University, UCL, Cardiff, Glasgow and Birmingham CTUs) and several pharmaceutical companies (e.g. AstraZeneca, Roche, Novartis, GSK, Chugai, and this year via a new overarching Master Agreement with Abbott Laboratories). To facilitate management of the extensive biomarker portfolio we have utilised a Laboratory Information Management System (LIMS) to establish a single database for all biomarker studies. The LIMS database will contain key information for more than 250 biomarker projects enabling effective tracking of all ongoing and future clinical biomarker studies.

Overview of CEP/AstraZeneca Serological Alliance

Since 2006 an Alliance has been in place between AstraZeneca (based at nearby Alderley Park) and CEP to analyse, report and advise on data obtained from blood borne serological biomarker assays validated for clinical use in our GCPL laboratories to support AZ's oncology portfolio. The CEP/AZ Alliance now encompasses a broad range of biomarkers (cell death, invasion, angiogenesis, CTC enumeration and characterisation) all established for high throughput clinical trial analysis where thousands of samples are shipped to CEP from clinical trial sites across the globe. Two highlights in 2011 were a) the development of a bespoke assay to measure androgen receptor (AR) protein expression in CTCs as a Proof of Mechanism biomarker in Phase I clinical trials of the Selective Androgen Receptor Down-regulator (SARD); and b) the development of a panel of highly sensitive ELISAs to quantitate circulating levels of the erbB ligands to trials of AZD8931 (pan-Erb inhibitor).

Publications listed on page 67

DNA Damage Response Group

http://www.paterson.man.ac.uk/dnadamage

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Rosa Morra

Scientific Officer
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Graduate Students
Eva Barkauskaite
Michael Tallis (from October)

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, the 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 proved to be a valuable strategy in searching for novel approaches and targets in cancer therapy.

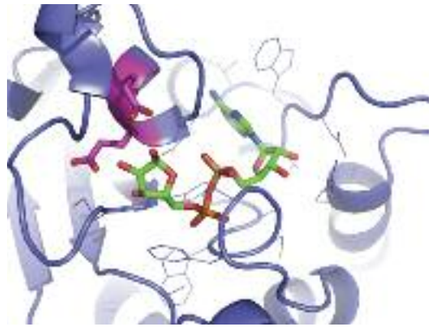
Poly(ADP-ribosyl)ation in regulation of DNA repair

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, 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 the chromatin structure and in apoptotic signalling. The recent development of potent PARP inhibitors provided 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 breast and ovarian cancers (Bryant *at al*, Nature 2005; 434: 913). 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 the identification of new pathways and protein functions regulated by poly(ADP-ribosyl)ation. 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 at 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 the 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).

Figure 1
Active site of the Thermomonospora curvata PARG enzyme with bound ADP-ribose. The two catalytic glutamate residues are shown in pink.



Another class of protein that remains a focus of our research is those containing a macrodomain. 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 mechanistic and structural data for the human PARG protein. Recently, we solved the structure of a bacterial PARG-like protein, which gave the first insight into the basic principles of PARG structure and its mechanism of catalysis (Slade *et al*, Nature, 2011) (Figure 1). This structure revealed that the PARG catalytic centre is a diverged type of macrodomain. Despite this advance, structural information on the human PARG is still lacking. Our goal is to solve the structures of human PARG in complex with the substrate analogues and inhibitors by means of X-ray crystallography 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.

Publications listed on page 69

Drug Discovery Group

http://www.paterson.man.ac.uk/drugdiscovery

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Emma Fairweather
Gemma Hopkins
Graeme Thomson
Helen Small
Mandy Watson
Mark Cockerill
Nicola Hamilton
Nikki March
Samantha Fritzl
Sarah Holt

During 2011 we have expanded the Drug Discovery team, consolidated our informatics platform and, most importantly, have progressed our discovery project portfolio. Five hit-to-lead phase projects are now underway, targeting a variety of cancer targets. These activities are underpinned by multiple collaborations both within and beyond the Manchester Cancer Research Centre (MCRC).

People

With the increased funding stream for this programme from April 2011, following a major recruitment campaign, we have expanded the team from nine to twenty four scientists. Alongside cell and molecular biologists we have also recruited synthetic, medicinal and computational chemists and a drug metabolism specialist. This multidisciplinary team allows us to pursue projects at all stages of drug discovery from target selection to lead optimisation. After much searching, we were particularly pleased to secure Dr Ian Waddell, a highly experienced academic and industrial scientist, as Head of Drug Discovery Bioscience.

Infrastructure

Our Dotmatics chemoinformatics and electronic notebook platforms are now fully deployed and, after significant efforts, are fully operational for integration and visualisation of all kinds of data. The more specialised computational chemistry platform allows us to integrate protein and chemical ligand structural information for target assessment and virtual screening. On the technology hardware front, we have been particularly impressed with the Echo acoustic dispensing technology and are planning to upgrade this facility to improve throughput and also handle aqueous solutions. We have also sought to improve existing laboratory equipment by adding simple automation such as plate stackers. These cost-effective modifications have allowed us to screen larger compound subsets efficiently.

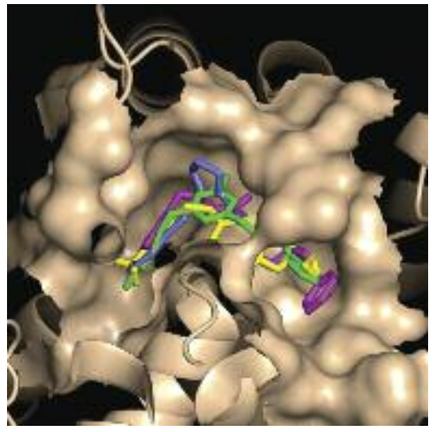
Drug Discovery Targets

We continue to review many cancer drug target opportunities and, during 2011, have worked closely with several Paterson Institute group leaders (John Brognard, Tim Somervaille & Ivan Ahel) in the identification, validation and prosecution of targets. It is very exciting to be able to access, and exploit pre-publication data and facilitate new basic discoveries by provision of tool compounds. We have also carried out a broad high level review of breaking cancer science and have prioritised key areas for more thorough target investigation. To support this we are looking to strengthen our links to Crispin Miller's bioinformatics group through a jointly funded post-doc who will focus on the drug discovery target selection process.

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. One approach is to conduct a high throughput screen (HTS), in which a simple drug target-related assay is interfaced with a collection of ~100,000 diverse chemicals (mw~400) in order to try and identify compounds ("hits") which interfere with the functional activity of the target. During 2011 we completed a successful HTS against one of our DNA repair enzyme targets in collaboration with the Cancer Research Technology Drug Discovery Laboratory (CRT-DL) laboratory in London. This has provided useful chemical start points which are now being investigated further by our chemists. Since we do not have our own HTS facility, we have also made significant investments in alternative hit finding strategies that we can use locally. These include Virtual Screening – described in detail in last year's report - and, recently, functional fragment screening. The latter approach involves testing a collection of ~1200 smaller compounds (mw~300) against our targets, at higher concentrations than used those normally used in HTS. Using the Echo acoustic dispensing technology we have been able to run screens against five potential drug target molecules using a small sample of a fragment library (kindly provided by the Beatson Institute Drug Discovery Unit). In just three months we have identified early hits against all of these targets, some of which have proved elusive to other hit finding technologies. One advantage of screening with fragments is that the compounds are smaller and can fit into protein pockets inaccessible to larger (HTS) inhibitors. A potential downside is that fragment hits generally have a lower affinity and require more optimisation for potency. We have also secured access to a focused ~10,000 compound kinase inhibitor collection from CRT-DL and will be using this for screening selected targets in house. An additional, unanticipated hit finding activity has been to engage with pharmaceutical companies regarding the opportunity to work on validated hits (and sometimes associated assay technologies) from their deprioritised cancer projects, but only if they meet our strict target criteria.

Figure 1
Inhibitors of the enzyme PARG represent a promising new approach to disrupting DNA repair pathways in tumour cells. Knowledge of the 3D atomic structure of PARG enables DDU scientists to design novel compounds with the potential to block the action of the enzyme. The figure shows the surface of the bacterial PARG catalytic site as determined using X-ray crystallography by Ivan Ahel's DNA Damage Response Group, with several small drug-like compounds that have been computationally modelled into the binding site. Using virtual screening methods, very large databases of commercial compounds can be analysed to find the most promising candidates for purchase and testing.



Project Portfolio

Most drug discovery projects do not progress all the way to the clinic so our major task is to deliver quality data, as quickly as possible, to identify and stop the "losers" early and redeploy our resources onto potential "winners". During 2011, we reached stop decisions on three projects and have started three others. Our current portfolio includes five hit identification projects against metabolic, DNA repair (x2), redox modulation and oncogene signalling targets. Two of these projects are approaching consideration for progression to the lead identification stage. For the most advanced project, HTS screening has identified hit compounds whose potency has been improved ten fold by our chemists in the last few months. The *in vitro* pharmacology of these more potent compounds is currently being explored in cells by us and key expert collaborators. 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 have actively participated in broader Cancer Research UK drug discovery activities and are now involved in collaborations, some of which have been described in this report, with most of the Cancer Research UK drug discovery units. During the summer of 2011 we underwent a successful annual review with the Drug Discovery Advisory Group.

The Future

During 2011 we have expanded out team, progressed our project portfolio and, pleasingly, increased the depth of our interactions with Paterson Institute scientists. In 2012 we look forward to starting and stopping more projects and progressing the best ones to the next stage.

Publications listed on page 69

Immunology Group

http://www.paterson.man.ac.uk/immunology

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Owen McGinn
Rasilaben Vaghjiani
Julie Brazzatti (from November)
Darren Roberts (from February)

Clinical Fellow
Saladin Sawan

Scientific Officer
Jian Li

Graduate Students
Andrzej Rutkowski (with Crispin Miller)
Georgi Marinov

Undergraduate student
Milena Kalaitsidou (until September)

Historically, a principle goal was to harness immunity to 5T4 oncofoetal antigen for cancer therapy. Two approaches are in late phase clinical trial: TroVax, a recombinant viral vaccine (Oxford BioMedica, UK) and Anyara, an antibody targeted super-antigen (Active Biotech, Sweden); 5T4 antibody targeting of a toxin is in clinical development (Pfizer).

We have recently exploited our 5T4 knock out (5T4KO) mouse to investigate how endogenous expression of 5T4 influences 5T4 vaccine induced T cell immunity and tolerance in comparison to wild type (WT) mice. The regulation of CXCL12/CXCR4 chemotactic responses by 5T4 molecules provides a functional role for 5T4 in the spread of tumour cells from the primary site (PLoS ONE 5(4): e9982. doi:10.1371/journal.pone.0009982). Further work has now shown that 5T4 molecules integrate at least two distinct signalling pathways of high relevance to the growth and movement of developing or malignant cells.

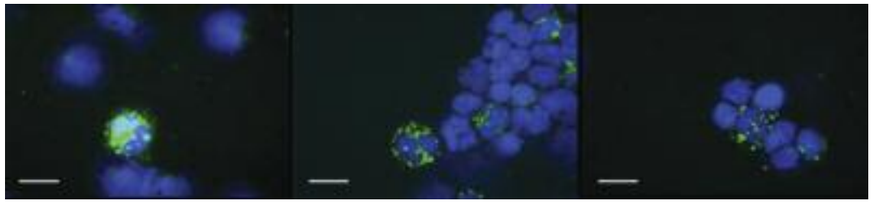
Regulation of autologous immunity to the mouse 5T4 oncofoetal antigen; implications for immunotherapy

Effective vaccination against the 5T4 oncofoetal glycoprotein may be limited by the nature of the T cell repertoire and the influence of immunomodulatory factors, in particular T regulatory cells (Tregs). Work led by Fernanda Castro identified mouse 5T4-specific T cell epitopes using a 5T4KO mouse and evaluated corresponding WT responses as a model to refine and improve immunogenicity. We showed that 5T4KO mice vaccinated by replication defective adenovirus encoding mouse 5T4 (Adm5T4) generate potent 5T4 specific interferon(IFN)-γ CD8 and CD4 T cell responses which mediate significant protection against 5T4 positive tumour challenge. 5T4KO CD8 but not CD4 primed T cells also produced IL-17. By contrast, Adm5T4 immunized WT mice showed no tumour protection consistent with only low avidity CD8 IFN-γ and no IL-17 T cell responses and no detectable CD4 T cell effectors producing IFN-γ or IL-17. Treatment with anti-folate receptor 4 (FR4) antibody significantly reduced the frequency of Tregs in WT mice, enhancing 5T4 specific IFN-γ while reducing IL-10 T cell responses but did not reveal IL-17 producing effectors. Treatment with FR4 antibody after Adm5T4 vaccination altered the balance of effectors and provided modest protection against autologous B16m5T4 melanoma challenge. We conclude that the efficacy of 5T4 and some other tumour associated antigen (TAA) vaccines may be limited by the combination of TAA specific Tregs, the deletion and/or alternative differentiation of CD4 T cells as well as the absence of distinct subsets of CD8 T cells. Comparison and adoptive transfer of effector T cells between WT and 5T4KO mice can provide a useful platform to explore and improve immune therapeutic modalities in the context of an autologous TAA which provides a more realistic model for assessment of immunogenicity, efficacy and safety (Cancer Immunology, Immunotherapy doi:10.1007/s00262-011-1167).

5T4 oncofoetal glycoprotein, CXCL12 receptor preference, signal transduction and biological response

CXCL12 is a pleiotropic chemokine capable of eliciting multiple intracellular signal transduction cascades and biological functions, via interaction with either CXCR4 or CXCR7. Factors that determine CXCL12 receptor choice, 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 CXCR4 expression leading to CXCL12 mediated chemotaxis in mouse embryonic cells. Using WT and 5T4KO murine embryonic fibroblasts (MEFs), Owen McGinn has led a study which has 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. However, 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. Thus the surface expression of 5T4 marks the use of the CXCR4 rather than the CXCR7 receptor, with distinct consequences for CXCL12 exposure, relevant to the spread and growth of a tumour. Consistent with this hypothesis we have identified some human tumour cell lines with similar 5T4/CXCR7 reciprocity that is predictive of their biological response to CXCL12.

Figure 1
Expression of 5T4 by B-ALL blasts in bone marrow samples from three relapse patients; magnification x 40, scale bar 5 μm.



5T4 oncofoetal antigen is expressed in high risk of relapse childhood pre-B acute lymphoblastic leukemia (ALL) and is associated with a more invasive and chemotactic phenotype

While mature hematopoietic cells lack the expression of 5T4, open access microarray data suggests that 5T4 is present during the early stages of normal B cell development in particular at the pro- and pre-B cell phase. Building on our collaboration with the Children's Cancer Group (Blood 118: 638-49, 2011), Fernanda Castro and Owen McGinn have led the research showing that lymphoblasts from patients of the high risk of relapse subset of childhood pre-B ALL express higher levels of 5T4. Gene expression profiling of 85 diagnostic pre-B ALL bone marrow samples revealed higher 5T4 oncofoetal antigen transcript levels in the cytogenetic high-risk subgroup of patients ($p < 0.001$). The figure shows immunofluorescence staining for 5T4 on DAPI labelled cytospins of bone marrow samples from pre-B ALL relapse patients. Using $5T4^{+ve}$ and $5T4^{-ve}$ cells derived from a high risk cytogenetics *BCR-ABL*+ pre-B ALL line, we have shown that 5T4 expression correlates with increased invasion, adhesion and CXCR4/CXCL12 chemotaxis *in vitro* and differential infiltration of extramedullary sites following intraperitoneal challenge of immunocompromised mice. Even with the current successful B-ALL treatments, approximately 1 in 4 children will relapse. Recurrence is frequently characterised by the occurrence of disease at extramedullary sites such as the central nervous system and gonads. 5T4 expression may be a prognostic marker of such spread but also could be mechanistically involved. We have also shown that 5T4 positive pre-B-ALL cells are susceptible to 5T4 specific superantigen antibody-dependent cellular cytotoxicity *in vitro* and *in vivo* providing support for high risk pre-B-ALL 5T4 immunotherapy.

5T4 Inhibits Wnt/β-Catenin Signalling and Activates Noncanonical Wnt Pathways by Modifying LRP6 Subcellular Localization

Wnt proteins can activate distinct signalling pathways, but little is known about the mechanisms regulating pathway selection. In collaboration with the Weidinger group in Dresden, we have shown that the Zebrafish homologue of mammalian 5T4, Wnt-activated inhibitory factor 1 (Waif1) interferes with Wnt/β-catenin signalling and concomitantly activates noncanonical Wnt pathways. Waif1 inhibits β-catenin signalling in zebrafish and *Xenopus* embryos as well as in mammalian cells, and zebrafish *waif1a* acts as a direct feedback inhibitor of wnt8-mediated mesoderm and neuroectoderm patterning during zebrafish gastrulation. Waif1a /5T4 binds to the Wnt coreceptor LRP6 and inhibits Wnt-induced LRP6 internalisation into endocytic vesicles, a process that is required for pathway activation. Thus, Waif1a/5T4 modifies Wnt/β-catenin signalling by regulating LRP6 subcellular localisation. In addition, Waif1a/5T4 enhances β-catenin-independent Wnt signalling in zebrafish embryos and *Xenopus* explants by promoting a noncanonical function of Dickkopf1. These results suggest that Waif1/5T4 modulates pathway selection in Wnt-receiving cells. Interestingly, β-catenin -independent signalling can enhance motility and invasion of cancer cells raising the possibility that Waif1/5T4 promotes invasion of cancer cells by enhancing noncanonical Wnt signalling (Developmental Cell doi: 10.1016/j.devcel.2011.10.015).

Publications listed on page 70

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Phosphoinositides are a family of lipid second messengers that are regulated in response to environmental changes by the activities of 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 as it is the substrate for phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PIC) both of which are deregulated in human tumours (Figure 1). Furthermore, PtdIns(4,5)P_2 is itself a regulator of cytoskeletal dynamics, cell survival and cell polarity.

PIP5Ks and PtdIns(4,5)P_2

PtdIns(4,5)P_2 is present in the plasma membrane and in the nucleus where its levels in these two compartments can be regulated distinctly. PtdIns(4,5)P_2 is synthesised by two different families of kinases using 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 can also localise to other subcellular compartments, such as the golgi (PIP4Kβ), the nucleus (PIP4Kα and γ), focal adhesions (PIP5Kγ) and the cytokinetic furrow (PIP4Kα). We have identified two components that coordinate the localisation of PIP5K to the plasma membrane. The first is the small molecular weight G protein Rac and the second is the plasma membrane level of PtdIns(4,5)P_2. We identified and characterised a binding site for Rac that is highly conserved between all isoforms of PIP5K. Mutation of this site, which attenuates interaction with Rac, reduces but does not abolish membrane localisation of PIP5K (Figure 2). We also observed that a mutation, which attenuates PIP5K activity, also leads to its reduced localisation at the membrane. Interestingly, a combination of both mutations led to the complete delocalisation of PIP5K from the plasma membrane (Figure 2). We also studied the intracellular localisation of PIP5K under conditions where PtdIns(4,5)P_2 was reduced, by PLC mediated hydrolysis (ionomycin/calcium addition), or when PtdIns(4,5)P_2 was being resynthesised (EGTA addition). Biochemical analysis demonstrated that ionomycin/calcium led to a rapid and sustained depletion in both PtdIns4*P* and PtdIns(4,5)P_2 and that the addition of EGTA led to a resynthesis of both phospholipids. A reduction in intracellular PtdIns(4,5)P_2 is apparent from the delocalisation from the membrane into the cytosol of the RFP-PH domain, a specific *in vivo* fluorescent reporter of PtdIns(4,5)P_2, (Figure 3). Under conditions of low PtdIns(4,5)P_2, GFP-PIP5K was delocalised into the cytosol and upon PtdIns(4,5)P_2 resynthesis became relocalised to the plasma membrane (Figure 3). In order to implicate PtdIns(4,5)P_2 resynthesis we treated cells with a specific inhibitor of PIP5K (see PIP5K as a drug target) and monitored GFP-PIP5K localisation. Biochemical analysis demonstrated that treatment with the inhibitor prevented the resynthesis of PtdIns(4,5)P_2 but not of PtdIns4*P*. Ionomycin treatment led to the delocalisation of GFP-PIP5K from the membrane, however the relocalisation of PIP5K was prevented upon blocking the resynthesis of PtdIns(4,5)P_2 (Figure 3). The lack of RFP-PH domain at the plasma membrane demonstrates that the inhibitor prevented PtdIns(4,5)P_2 resynthesis (Figure 3). Future studies are aimed at understanding how changes in PtdIns(4,5)P_2 regulate membrane localisation of PIP5K and how PIP5Ks localise to other specific cellular locations.

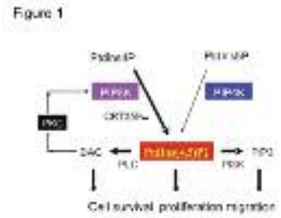

Figure 1

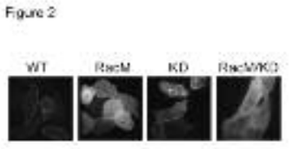

Figure 2

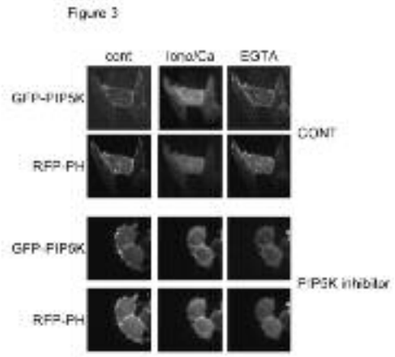

Figure 3

Figure 1
There are two pathways for PtdIns(4,5)P_2 synthesis however the major synthetic pathway is through PIP5K. PIP4K regulates the levels of PtdIns5P. 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
The intracellular localization of wild type GFP-PIP5K (WT), a mutant unable to interact with Rac (RacM), a kinase inactive mutant (KD) and of GFP-PIP5K mutated to reduce its interaction with Rac and block its kinase activity (RacM/KD) were determined in live cells using confocal microscopy.

Figure 3
Cells were transfected with constructs as indicated on the right and imaged live as controls (cont), after activation of PLC and reduction in PtdIns(4,5)P_2 (iono/Ca) or after the initiation of PtdIns(4,5)P_2 resynthesis (EGTA). PIP5K inhibition was carried out by treating the cells with a specific PIP5K inhibitor (bottom two panels). Iono/ca leads to a dramatic decrease in PtdIns(4,5)P_2 levels shown by the decrease in membrane localisation of the RFP-PH domain, whereas EGTA addition stimulates PtdIns(4,5)P_2 resynthesis and relocalisation of the PH domain to the membrane.

PIP5K as a target for drug development

PIP5K inhibitors were identified using a novel high throughput *in vitro* assay for PIP5K (Cancer Research Technology). To investigate if these inhibitors could attenuate PIP5K activity *in vivo*, we have developed a novel assay based on the inducible cellular expression of fluorescent probes that interact specifically with and report the levels of PtdIns(4,5)P_2 in living cells. Using this and other assays we have assessed the structure/function relationships of inhibitors *in vivo* in order to drive rational chemical synthesis of new compounds with enhanced affinity and specificity. Furthermore we have shown that these inhibitors attenuate PtdIns(4,5)P_2 synthesis *in vivo* and in doing so reduce growth factor induced activation of both the PLC and PI3Kinase pathway (Figure 1). These data suggest that PIP5K inhibitors likely will have added therapeutic value compared to the inhibition solely of PI3Kinase.

PIP4K and PtdIns*5P*

There are three isoforms of PIP4Ks of which α is cytosolic, β is cytosolic and nuclear and γ localises to internal membrane compartments. PIP4Ks homo and heterodimerise and the heterodimerisation between α and β localises the α isoform to the nucleus. The heterodimerisation is important as the majority of PIP4K activity associated with PIP4Kβ is accounted for by its interaction with PIP4Kα. Using tissue microarrays of advanced human breast tumour samples, we have found that PIP4Kβ expression is both up and down regulated and interestingly, high expression correlates with better patient survival (with Professor Landberg, Breakthrough Breast Cancer and University of Manchester). Studies in human breast tumour cell lines are ongoing to define signalling pathways regulated by PIP4Kβ expression. Our hypothesis is that PtdIns*5P* is a key signalling intermediate as we have shown that genetic manipulation, to reduce the levels of PIP4K in different organisms, leads to an increase in the levels of PtdIns*5P* rather than a substantial decrease in PtdIns(4,5)P_2 and from our previous studies that PIP4Ks are important during stress signalling. Oxidative signalling plays a key role in aging and cancer, exemplified by the development of pathologies in mice genetically manipulated to mismanage reactive oxygen species. We find that oxidative stress leads to a rapid increase in the levels of PtdIns*5P* and that PtdIns*5P* signalling impinges on the oxidative stress sensitivity of the cell. Pin1 null mice show phenotypes of premature aging and in cancer development and MEFs derived from these mice show growth insensitivity to oxidative stress. Interestingly, we find that Pin1 null MEFs exhibit increased levels of PtdIns*5P* in response to hydrogen peroxide stimulation and that removal of this increased PtdIns*5P*, by overexpression of a PIP4K, re-instigates oxidative stress sensitivity. Pin1 is a proline-directed phosphorylation-dependent regulator of protein conformation. In response to stress signalling we show that Pin1 interacts with and regulates PIP4Ks which in turn regulates the levels of PtdIns*5P* and the cellular response to oxidative stress. How PtdIns*5P* regulates cellular processes is far from clear and we are using lipid affinity purification and mass spectrometry to identify proteins that interact with and therefore are likely to function as downstream targets for PtdIns*5P* signalling. For example, the PHD finger is a cross-braced Zinc finger, present in numerous chromatin regulating proteins that can interact with and decode histone modifications and can also interact with phosphoinositides. We found that the PHD finger of TAF3, a regulator of the basal transcription complex and cell differentiation, interacts with phosphoinositidies and mutants that ablate this interaction compromise basal transcription and muscle differentiation. Future studies are aimed at identifying novel pathways regulated by PIP4Ks.

Phosphoinositide and their therapeutic potential

In order to define new targets for potential therapeutic development we have utilised a small shRNAi lentiviral library targeting all the known genes involved in phosphoinositide metabolism. This library had been used to identify genes potentially involved in migration and in leukemogenesis. Hits have been validated and future work is aimed at understanding how these genes modulate phosphoinositide metabolism to regulate cell responses.

Publications listed on page 70

Leukaemia Biology Group

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Tim Somerville

It has become apparent in the past several years that epigenetic dysfunction has a central role in the pathology of myeloid cancers. This is illustrated by the discovery of recurrently occurring mutations targeting genes that code for key epigenetic regulators such as the methylcytosine hydoxylase TET2, the DNA methyltransferase DNMT3A, the Polycomb-related complex 2 H3 K27 methyltransferase EZH2 and the Polycomb-related protein ASXL1. Mutations in IDH1 and IDH2 likely also affect the epigenome through neomorphic generation of 2-hydroxyglutarate which inhibits TET2 and Jumonji-domain histone demethylases. Further, sub-types of acute myeloid leukemia (AML) exhibit distinct and abnormal patterns of DNA methylation.

Novel oncoproteins generated by recurrently occurring chromosomal translocations in myeloid leukaemia also induce epigenetic dysfunction. For example, the gene coding for the H3 K4 methyltransferase MLL, itself a key epigenetic regulator, is mutated by translocation in about 4% of human AML. This results in constitutive transcription at MLL target genes through aberrant recruitment by MLL fusion partners of complexes associated with transcription elongation including pTEFb, PAFc and the DOT1L complex, which contains a H3 K79 methyltransferase. By contrast, fusion of *RUNX1* (AML1) to *RUNX1T1* (ETO), which occurs in 7% of patients with AML, generates a novel constitutive transcriptional repressor.

These and other observations have led to speculation that regulators of the structure and function of chromatin, such as enzymes which regulate turnover of DNA methylation, histone methylation or histone acetylation, might be attractive therapeutic targets in myeloid malignancy. To date only the DNA demethylating agent azacitidine has provided a survival benefit in phase III trials, in patients with high-risk myelodysplasia. While this emphasises that epigenetic therapies hold significant potential in the treatment of myeloid malignancy, it also highlights the necessity for identification and evaluation of other therapeutic targets.

One such candidate is KDM1A (also known as AOF2, LSD1, KIAA0601 or BHC110), a flavin adenine dinucleotide (FAD) dependent lysine-specific demethylase with monomethyl- and dimethyl-histone H3 lysine-4 and lysine-9 substrate specificity. It is a component of the MLL supercomplex associated with transcription activation. It is also a component of complexes associated with transcription repression such as the CoREST-HDAC and CtBP co-repressor complexes and the nucleosome remodelling and deacetylation complex (NuRD). While KDM1A expression has been correlated with poor prognosis in high-risk prostate and breast cancer and poor differentiation in neuroblastoma, to date there is no information as to its functional role in myeloid leukaemia, nor whether it represents a viable therapeutic target.

In the past year, focusing on the subtype of human AML associated with translocations targeting *MLL*, we have generated a significant body of data which suggests that KDM1A is a critical regulator of MLL leukaemia stem cells (LSCs). Knockdown experiments demonstrate that KDM1A is required to prevent

Figure 1
Survival curve of mice transplanted with MLL-AF9 AML cells transduced with non-targeting control (NTC) or Kdm1a knockdown (construct #4) lentiviruses. Knockdown of Kdm1a confers a significant survival advantage in these mice relative to control mice.

Figure 2
Primary human MLL-AF9 AML blast cells treated with a novel tranylcypromine analogue undergo terminal macrophage differentiation. Left panel shows vehicle treated cells, right panel shows an-TCP treated cells.

Figure 1

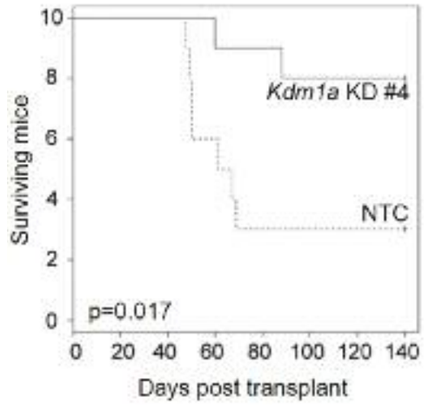
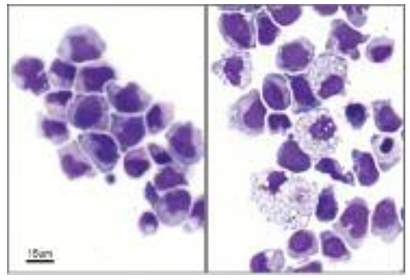


Figure 2



differentiation and apoptosis of LSCs from mice with experimentally initiated MLL-AF9 AML. Similar findings were obtained in knockdown experiments using human MLL-AF9 cells lines and primary MLL AML cells from patients treated at The Christie NHS Foundation Trust. KDM1A knockdown cells are unable to initiate AML in syngeneic or xenogeneic transplant assays. Exon array analysis demonstrates that KDM1A is required to sustain expression of an MLL-AF9 associated oncogenic program.

KDM1A may be pharmacologically inhibited using tranylcypromine (TCP), a monoamine oxidase inhibitor used in the clinic for many years to treat depression. Use of TCP, at concentrations close to the IC_{50} for KDM1A, phenocopies knockdown in both murine and primary human MLL-AF9 AML cells. However, the poor selectivity for KDM1A and the high IC_{50} likely preclude its use as a useful therapy for AML. Instead, in collaboration with the Drug Discovery Group at the Paterson Institute (led by Donald Ogilvie) we have synthesised a recently patented tranylcypromine analogue (an-TCP) reported to have substantially higher selectivity and potency versus KDM1A. Active in the low nanomolar range, use of an-TCP also phenocopied KDM1A knockdown in both murine and primary human MLL-AF9 AML cells. Furthermore, treatment of mice with experimentally initiated MLL-AF9 AML with an-TCP was sufficient to promote *in vivo* loss of clonogenic potential and induction of differentiation of MLL-AF9 AML cells while sparing the clonogenic potential of the residual normal bone marrow stem and progenitor cells. In keeping with this, death from AML was delayed in mice treated with the KDM1A inhibitor.

Thus our data establish KDM1A as a realistic new target for differentiation therapy in human myeloid leukaemia. This work will be published in 2012.

Publications listed on page 70

Signalling Networks in Cancer Group

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Zoe Edwards (since October)

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 drug resistance and emphasising 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 and/or therapeutic resistance.

Drivers of Lung Cancer

The lab combines cancer genomic sequencing with siRNA screening technology to screen lung cancers and identify kinases that harbour activating mutations. This approach streamlines the identification of mutationally activated kinases that are likely to be robust drivers of lung tumorigenesis and therefore potentially druggable targets. Additionally, these studies allow us to begin to illuminate a basic understanding of how oncogenic mutations interact with other mutations in a specific tumour or cancer cell and we will be able to identify if the mutant protein interactions will be additive or synergistic in promoting tumorigenic phenotypes. We will determine if inhibiting multiple pathways that are aberrantly activated by driver mutations in kinases in a specific cancer will result in better and more specific killing of cancer cells (Figure 1), providing a snapshot into the future of personalised medicine. We will also be able to gain insight into when a mutant protein is a strong driver and should be targeted with small molecule inhibitors. For example, we are discovering that in some lung cancers activating K-ras mutations are essential for maintenance of tumorigenic phenotypes, while in other lung cancers with similar activating mutations in K-ras they are dispensable, as targeted depletion of the mutant K-ras does not dramatically effect cancer cell survival or proliferation in these specific cancer cells. Thus the background of mutations present in a specific tumour dictates the relative importance of a putative driver mutation. In summary, with this approach we will be able to (1) identify novel kinases with activating mutations and determine their importance in a larger cohort of lung cancer samples and (2) determine the "linchpin" mutant proteins that are essential to maintain tumorigenic phenotypes and demonstrate that targeting these cancer drivers will result in specific killing of cancer cells (Figure 1). As we enter the age of personalised medicine where every cancer patient's genome will be sequenced, these studies will illustrate how we can manage these data to deliver the right drugs to the right patients.

Novel Cancer Associated Kinases

Another primary objective of the laboratory is to use bioinformatic tools to analyse kinases with somatic mutations reported in various cancer kinome-sequencing studies for those most likely to contribute to tumorigenesis or drug resistance. Candidate cancer-associated kinases that our lab will study are determined based on whether the kinase has limited characterisation, a high probability of possessing a driver mutation, and if mutations are predicted to be cancer mutations based on bioinformatic analyses (CanPredict, PMUT, polyphen2, mutationtaster, snpeffect, puppasuite and SNPs and go). Additionally, we model many of the mutations that score highly in our analysis to determine the structural consequences of the putative driver mutations (Figure 2A).

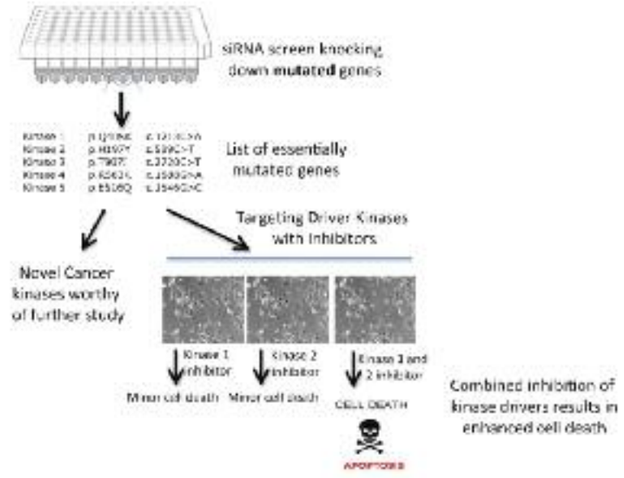

Figure 1
A high-throughput approach to find the drivers of lung cancer. Using lung cancer cells where all mutations are identified through cancer genomic sequencing, we deplete cells of the mutated proteins to identify the essential driver mutations. We then use this as a guide to assess which small molecule inhibitors should be used for the treatment of each unique and individual cancer (ie personalizing and tailoring treatment to a specific cancer).

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 (Figure 2B). We will 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 (Figure 2B). Next we will 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 will 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 will 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 tumorigenesis and identify convergent and essential targets that could be exploited for the development of novel therapeutics.

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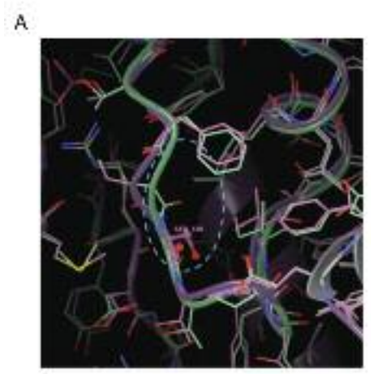
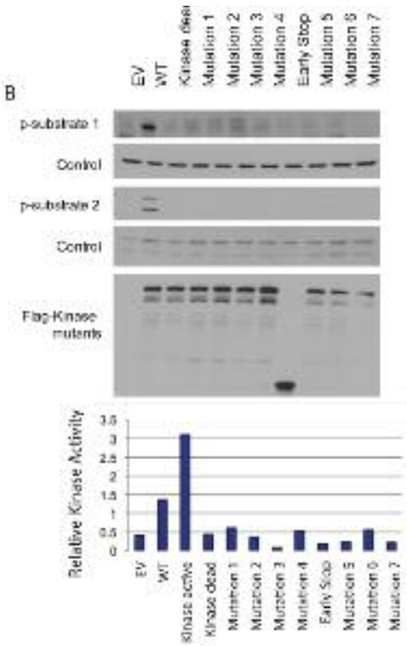
Figure 2
Evaluation of novel loss-of-function mutations in a cancer associated kinase. A. Structurally modelling cancer kinase mutations allows us to predict which mutations will alter the conformation of a kinase in such a manner that it has greater or reduced catalytic activity. B. Biochemical characterization of mutations identified in cancer patients demonstrating a majority of mutations in this particular kinase are loss-of-function mutations.





Stem Cell Biology Group

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Olga Tsoulaki (until April 2011)

The major interest of our lab is to decipher the cellular and molecular mechanisms that control the development and maintenance of the haematopoietic system. In this context, we study the functions of the transcription factor AML1/RUNX1 and of the transcriptional co-activator MOZ.

AML1/RUNX1is one of the most frequent targets of gene rearrangements and mutations in acute leukaemia. Similarly the gene *MOZ* is involved in myeloid chromosomal translocations. Understanding the function of these transcription regulators during normal haematopoiesis should result in a better comprehension of how perturbations of their functions lead to development of leukaemia.

Generation of blood cells

There is a worldwide shortage of matched donors for blood stem cell transfers for leukaemia or lymphoma patients. The generation of blood cells upon *in vitro* differentiation of embryonic stem cells or induced pluripotent stem 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. Support for the haemangioblast concept was initially provided by the identification during embryonic stem (ES) cells' differentiation of a clonal precursor, the blast colony-forming cell (BL-CFC), which gives rise after 4 days to blast colonies with both endothelial, smooth muscle and haematopoietic potential. Recent studies have now provided evidence for the presence of this bipotential precursor *in vivo*.

We have recently demonstrated that the haemangioblast generates haematopoietic cells through the formation of a haemogenic endothelium intermediate, providing the first direct link between these two precursor populations. This haemogenic endothelial cell population is transiently generated during blast development and is also detected in gastrulating embryos. At the molecular level, we have demonstrated that the transcription factor SCL/TAL1 is indispensable for the establishment of this haemogenic endothelium cell population from the haemangioblast whereas RUNX1/AML1 is critical for the generation of haematopoietic cells from this haemogenic endothelium.

Haemogenic endothelium and transcriptional targets of RUNX1/AML1

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 cells at different stages of haematopoietic development. We have also developed strategies to identify genome-wide RUNX1 binding sites at these different stages of blood development by either chromatin immunoprecipitation or DamID (a method developed by Professor Bas van Steensel, NKI, Amsterdam). These studies are performed in collaboration with the Applied Computational Biology and Bioinformatics Group led by Dr Crispin Miller. We are currently evaluating the potential of some of these identified transcriptional targets to rescue the development of the haematopoietic system in absence of RUNX1. We are also further documenting the regulation of the expression of these genes by RUNX1 using reporter assays.

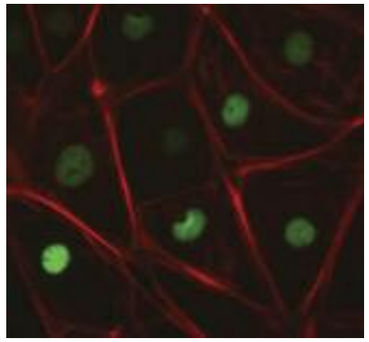

Figure 1
Smooth muscle cells generated from differentiated ES cells. The red staining corresponds to smooth muscle actin (SMA) whereas the green nuclear staining corresponds to expression of H2B-venus under the control of the SMA locus.

Generation of blood, endothelial and smooth muscle cells

It is now well established that clonal haemangioblast precursors generate blast colonies containing vascular smooth muscle, endothelial and haematopoietic potentials. However the order of restriction to these developmental potentials is not well understood. To enable us to track the cells committed to these different cell fates, we have created reporter ES cell lines expressing fluorescent protein under the control of early genes specifically expressed in these different lineages. We are currently investigating the pattern of development of these different lineages and examining the influence of different signalling pathways on their generation.

Expression and Function of Runx1/AML1 isoforms

Runx1/AML1 is expressed as multiple naturally occurring spliced isoforms that generate proteins with distinct activities on target promoters. We have generated ES cells and mouse lines containing a reporter gene knock-in in the different isoforms and produced knockouts altering the specific expression of these isoforms. We have previously demonstrated that the expression of these isoforms is differentially regulated during early haematopoietic development both *in vitro* and *in vivo* and that their expressions define specific stages of haematopoietic development. We are currently investigating the pattern of expression of the different RUNX1 isoforms in the different compartments of the adult hematopoietic hierarchy. We are also further evaluating the requirement for these different isoforms in adult haematopoiesis.

Figure 2
Embryoid bodies generated from ES cells containing red blood cells.

Model of leukaemia

Human acute leukaemia is characterised by the presence of recurrent chromosomal abnormalities, which frequently result in the formation of chimeric transcription factors. The core binding factors AML1/RUNX1 and CBFβ are the most frequent targets of these genetic alterations. The t(8;21) translocation resulting in the AML1-ETO fusion and the inv(16) generating the SHMMC-CBFβ fusion accounts together for more than 20% of all AML cases. Animal models have indicated that the full length AML-ETO, expressed either upon viral transfer or as a transgene, is not able by itself to induce leukaemia in mice. However an alternatively spliced form of AML1-ETO, *AML1-ETO9a (AE9a)*, has recently been shown to cause a rapid development of leukaemia in mice following retroviral transfer. Using an ES cell line with a doxycycline *AE9a IRES GFP* inducible cassette, we first validated *in vitro* that the induced expression of *AE9a*, thought to act as a dominant inhibitor of AML1/RUNX1, blocked the generation of haematopoietic cells during blast development. Based on these results, we subsequently generated a mouse line from these ES cells. The mice expressing *AML1-ETO9a* developed extra medullary haematopoiesis followed by the development of acute myeloid leukaemia. The disease latency was shorter when *AML1-ETO9a* expression was induced on a $p53^{-/-}$ background. Altogether these results indicate that we have now established a new model of leukaemia development, which will allow us to investigate further the molecular and cellular events associated with t(8;21) leukemogenesis.

Function of the HAT activity of MOZ

The *Moz* gene is involved in leukaemia in three independent myeloid chromosomal translocations fusing *Moz* to the partner genes CBP, P300 or TIF2. All these genes encode enzymes containing a histone acetyl transferase domain (HAT) suggesting that aberrant modification of histones or other factors could provide the first step in the route to oncogenicity. We specifically addressed the role of the HAT activity of MOZ during haematopoiesis by generating a mouse strain that carries a single amino acid change in the HAT domain of MOZ. Analysis of these mice has revealed a profound defect in haematopoiesis. The numbers of haematopoietic stem cells and their potential is dramatically affected in homozygous mice. We also uncovered that the balance between proliferation and differentiation of blood progenitors is hampered in the absence of MOZ driven acetylation. We are currently investigating further the molecular mechanisms affected in the absence of the HAT activity of MOZ leading to such a phenotype.

Publications listed on page 71

Stem Cell and Haematopoiesis Group

http://www.paterson.man.ac.uk/sch

Group Leader
Valerie Kouskoff

Postdoctoral Fellows
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Maud Fleury
Sarah Lewis

Scientific Officer
Stella Pearson

Graduate Students
Guilherme Costa
Sara Cuvertino
Andrzej Mazan

The overarching goal of the Stem Cell and Haematopoiesis group is to further delineate and understand the transcriptional networks that orchestrate the formation of the haematopoietic system during embryonic development. Many transcriptional regulators implicated in haematopoietic specification have been 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.

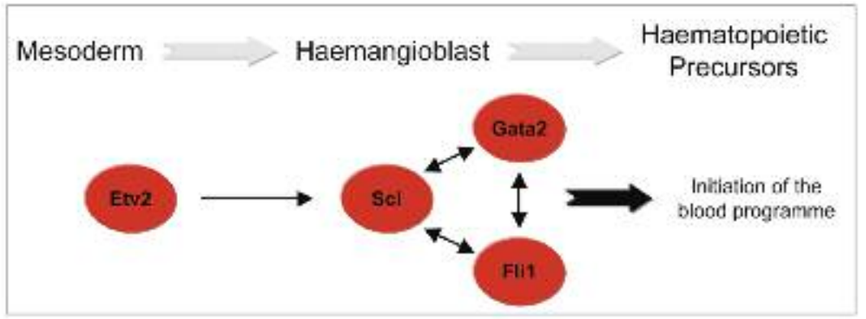
Molecular and cellular control of blood specification

During embryonic life, the onset of haematopoiesis occurs soon after gastrulation and is one of the first programmes to be specified from the mesodermal germ layer. Mesoderm precursors within the primitive streak become progressively committed to the blood programme as they migrate toward the extra-embryonic region of the developing embryo where they later form the yolk sac blood islands. The first identified step toward haematopoietic commitment occurs when mesodermal cells up-regulate the tyrosine kinase receptor FLK1 marking the formation of the haemangioblast, a bi-potential precursor giving rise to blood and endothelial cells upon further maturation. These haemangioblast precursors rapidly form specialised endothelium cells, termed haemogenic endothelium, that generate blood precursors upon further maturation. The transition from haemogenic endothelium to blood precursors is marked by the acquisition of CD41, the αIIb integrin that defines the earliest fully committed blood precursors. While the cellular basis underlying the formation of the first blood cells from the mesoderm germlayer are well characterised, the molecular mechanisms orchestrating this developmental process still remain poorly understood. Several transcription factors have been implicated at various steps of this developmental programme. Early studies on the function of RUNX1 revealed its critical role in the formation of definitive haematopoietic precursors but not in the emergence of primitive erythropoiesis. More recently this transcription factor has been identified as a master regulator controlling the transition from haemogenic endothelium to blood precursors. The transcription factor SCL is critically required for blood cell formation as SCL deficiency led to a complete absence of both primitive erythroid and definitive blood precursors and early embryonic lethality. Further studies have revealed a role for SCL in the formation of haemangioblast and haemogenic endothelium precursors. A newly identified player in haematopoietic specification, the Ets transcription factor ETV2, appears to be equally critical for haematopoiesis since its deficiency also leads to the absence of all blood progenitors and early embryonic lethality.

ETV2, an Ets factor at the top of the hierarchy

To further evaluate the role of ETV2 at the onset of blood specification, we generated an *Etv2*-deficient ES cell line in which an *Etv2* transgene could be re-expressed upon doxycycline induction using a Tet-on inducible system. The *in vitro* differentiation of this ES cell line deficient for Etv2 expression revealed a complete absence of all haematopoietic progenitors in the absence of doxycycline, a phenotype similar to the one described for Etv2-deficient embryos. Addition of doxycycline during the *in vitro* differentiation allowed a complete rescue of haematopoietic development. In search of the molecular

Figure 1
Schematic representation of the molecular circuitry controlling haematopoietic specification.



network downstream of ETV2, we performed a comparative gene expression survey upon ETV2 re-expression, analysing the transcriptome of *Etv2*-deficient cells stimulated or not with doxycycline. The most prominent category of genes up-regulated upon ETV2 induction belonged to the cardiovascular system, a developmental network including many genes, such as *Ve-cadherin*, *Gata2*, *Scl* or *Hhex*, all known to be implicated and important in vasculogenesis and haematopoiesis. The presence of *Scl*, *Fli1* and *Gata2* within this list of genes was of particular interest given that these three factors form a recursive loop controlling multiple blood genes during development. Strikingly, however, *Scl* is the only one of those three genes whose deficiency results in a complete haematopoietic defect similar to *Etv2* deficiency. These observations led us to investigate the degree of interconnection between the downstream programmes controlled by ETV2 and SCL. We assessed whether SCL expression might rescue to some extent the haematopoietic defect resulting from ETV2 deficiency. This hypothesis was tested using a doxycycline-inducible *Scl* transgene introduced into the *Etv2*-deficient ES cells. Strikingly, induction of SCL expression in *Etv2*-deficient cells was sufficient to fully rescue the production of all haematopoietic precursors. A time course analysis of haematopoietic progenitor formation revealed a pattern very similar to the one previously observed in wild-type cultures. Taken together, these findings demonstrate that SCL acts downstream of ETV2 in the specification of the haematopoietic programme. Given that FLI1 and GATA2 were also found to be dependent on ETV2 expression, we set out to test whether re-expression of either of these two transcription factors would also be sufficient to rescue ETV2 deficiency. In a similar doxycycline inducible system, FLI1 or GATA2 were expressed in ETV2$^{-/-}$ differentiating cells and replated in a clonogenic assay. However, unlike the rescue observed upon SCL induction, very few haematopoietic progenitors were detected upon FLI1 or GATA2 expression. Altogether, our data suggest that SCL is a limiting factor for blood specification in ETV2-deficient mesodermal precursors since neither GATA2 nor FLI1 expression is able to significantly rescue the formation of haematopoietic progenitors.

Haematopoietic specification: an ETV2-SCL linear module

The regulation of *Scl* transcription in haematopoietic and endothelial lineages is controlled by a 5'-enhancer region containing five Ets binding sites. Luciferase assays revealed that ETV2 was able to efficiently activate the transcription from this 5'-enhancer region whereas mutations in the five Ets binding sites contained within this enhancer region completely abolished the transcription driven by ETV2. Furthermore, chromatin immuno-precipitation experiments showed that ETV2 occupancy was enriched on the 5' enhancer of *Scl* when compared to intergenic regions further away from the transcription start site of *Scl*. Taken together, these data strongly suggest that ETV2 directly controls the transcription of *Scl* through binding to Ets sites within the 5' enhancer of *Scl*. Our study identifies the molecular switch that drives blood specification, showing that the SCL-FLI1-GATA2 recursive loop controlling the expression of many haematopoietic genes is activated by ETV2 via *Scl* as an entry point (Figure 1). Our data also indicate that GATA2 and FLI1 are not able to initiate this recursive loop on their own. While our findings further define how ETV2 controls haematopoietic specification, the transcriptional control of endothelium specification by this factor remains to be explored given that vascular defects are much more profound in *Etv2*-deficient embryos than in *Scl*-deficient embryos. It will be interesting to define whether, similar to blood specification, the control of vascular development is also regulated by a very limited set of ETV2 transcriptional targets.

Publications listed on page 71

Stromal-Tumour Interaction Group

http://www.paterson.man.ac.uk/stromal/

Group Leader
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Postdoctoral Fellow
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Graduate Student
Ahmet Acar

Human tumours are highly complex tissues and the non-neoplastic cell compartment of tumours, which is often termed the "stroma", is itself quite complex histologically. Carcinoma cells initially recruit and/or activate these various stromal non-neoplastic cells, including fibroblasts, myofibroblasts, immune cells, endothelial cells, bone marrow-derived cells. The resulting stromal cells reciprocate by fostering carcinoma cell growth and survival and neoangiogenesis during the course of tumour progression.

Studying the heterotypic interactions between the neoplastic cells and the supporting stroma is essential for understanding the nature of the bulk of carcinoma mass. We study how the evolution of stromal fibroblasts facilitates tumour cells to become invasive and metastatic during the course of tumour progression.

Evolution of stromal myofibroblasts in tumours

Neoplastic epithelial cells coexist in carcinomas with a stroma composed of various types of mesenchymal cells as well as extracellular matrix (ECM), both of which create the complexity of the tumour microenvironment.

Noticeable numbers of myofibroblasts, which are characterised by their production of α-smooth muscle actin (α-SMA), have been observed repeatedly in the stroma of the majority of invasive human breast cancers. However, the specific contributions of these cells to tumour progression are poorly defined. Stromal fibroblasts and myofibroblasts, collectively termed carcinoma-associated fibroblasts (CAFs), were extracted from various human carcinomas. CAFs, in comparison with their control fibroblasts, when co-injected with carcinoma cells into immunodeficient mice, are known to substantially promote carcinoma growth and neoangiogenesis. CAFs retain their myofibroblastic properties and tumour-promoting phenotypes, after they have been passaged for ten population doublings (PDs) *in vitro* in the absence of ongoing contact with carcinoma cells. Accordingly, even though the CAFs appear to have initially acquired their unique phenotypes under the influence of carcinoma cells, once it is acquired, they display this trait independent of further signalling from the carcinoma cells. We have reported that normal human mammary fibroblasts, when co-inoculated with breast carcinoma cells into immunodeficient mice, convert stably into tumour-promoting myofibroblasts within the resulting tumours (Kojima *et al*., 2010, Proc. Natl. Acad. Sci. USA., 107, 20009-20014). During tumour progression, these fibroblasts progressively elevate two autocrine signalling loops mediated by the TGF-β and SDF-1 cytokines in self-stimulating and cross-communicating fashions, thereby enhancing both their transdifferentiation into myofibroblasts and the associated tumour-promoting capability. Taken together, these findings indicate that the establishment of cross-communicating TGF-β and SDF-1 autocrine signalling gives rise to myofibroblast differentiation and mediates the evolution of residual fibroblasts into tumour-promoting myofibroblasts.

Stroma-derived signalling crucial in promoting tumour metastasis

The tumour invasion-metastasis cascade is a complex multistep process that includes localised invasion of carcinoma cells, entrance into the systemic circulation, survival during transportation, extravasation and colonisation in establishing micro and macro metastases in distant organs. It has long been assumed that dissemination of metastatic carcinoma cells depends largely on their cell-autonomous effects, due to epigenetic and/or genetic alterations that accumulate within these malignant cells.

However, emerging evidence now proposes a different scheme in which metastatic spread is not totally dependent on the acquisition of genetic alterations within carcinoma cells. The tumour microenvironment also serves as an important determinant that encourages carcinoma cells in the primary tumour to become motile and invasive, and to disseminate into distant organs. Interaction of carcinoma cells with the tumour-associated stroma facilitates the invasion-metastasis cascade.

Tumour-associated stromal fibroblasts play a significant role in regulating migratory and invasive behaviours in carcinoma cells. This is supported by evidence indicating that stromal myofibroblasts are frequently present at the invasive front of human carcinomas. In addition, it has been shown that CAFs increase the migratory and invasive propensity of the cancer cells co-cultured in collagen gels. Tenascin C, HGF and SDF-1, which are secreted by CAFs, also play a role in mediating CAF-stimulated invasion of cultured carcinoma cells. It remains, however, unclear as to how CAFs influence each step of the metastatic cascades and what paracrine signalling from CAFs is essential for facilitation of metastatic dissemination of carcinoma cells *in vivo* and what molecular alteration(s) is provoked in such metastatic carcinoma cells. Studying crosstalk between tumour cells and mesenchymal cells during tumour progression could help understand the nature of biology of a bulk of human carcinomas and facilitate the development of novel stroma-targeted therapeutic approaches.

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Figure 1
Co-evolution of stromal fibroblasts with carcinoma cells during tumour progression Resident stromal fibroblasts within the tumour increasingly acquire two autocrine signalling loops involving TGF-β and SDF-1 during the series of tumour progression. These autocrine signalling loops are very likely conferred by the interaction with nearby carcinoma cells during the series of tumour progression and they mediate transdifferentiation of stromal fibroblasts into tumour-promoting CAF myofibroblasts. Apposed carcinoma cells are also subject to acquire invasive and metastatic propensities through the interaction with CAFs during tumour progression. The molecular mechanism(s) mediating the metastasis-promoting ability of carcinoma cells remains unclear.

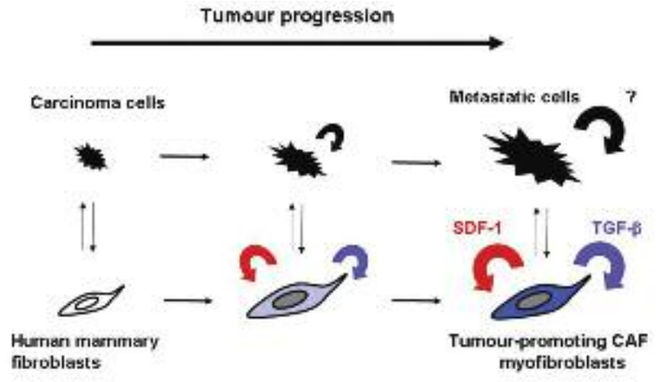
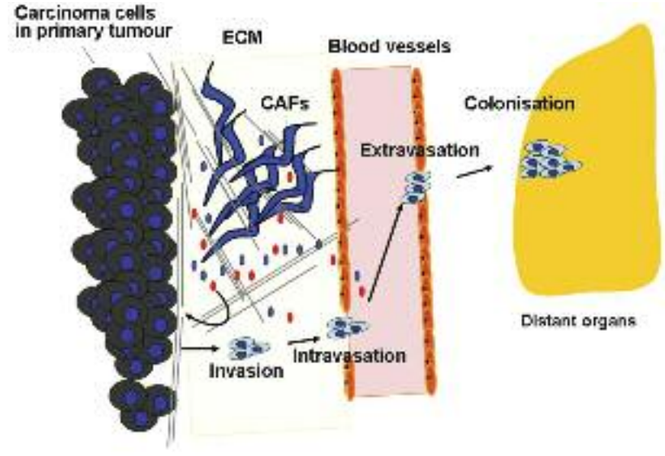


Figure 2
How do CAFs influence the tumour invasion-metastasis cascade? CAFs likely provide nearby carcinoma cells with an invasive propensity in vivo that results in an increase in tumour cell intravasation. Carcinoma cells educated by CAFs within primary tumours may stably or transiently maintain their phenotypes during extravasation and colonisation in distant organs. The molecular mechanism(s) underlying the potential CAF-induced tumour invasion-metastasis cascade remains unclear.



Research groups
The University of Manchester School of Cancer and Enabling Sciences

Children's Cancer Group

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Postdoctoral Fellows
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Mark Holland
Suzanne Johnson
Jizhong Liu

Clinical Fellow
Ashish Masurekar

Scientific Officer
Seema Alexander

Graduate Student
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Clinical MRes Students
Caroline Fong
Zahaan Pinto

Clinical Trials Manager
Catriona Parker / Adiba Hussain

Administrator
Charlotte O'Horo / Parisa Mahjoob

Our group investigates 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 provides us with the data and clinical material for hypotheses based laboratory investigations.

Clinical

We have previously reported on the degradation and inactivation of the key anti-leukaemic drug L-Asparaginase by lysosomal cysteine proteases expressed by lymphoblasts. This led us to devise protease resistant L-Asparaginase and show that the glutaminase activity of the drug was essential for cytotoxicity (Blood, 2011, 117:1614). Until this discovery, resistance to L-Asparaginase was thought primarily to be either intrinsic (due to unknown mechanisms) or as a result of neutralising antibodies developed post-exposure. Our findings suggested that lysosomal proteases could contribute to an inadequate therapeutic effect.

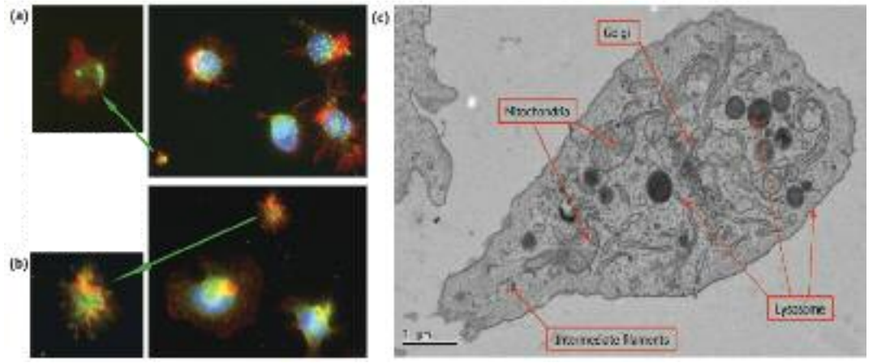
Ashish Masurekar, along with Caroline Fong and Adiba Hussain investigated this in a prospective fashion in the national frontline trial, ALL2003. They serially analysed ~500 patients for Asparaginase activity and antibody levels and correlated this with known risk factors and surrogate markers of outcome. They found that 70% of patients had good activity throughout their treatment. Those who did not mostly had non-detectable levels. This is an important finding. The pegylated version of the drug used in the UK is expensive. Based on our previous experience, we use 1000 u/m². Most groups worldwide use 2000-3000 u/m². Our results show that this is unnecessary and that increasing the dose is unlikely to improve activity in those who do not achieve adequate levels. We also found that 10% of patients with good initial activity, subsequently showed low activity. Most of these patients had developed anti-asparaginase antibodies. Of the remaining 20%, all had inadequate activity initially, though half showed adequate activity later on. None of these patients had detectable antibodies, nor could we correlate this with the levels of protease expression, suggesting unknown and perhaps complex mechanisms of inactivation. Further analysis showed for the first time, that inadequate L-Asparaginase activity during induction significantly affects disease clearance in standard risk patients. This suggests that modification of dosage schedule and the addition of other drugs have the potential to further improve outcome in childhood ALL and that in future, the monitoring of L-Asparaginase activity during therapies will become incorporated into national trials.

The results of the international clinical trial in relapsed ALL (ALLR3), reported last year, continue to show some of the best outcomes in the world (Lancet 2010; 376:209). A new trial, IntReALL is due to open next year that will now include 20 different countries making it the largest such study worldwide.

Laboratory

We wanted to understand how leukaemic lysosomal proteases interacted with L-Asparaginase. We initially identified these lysosomes at the periphery of the cell localised to vesicles contiguous with the cell membrane (JCI 2009;119:1964). We subsequently identified that these vesicles are shed as microvesicles (MVs). Suzanne Johnson showed that the MVs stain positive for actin, talin and vinculin (Figure 1A and B), are motile and show a complex anucleate structure (Figure 1C). MVs taken up by other lymphoblasts, develop an activated phenotype typical of the cell of origin (Figure 2). Bone marrow stromal cells actively take up MVs, suggesting horizontal transfer of RNA, DNA and protein. Thus this suggests a mechanism by which leukaemic cells regulate the microenvironment and is possibly a general mechanism by which cancer cells establish a cancer specific niche.

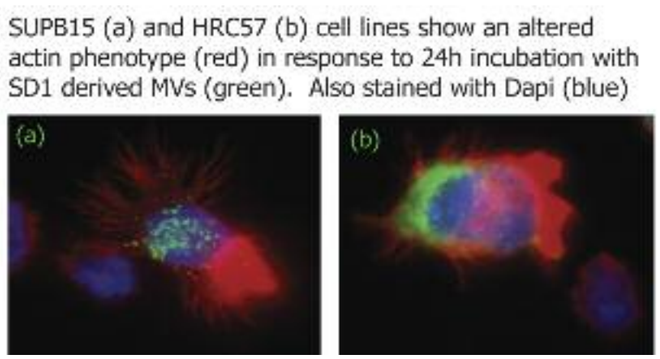
Figure 1
SD1 cells seeded onto Cell Tak and probed for (a) LAMP1 or (b) talin and co-stained for actin using Alexa Fluor 555 (Red) and Dapi (Bue). Preparations also show microvesicles. (c) TEM image at x2900



Ashish Masurekar has developed a technique to capture leukaemia specific MVs from patient samples. Suzanne Johnson and Zahaan Pinto are now exploring the effect of MVs on stromal cells *in vitro* and *in vivo*.

Jizhong Liu has been investigating the converse, i.e. how the microenvironment protects the leukaemic cell from chemotherapy. He first created an organotypic, orthotropic model of the bone marrow microenvironment using mesenchymal cells obtained from bone marrow aspirates. Under culture conditions these cells showed differentiation into mesenchymal lineage. This model is able to support the leukaemic cells in co-culture without the addition of exogenous factors. Conditioned medium from this stromal cell culture model provided broad-spectrum chemoprotection to a wide variety of cancer cell lines. The protective soluble factors include miRNA enriched exosomes that regulate the PI3K/AKT and aerobic glycolysis pathways. This produces a quiescent state in leukaemic cells, leading to a low tonic expression of pAKT and ROS. Stimulation of ROS generation using PEITC or piperlongumine is cytotoxic to multidrug resistant leukaemic cells. While the cells are resistant to L-Asparaginase, the drug is additionally cytotoxic when given along with PEITC. This may be due to the glutaminase activity of the drug. We are verifying this *in vivo* and if reproducible will plan to test this in an early phase clinical trial.

Figure 2



We reported earlier this year that lymphoblasts from patients with high risk ALL express adhesion molecules, such as ICAM1 and LFA-1, and produce central nervous system (CNS) leukaemia in NSG mice (Blood 2011;118:638). Mark Holland used a semi quantitative discovery approach to examine the plasma membrane proteome of these cells. Almost half the proteins unique to the plasma membrane of the cells transgressing into the CNS were associated with adhesion, invasion and cytoskeleton re-organisation and included ICAM1 and LFA-1. Pathway analyses suggested a model by which cellular adhesion proteins ICAM1 and LFA-1 activated RAC2 leading to the development of an organised cytoskeleton and diapedesis. Seema Alexander showed that inhibition / silencing of RAC2 abrogated *in vitro* invasion and that the invasive cell lines had a well-organised filamentous actin structure. Using a NSG mouse model they were then able to show that while non-invasive cell lines produced leukaemia in mice, the invasive cell lines led not only to leukaemia but the establishment of CNS disease, which was partially inhibited by silencing RAC2. Overall the work suggests a model by which cells migrate to extramedullary sites, adhere to the host cells then are able to invade across the blood and CSF brain barriers into the central nervous system. Stephanie Harrison, a new PhD student who joined the group in October, is now further investigating the role of ICAM1 and LFA-1 in CNS disease. Clare Dempsey has been working with them to devise suitable in vivo models.

We are also pleased to report that Shekhar Krishnan and Seema Alexander successfully defended their doctoral dissertations in 2011 and Catriona Parker gave birth to a bouncing baby boy in September. Charlie moved on in October and we wish her a very successful career in her new management role. We welcome Parisa Mahjoob-Afag into the role of department administrator.

Publications listed on page 72

Medical Oncology: Clinical and Experimental Immunotherapy Group

Group Leader
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Fiona Thistlethwaite

Senior Fellow
David Gilham

Research Fellow
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ATTRACT Clinical Fellow/ Marie Curie Fellow
Symeon Eleftheriades

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Vicky Sheard
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Lidan Christie (Cellular Therapeutics Unit, until July)

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Vickie Hambleton

Clinical Fellow
Rob Brown

Marie-Curie Training Fellow
Vania Baldan

Project Managers
Nikki Price
Helena Kondryn

Immunotherapy utilises the immune system to control and potentially eradicate cancer. Whilst the group is involved in a number of innovative approaches in clinical trials, at a pre-clinical level we focus on developing adoptive cell therapy.

Clinical Trials

The lack of an approved Cell Therapy Unit for much of the year has meant there was no recruitment to cell therapy trials – now the unit is open (see below) the trial targeting CD19 with engineered T cells should re-open shortly. Trials in melanoma also form a major part of our future plans. The initial patient treated with tumour infiltrating lymphocytes (TIL) was successful and we hope to obtain NHS funding for this treatment as well as NIHR funding for trials to assess improvements to the treatment. In addition, we are working with our EU partners to develop trials targeting NY-ESO1 to assess the potential benefits of using selected cells.

On-going analysis of previous immunotherapy trials continues to throw up clues as to the most appropriate biomarkers to use to select patients who are likely to benefit from treatment. This could be very useful for future trials and these biomarkers are increasingly being incorporated into cancer vaccine studies. Other targeted therapy trials are increasingly looking at angiogenic pathways other than the VEGF pathway.

Cellular Therapeutics Unit (CTU)

A recent major effort of the group has been the development of a unit capable of delivering clinical grade cells to treat patients. Since the incorporation of the provisions of the European Union Clinical Trials directive into UK law, there has been a requirement to deliver medicines and therapies produced in accordance with the principles of Good Manufacturing Process (GMP). In effect, this means that all medicines and therapies have to be produced to a level equivalent of that in the pharmaceutical industry. This includes validation, quality control, continuous environmental monitoring and rigorous product testing. This has had a major impact upon the ability to translate academic bench-based observations through to clinical testing due to the requirement to comply with GMP even in first into man early phase clinical trials.

Producing drugs in compliance with GMP is difficult. Producing individual patient cell products is extremely demanding. For example, handling of the cell product is only allowed with a continuously monitored category A environment (determined by a particle measurement of less than 1 >5μm particle present in $1m^3$ of air). To achieve this in our previous clinical trials of adoptive T cell therapy, all work was carried out in a dedicated suite of laboratories maintained by the National Blood Service (NBS) sited at Plymouth Grove, Manchester. A major issue was the lack of flexibility of the use of such laboratories; each single patient product required the exclusive use of one of the labs hence meaning low throughput and high cost associated with each cell product generated. Nonetheless, 23 patients were treated with cells generated in these clean rooms (17 gene modified; 6 non-modified, regulatory T cell depleted). However, the NBS took the decision to close the clean rooms in Manchester as a part of a large scale move of stem cell activities from Manchester to Liverpool. To continue cell therapy clinical studies, an alternative cell production unit was required.

After consultation with the Regulators (MHRA), we initiated the development of a new Cellular Therapeutics Unit (CTU) at the University Incubator Building in Grafton Street. In order to avoid the lack of flexibility of large clean-rooms, a design based upon the use of isolators was followed. In this scenario, the category A environment is maintained within the sealed isolator with sealed glove units allowing the operator to manipulate the cells within the sterile environment (Figure 1). Materials are moved into the isolator through a separate transfer unit which means that the area required to be maintained to this high level is significantly reduced from that of a standard clean room. Two isolators have been generated with a shared transfer unit which are sterilised by perfusion with hydrogen peroxide gas and permits up to four different T cell products to be manipulated per day. In addition to the use of closed culture and selection systems, this means that a higher throughput of cell products is possible which should translate to cost efficiencies.

Dr Ryan Guest has overseen the development of the CTU which was accredited for the production of Investigational Medicinal Products (IMP) and to produce 'Specials' (one-off patient products) in November, 2011. The first patient product produced by the Unit was a T cell product generated from a Malignant Melanoma Tumour (Tumour Infiltrating lymphocyte, TIL). TILs are generated from enzymatically digested tumour and cultured for a period of three weeks prior to a rapid expansion using donor lymphocytes, antibodies and cytokines. The successful generation of TILs is of major importance; this confirms that complicated handling procedures can be efficiently carried out in an isolator and that closed culture systems are suitable for expansion of these T cells. One further patient tumour has been processed with the T cells isolated in readiness for rapid expansion.

With the CTU now on-line, the immediate intention is to continue TIL therapy for Melanoma and also to resume the gene-modified T cell adoptive therapy targeted B-cell lymphoma which has been on-hold waiting for cell production to re-commence. Importantly, the Unit is well equipped including flow cytometry (Miltenyi MACSQuant) and clinical grade cell selection technology (Clinimacs) which is designed to provide a flexible service for investigators wishing to exploit any form of cell therapy in the clinical setting.

Figure 1

Pre-Clinical Research

The group continues to focus upon understanding and improving adoptive T cell therapy for cancer. In particular, our focus has been upon engineering T cells with anti-tumour specificity through genetic modification. Two routes have been explored – co-expression of the α and β chains of a tumour antigen specific T cell receptor (TCR) or the expression of an antibody-fusion receptor (Chimeric Antigen Receptor, CAR) on the T cell surface which circumvents the normal T cell recognition process and directs the functional activity of the T cell against cell surface protein antigens.

Both approaches have their own advantages and limitations. However, one key advantage of the CAR approach is that many different signalling domains can be engineered downstream of the antibody targeting domain. In this way, many varied signalling pathways can be activated though a single CAR binding event. The most commonly used CAR-based signalling combinations have been CD3ζ linked with the CD28 cytosolic domain. Upon antigen binding, the CD3ζ domain drives 'signal 1' which is effectively activation of the T cells' effector functions while the CD28 domain provides 'signal 2' which is a survival signal typified by the T cell producing cytokines such as IL-2 and up-regulating anti-apoptotic gene expression resulting in survival and proliferation of the activated T cell. The combination of two signalling domains into a single CAR have been termed '2nd generation CARs while those CARs containing the CD3ζ effector domain are termed '1st generation CARs.

Despite this clear separation of function, we have previously found that mouse and human T cells bearing a 1st generation CAR specific for CD19 produce IL-2 when co-cultured with B cell lymphoma cells. This activity is independent of the activity of the native CD28 receptor but is dependent upon the binding of the CD2 co-stimulatory receptor to its ligand on tumour cells (Cheadle *et al*., 2011, Gene Ther). Importantly, this may explain why T cells engrafted with a 1st generation CD19-specific CAR persist and maintain anti-tumour activity for an extended period of time in mouse models of systemic B cell lymphoma. This observation indicates that the ligands of the CD2 receptor present on the tumour target cell can play a role in determining engineered T cell response and suggests that manipulation of the CD2 signalling pathway may enhance engineered T cell function.

Publications listed on page 72

Medical Oncology: Translational Anti-Angiogenesis Group

Group Leader
Gordon Jayson

Senior Research Fellow
Egle Avizienyte

Postdoctoral Fellows
Claire Cole
Steen Hansen
Gavin Miller

Clinical Fellows
Danielle Shaw
Laura Horsley
Kalena Marti-Marti

Scientific Officer
Graham Rushton

Heparan sulphate (HS) is an essential regulator of the biological activity of the majority of angiogenic cytokines and chemokines. However, the key HS structural features involved in the inhibition of cytokine/receptor complexes remain unclear. Using our unique synthetic chemistry programme we have started to unravel HS structural requirements for inhibition of angiogenic cytokine-specific functions. We have identified precise HS structures that are capable of inhibiting multiple angiogenic cytokines. In addition we have focussed on defining the role of HS in human ovarian cancer angiogenesis. We are currently evaluating HS specific sulphation epitopes and their functional relevance in ovarian cancer tissue and circulating endothelial cells and their progenitors from ovarian cancer patient blood.

Evaluation of synthetic structurally-defined HS oligosaccharides

Most angiogenic growth factors and chemokines, including Fibroblast Growth Factors (FGFs), Vascular Endothelial Growth Factor 165 (VEGF165), Stromal Cell-Derived Factors (Sdfs) and interleukin-8 (IL-8), depend on HS for their biological activity. HS, which is a major component of HS proteoglycans, contains repeating disaccharide units which are composed from D-glucuronic acid (GlcA) or L-iduronic acid (IdoA) linked to N-substituted-glucosamine. In cells, initial N-sulphation is usually followed by conversion of some GlcA to IdoA, sulphation at the 2-O position in iduronic acid and at the 6-O and 3-O positions of glucosamine. Heavily sulphated HS regions (S-domains) that are usually separated by poorly sulphated regions (NA-domains) facilitate numerous signalling events by providing interaction sites for a number of growth factors and their receptors, chemokines and extracellular matrix proteins. Although the significance of HS in regulating angiogenic cytokine functions is well appreciated, the structural requirements for such regulation remain unclear. Some studies suggest that sulphation at specific positions in HS is important, while other studies indicate that charge density and domain organization are the key determinants. Our unique organic synthesis programme allows us to address these questions. Previously we showed that longer oligosaccharides that were uniformly 2-O and N-sulphated (ISNS) had superior inhibitory properties when compared to 2-O (IS) sulphated oligosaccharides of the same length (Cole *et al*., PLoS One, 2010). Specifically, ISNS dodecasaccharide showed significant activity against FGF2- and VEGF-induced endothelial cell migration, tube formation and signalling, while poorly inhibiting endothelial cell proliferation *in vitro*. In addition, we showed that ISNS dodecasaccharide was well tolerated by animals at a high dose, was detected in tumour xenografts at biologically active concentration, did not affect coagulation cascade components and reduced tumour xenograft microvessel density by 40% (unpublished data).
By varying the degree of sulphation along synthetic HS oligosaccharides, we have demonstrated unique inhibition of angiogenic cytokines, which was not observed when testing homogeneously sulphated species. Our data show for the first time that HS structural specificity is more important than charge density in regulating biological activity. Such dependency on a precise structure opens opportunities for the design of more potent inhibitors of angiogenic cytokines.

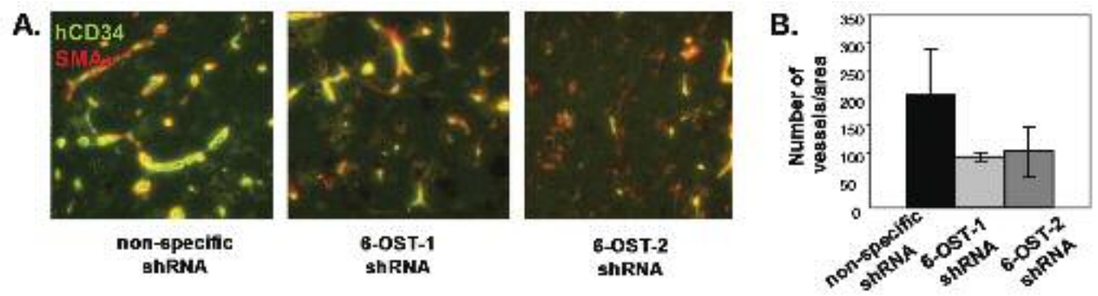
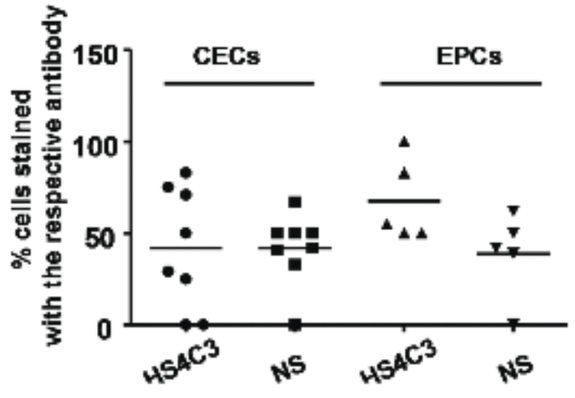


Figure 1
Endothelial HS 6-O sulphation levels regulate vasculogenesis in vivo. A. Human CD34 and SMAα staining of HUVEC spheroids at day 21 after implantation. B. Average number of human CD31-positive blood vessels per standardized area of a plug.

Endothelial HS 6-O sulphation levels modulate FGF2- and VEGF-dependent endothelial cell functions

Ovarian cancer is one of only a few diseases that respond to single agent VEGF inhibitors, such as bevacizumab. Recent data showed an improvement in progression-free and overall survival of ovarian cancer patients treated with bevacizumab in combination with chemotherapy. To determine the role of HS in ovarian cancer, we evaluated expression of HS sulphation patterns in ovarian tumours and normal ovaries. We found an increase in staining for 6-O sulphation specific epitopes in tumour endothelium, while 6-O sulphation in normal ovaries was often detected in the perivascular region of blood vessels. To test functional significance of 6-O sulphation in endothelial cells, we down-regulated 6-O sulphotransferases 1 or 2 (6-OST-1 and 6OST-2) and discovered that reduced 6-O sulphation in S-domains by 25-40% significantly compromised endothelial tube formation and sprouting in vitro and vasculogenesis in vivo (Figure 1). In addition, wild-type HS in neighbouring endothelial or smooth muscle cells did not rescue compromised FGF2- or VEGF-dependent sprouting and tube formation in cells with reduced 6-O sulphation. Although FGF2 or VEGF binding to HS or to receptors on cells was unaffected by reduced 6-O sulphation, FGF2 and VEGF-induced receptor phosphorylation was compromised (manuscript in preparation), suggesting that 6-O-sulphation of N-substituted glucosamine residues plays an important role in the regulation of angiogenesis *in vivo*.

Figure 2
Percentage of CECs and EPCs positive for staining with antibodies recognizing HS sulphation specific epitopes. NS antibody recognizes 2-O, N- and 6-O sulphates and HS4C3 antibody recognizes N-, 6-O and 3-O sulphates.



Clinical significance of HS

In preclinical animal models of cancer, endothelial progenitor cells (EPCs) have been identified as catalysts of vasculogenesis in primary or metastatic tumours. However, it is unclear how much they contribute to tumour vasculogenesis in humans. As a number of angiogenic cytokines that regulate EPC functions critically depend on HS, we aim to investigate HS sulphation patterns and their functional significance in EPCs from the blood of ovarian cancer patients and healthy controls. We developed a flow cytometry protocol for evaluation of EPC and circulating endothelial cell (CEC) staining with sulphation specific anti-HS antibodies. We discovered a higher proportion of EPCs bearing CD31+CD146+CD45-CD133+ immunophenotype that stained with anti-HS antibody specifically recognizing 6-O, N- and 3-O sulphation when compared to the number of CECs (CD31+CD146+CD45-CD133-) (Figure 2). These data indicate that specific HS sulphation patterns might be important in EPC functions, specifically in their response to cytokines that regulate EPC homing and differentiation in tumours.

Publications listed on page 73

Targeted Therapy Group

Group Leader
Tim Illidge

Postdoctoral Fellows
Ellie Cheadle
Simon Dovedi
Jamie Honeychurch

PhD students
Waleed Alduaij
Clara Chan
Monique Melis

MRes Student
Lauren Sidon

BSc Pathology Students
Charlotte Pollard

The overarching goal of the Targeted Therapy Group is to enhance the efficacy of RT (radiotherapy) in the treatment of cancer with immunotherapy. The research programme aims to further increase our understanding of the mechanisms underlying the host immune response to irradiated tumour cells and by using radio- immunotherapy combinations to increase the anti-tumour immune response and outcome in cancer.

The specific objectives of the group are i) to investigate the mechanisms of action of radio-immunotherapy and to define optimal combination strategies to take forward to early phase clinical trial design ii) to investigate how RT-induced tumour cell death is recognised and processed by different antigen presenting cells (APC) in the tumour microenvironment and how this impacts on the subsequent adaptive immune response. This is a translational research programme where the aim is to take our experimental research findings into the clinic and where the laboratory research is influenced by clinical insights and developments.

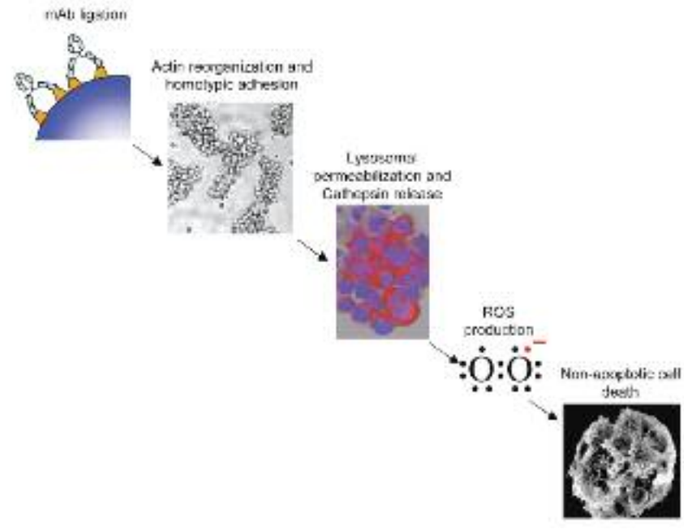
This last year has been highly successful for the Targeted Therapy Group and the highlights have included both Waleed Alduaij and Monique Melis being awarded PhD degrees. Waleed Alduaij was awarded the highly prestigious Royal Society of Medicine Oncology Section - Sylvia Lawler Prize open to all clinicians and scientists in training for the best PhD project in oncology. Both Waleed and Monique gave oral presentations at the 2011 Keystone cancer meeting. Oral presentations have also been given at international leading research meetings including ASCO, ASTRO and ASH the UK National Cancer Research Institute meeting as well as some notable high impact publications.

Novel Mechanisms of antibody induced cell death

We recently demonstrated that certain mAbs (type II anti-CD20 and anti-HLA DR mAbs) potently evoked a direct Programmed Cell Death (PCD) pathway through an actin-dependent, lysosome-mediated process using both lymphoma cell lines and primary chronic lymphocytic leukemia (CLL) cells (Ivanov *et al.*, J Clin Investigation, 2009). More recently we have been further investigating the mechanisms of action involved in this death pathway. We have focused our work on a novel third generation humanised type II anti-CD20 mAb called GA101, which has entered a large clinical trial development programme. We have discovered that on binding the CD20 antigen GA101 initiates large amounts of non-apoptotic PCD in a range of B-lymphoma cell lines and primary B-cell malignancies. Our recent studies performed by Jamie Honeychurch and Waleed Alduaij have demonstrated that the induction of PCD by a range of anti B cell mAbs against HLA DR antigens and the type II anti-CD20 mAb such as 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, and that inhibition of ROS using various ROS scavengers blocks cell death. ROS generation is also independent of Bcl2 or blockade of caspase activity confirming that ROS production (and subsequent cell death) is apoptosis-independent. Moreover, ROS were generated downstream of mAb-induced actin cytoskeletal reorganisation and lysosome membrane permeabilisation as confirmed through use of specific pharmacological inhibitors.

Through a collaboration with Professor Peng Huang and Helene Pelicano at the MD Anderson, University of Texas we have been able to demonstrate that the source of mAb induced ROS is extra-mitochondrial as Type II anti-CD20 mAb induce cell death and ROS in respiratory deficient Raji sub-clones (ϱ- cells), which lack functional mitochondria. ROS generation was instead found to be mediated by NADPH oxidase localised in the cell membrane using pharmacological inhibitors. The potential role of NADPH oxidases in this pathway was confirmed using siRNA knockdown of one of the key B-cell associated NADPH oxidases, NOX2, which resulted in a significant reduction in mAb-induced cell death. These findings provide further insights into a previously unrecognized 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. This work is currently under review at the journal, Blood.

Figure 1
Schematic diagram illustrating the proposed sequence of events in the proposed cell death pathway evoked by type II anti-CD20 and anti-HLA DR mAb. mAb ligation results in HA and actin reorganisation followed by lysosomal membrane permeabilisation, release of cathepsins and generation of ROS via NAPDH oxidase which ultimately culminates in non-apoptotic cell death.



Immune response to RT induced dying tumour cells

Recent work done by Simon Dovedi has demonstrated for the first time that the efficacy of external beam radiotherapy (EBRT) can be significantly enhanced by combination with the selective TLR7 agonist R848. Our data demonstrate that intra-venous administration of R848 can lead to the activation of dendritic cells (DC) and lymphocytes and induce the expression of several pro-inflammatory cytokines measurable in the serum. Furthermore, our data demonstrate that combination therapy with EBRT and weekly systemic dosing of R848 leads to the induction of a tumour-specific CD8+ T cell response capable of increasing the survival of mice bearing established tumours. Importantly, a proportion of mice treated with this novel therapeutic combination are able to completely reject the primary tumour and are protected against subsequent tumour rechallenge by the induction of a tumour-specific memory response. We are currently expanding this study to include additional syngeneic models of solid tumours. 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 TLR7 and EBRT.

Monique Melis in collaboration with Kathryn Simpson (Clinical Experimental Pharmacology Group) has developed a number of doxycycline (Dox)-dependent caspase-3 "death switch" syngeneic murine tumour models. Tumour cells transfected with the "death switch" can be conditionally induced to undergo rapid, synchronous apoptosis, and provides a unique and useful tool to compare the immunogenicity of classical apoptotic cell death with that induced by RT or chemotherapy. In particular, this model can be used to assess immune responses to cell death *in vivo* in established tumours. Following induction of the caspase-3 death switch, tumours rapidly regress and >50% disappear leading to long term remission and *in vivo* immunity. Tumour clearance was observed in immuno-competent mice but not in immuno-deficient mice. This indicates that an intact immune system is required for long term tumour clearance. Interestingly, when cells undergoing caspase-3 death switch induced apoptosis were used as prophylactic cellular vaccination they were unable to protect against subsequent tumour challenge. In contrast, cells dying after RT and Adriamycin treatment were capable of inducing a protective immune response in a prophylactic tumour vaccination approach, but not in a therapeutic setting when treating established tumours. This work suggests that there are important differences between tumour cells dying after RT and Adriamycin compared to classical apoptotic cells. It appears that the supposedly immunogenic apoptotic cell death induced by RT for example, is on its own insufficient to generate an immune response in a therapeutic setting. Finally, this study demonstrates that the addition of immunotherapy such as immunostimulatory anti-CD40 monoclonal antibody may enhance the therapeutic response towards dying tumour cells.

In summary, the 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 74

Translational Radiobiology Group

Group Leader

Catharine M.L. West

Postdoctoral Fellows
John Hall
Amanda Williamson

Bioinformatician
Janet Taylor (joint with Applied Computational Biology & Bioinformatics)

Clinical Fellows
Jonathan Bernstein
Guy Betts
Navin Mani
Ahmad Mirza (until July)

Scientific Officers
Joely Irlam-Jones
Helen Valentine

MRes Students
Rohan Iype (until July)
Kenneth Oguejiofor (from September)
Junaid Iqbal Wahid (from September)

Scientific Administrator
Rebecca Elliott

The goals of the Translational Radiobiology Group are to derive and validate molecular profiles that predict a cancer patient's response to radiotherapy. We aim to develop signatures associated with tumour radiosensitivity, tumour hypoxia and normal tissue radiosensitivity. In order to facilitate signature derivation, associated research investigates approaches for unlocking information contained in clinical samples.

Some tumours respond well to radiotherapy, whereas others do not. The underlying biology that accounts for differences in response to radiotherapy is poorly understood. There is evidence that intrinsic sensitivity to radiation, hypoxia and proliferation are important.

Profiling of archival formalin fixed paraffin embedded tumours

There are estimated to be almost one billion tumour samples archived world-wide, the majority formalin-fixed paraffin-embedded (FFPE). A wealth of biological information is associated with these samples, but degradation inherent to the processes of fixation and embedding results in fragmented and modified RNA that is poorly compatible with modern high-throughput RNA techniques. We showed previously that FFPE tumour samples in conjunction with Exon array profiling can be used to derive a gene signature in FFPE, without the requirement for matched fresh-frozen samples. The signature was applied to an independent fresh-frozen cohort and successfully stratified histologically distinct subtypes: adenocarcinoma (AC) and squamous cell carcinoma (SCC) (Hall *et al*., 2011, Br J Cancer, 104:971-81). RNA degradation in FFPE blocks is not a single event, but a continuum of events that persist beyond the processing of the blocks. Profiling of >150 human tumour samples revealed that the age of the FFPE block at RNA extraction correlated inversely with the technical success of the Exon arrays (R^2= -0.72; age vs. percentage detection above background [DABG]). Biological signals decrease with increasing sample age. In our cohort, older samples failed to discriminate between AC and SCC, whether using our gene signature or a single well-characterised gene (Figure 1a). This may explain why most microarrayed FFPE cohorts in the literature are generally <10 years old. As such, the majority of archived FFPE specimens, including those with the longest clinical follow-up, remain incompatible with high-throughput technologies. MicroRNAs are thought to be more stable in FFPE blocks. We reasoned that our cohort would be an ideal series to investigate whether miRNA stability overcomes near total mRNA degradation associated with prolonged storage. TaqMan real-time qPCR for hsa-miR-205, a microRNA expressed in SCC in a similar manner to TP63 discriminates between AC and SCC, regardless of sample age (Figure 1b). This finding demonstrates that mature miRNAs are less sensitive to the degradation incurred by mRNA and should be a preference in the profiling of older FFPE blocks. Ongoing work confirmed the result using Affymetrix miRNA v2 arrays, which have complete miRBase coverage and are ideal for miRNA profiling in FFPE samples. Future work aims to exploit this new knowledge to derive a miRNA signature associated with tumour radiosensitivity.

Presence of tumour necrosis predicts benefit from hypoxia modifying therapy

Over the past year we have investigated the ability of tumour necrosis to predict benefit following radiotherapy with and without hypoxia-modifying treatment in high grade urothelial cell carcinoma of the urinary bladder. Tissue samples were obtained for 231 of the 333 patients enrolled in the BCON phase III randomised trial comparing radical radiotherapy (RT) with and without carbogen and nicotinamide (CON). Tumour necrosis was scored on whole tissue sections as absent or present. Necrosis was identified in 121 of the 231 patients (52%). For the subset of BCON patients for whom tissue material was available, 5-year overall survival estimates were 41% for the RT arm and 48% for the CON arm (log rank p=0.14). When stratified using tumour necrosis, the 5-year overall survival rates were 48% (RT) and 39% (CON) (log rank p =0.32) in patients with no evident tumour necrosis and 34% (RT) and 56% (CON) (log rank p=0.004) in patients with tumour necrosis. Multivariate analyses showed that the presence of tumour necrosis was a significant independent predictor of benefit from CON with the risk of dying 57% lower compared to RT alone (HR 0.43, 95% CI 0.25-0.73, p=0.002). This trend was not observed when there was no evident tumour necrosis (HR 1.64, 95% CI 0.95-2.85, p=0.08). The work has shown that simple pathology reporting of tumour necrosis predicts which bladder cancer patients are likely to benefit from hypoxia-modifying treatment in combination with radiotherapy. Ongoing work is comparing the predictive ability of necrosis with other measures of hypoxia including our gene signature.

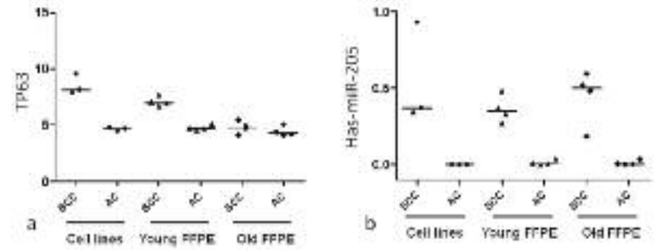


Figure 1
a: Graph showing expression of TP63 probeset data from Affymetrix Exon arrays. Un-degraded cell line RNA discriminates between SCC and AC samples. The difference was seen in young (median age 11.5±1.7 years) but not old (median age 17.5±1.2 years) FFPE blocks. b: Graph showing hsa-miR-205 (similar specificity to TP63) expression measured using TaqMan qPCR on the same samples. hsa-miR-205 expression discriminates between SCC and AC, regardless of the age of the block.

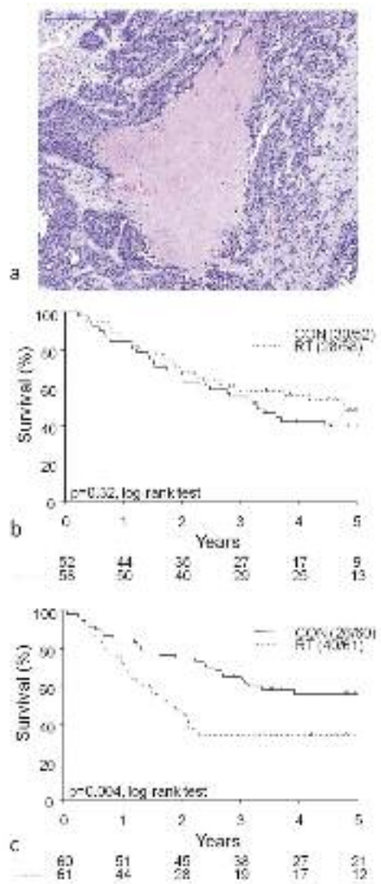


Figure 2
Tumour necrosis predicts benefit from hypoxia modifying therapy in patients with bladder cancer enrolled in the UK phase III BCON trial. 2a: A bladder tumour with a large area of necrosis (pale pink). 2b: In 110 patients with no tumour necrosis, addition of carbogen and nicotinamide (CON) to radiotherapy (RT) did not improve overall survival. 2c: In 121 patients with tumour necrosis, the hypoxia-modifying therapy improved survival.

Normal tissue radiosensitivity

RAPPER (Radiogenomics: Assessment of Polymorphisms to Predict the Effects of Radiotherapy) and our long-running collaboration with researchers at Cambridge University has now yielded results for a genome wide association study (GWAS) of the genetic variation underlying a patient's risk of toxicity following radiotherapy. The Stage 1 GWAS involved 2.4×10^6 genotyped and imputed SNPs in 1,853 patients who underwent radiotherapy for breast (n=1,217) or prostate (n=636) cancer. The quantile-quantile (Q-Q) plots showed no obvious bias due to population stratification or genotype error. No single nucleotide polymorphisms (SNPs) were identified as being associated with an overall measure of late (2 years) radiotherapy toxicity in all 1,853 patients. A number of potential associations were found with tissue specific toxicity at P-values $<10^{-7}$ with more SNPs associated with toxicity in prostate than in breast patients. We are conducting a rapid replication of the top 90 SNPs in 463 prostate samples from a group in Spain and a further ~500 cases from the USA. Some SNPs apparently replicate but caution is required since they have lower minor allele frequencies than the study was designed to detect and so might represent false positives.

Publications listed on page 74

Research Services

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

Head of Research Services
Stuart Pepper

Over the last few years the Institute's recruitment programme has been very successful. For the Research Service units, the corresponding increase in scientific activity has meant that the demand for scientific services has steadily increased to a point where service units have needed to expand again to match the needs of users. In the case of the Biological Resources Unit, this has meant an ambitious expansion into new space made available at the University, whereas for Laboratory Services the expansion has taken the form of an extra post and extra equipment. Mass Spectrometry has acquired a new ultra-high pressure nano LC system which, along with internal development work, is allowing an increased throughput of samples through the existing equipment.

Along with the expansion of existing services we have also been able to procure equipment that has allowed new developments. The Advanced Imaging Facility (AIF) acquired a macro confocal imaging system as well as a new histology scanning platform. The histology service has benefitted from a new Bondmax immuno-histochemistry (IHC) platform and the Molecular Biology Core Facility (MBCF) had a new 5500 clonal sequencer installed. The following sections give further details of these developments along with a variety of other technical advances that have taken place over the last year.

Advanced Imaging Facility

Steve Bagley, Achille Dunne

The remit of the Imaging Facility is to provide state of the art imaging tools for the visualisation of cancer cells (from molecular interactions through to tissue- wide responses), develop new imaging methods and to train scientists to apply these to their research. The facility is one that responds to the requirements of the researchers and develops solutions to aid in providing visual and numerical clues for both fundamental and translational oncology. Over the last year, 39 new users have been trained in the use of microscopes, taking the total up to 161 individuals, working with 28 research groups and generating 21 terabytes of data.

Over the past year the facility has brought in new equipment, redeveloped older equipment and introduced a range of novel techniques, which are detailed below.

Macro Confocal Imaging with Spectrophotometric Detection

When imaging primary tissue from patients, endogenous fluorescence (auto-fluorescence) from the sample itself masks our ability to study biochemical processes. This background fluorescence is often the limiting factor in our studies when trying to discern fluorescent labels attached to a process of interest. It has been estimated that auto-fluorescence can be equivalent to 34,000 fluorescent molecules per cell, sources of which include endogenous NAHD, flavins and flavoproteins.

A macro confocal has been acquired and developed for the visualisation of large areas of tissue. This is in contrast to a conventional microscope where only portions of the total tissue can be imaged. The confocal properties allow a three dimensional volume of tissue to be rendered and the localisation of multiple processes studied. Rather than using a camera, this system captures data via a spectrophotometer, which permits the exclusion of auto-fluorescence consequently increasing contrast and clarity. Although these techniques are still in development the system has already enabled us to examine patient material that previously has been difficult to study.

Histology Scanning

Over the last four years a new development for the facility has been integrating automated histology scanning into the laboratory so that tissue on glass slides could be scanned in batches in a tightly controlled manner. At the time, the techniques were rudimentary and the technology in its infancy, however the system quickly became became over-subscribed as the process of using immuno-histochemically labelled tissues was accelerated. The facility has collaborated with the Institute's Histology service in order to fine tune the process of sample preparation for scanning and numerical analysis. Recently two developments have improved how histological research can now be carried out within the Institute:

- Purchase of a second system via a joint venture between Breakthrough Breast Cancer and Cancer Research UK. This system is faster than the original and allows for batches of 384 slides to be imaged so to allow greater throughput of data.
- The original system has been redeveloped to allow fluorescence imaging, with the aim to be able to image up to six fluorescent tagged proteins simultaneously. This will make possible the development of providing biochemical signatures of biopsy material.

Generally image analysis of histological data has not been as well developed as fluorescent imaging mainly due to the complex nature of the data, the lack of viable software tools and the size of the data (each scanned image is 12 x 25 metres in size). Analysis had been carried out with software developed in the microscopy community but validation of these techniques has been somewhat lacking thus calling into question the final result. The facility has purchased a fully validated software solution, which can describe the data with up to 130 different measures and then relate those measures to each other so to numerically describe, and with the goal of developing methods to mathematically model, the data.

Induction of DNA Damage Microscopy

Working in conjunction with the requirements of the DNA Damage Response Group (Ivan Ahel) a system was developed three years ago for cell based assays of the mammalian DNA damage response pathway using a spinning disk confocal microscope. An additional laser induced DNA damage to a sample which could be strictly regulated so that monitoring of the repair processes was possible. Recently this technology has been modified in order to produce a laser

line that approaches the diffraction limit for fine control of DNA damage induction. Work is on-going into 2012 to develop the system further so to simultaneously study multiple proteins at the site of DNA repair.

Installation of Local Data Servers
The analysis of microscopy data is problematic due to the size of the data and the amount of data generated within the laboratory. As the number of techniques has increased to automatically scan and assess data so has our requirement for temporary storage. This year saw the introduction of 32TB of local temporary network-attached storage to hold microscopy data and to allow space for image analysis.

Biological Mass Spectrometry Facility

Duncan Smith, Yvonne Connolly, John Griffiths

The role of this facility is to enable PICR scientists to access cutting-edge proteomic workflows to enhance their research capabilities. 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 have a constantly evolving portfolio of capabilities to facilitate the qualitative and quantitative characterisation of proteins and their post-translational modifications (PTMs).

Our current portfolio of PTM analyses has been boosted with the successful development and implementation of site mapping of nitrosylated tyrosine, di-methylated lysine and tri-methylated lysine. In addition, the facility has developed a completely novel approach to the high efficiency mapping of sites of Sumoylation. This research and development project has lead to Institute scientists having access to a unique biochemical and mass spectrometry workflow able to deliver biological answers previously beyond the field. This approach has enabled the Cell Cycle group to answer sumo-specific questions about their target of interest.

During 2011, the demand for large scale global protein profiling approaches has increased significantly. These analyses are demanding due to the massive complexity of protein mixtures that represent a typical sample. The facility has supported this demand by strengthening workflows in both online nano LC separations and offline HPLC pre-fractionation. Both new approaches are designed to maximise the capability of our existing LTQ-Orbitrap. In August 2011, the Institute invested in the next generation of ultra-high pressure nano LC systems. This system enables higher resolution separations 'off the shelf' with the use of higher efficiency nanocolumns. This commercial configuration involves shifting from conventional 15cm long to 50cm long columns that increases resolution by a factor of approximately three-fold. This delivers significant improvements in the number of peptide identifications from a complex sample. Furthermore, we have demonstrated that separations on a 1.5M long nano column delivers performance way beyond the commercial product's capacity and pushes complex mixture analysis to the very limits of our hardware's capabilities.

In addition, the facility has developed a novel peptide fractionation workflow exploiting the unique physiochemical properties of polygraphitic carbon designed to reduce the complexity of samples prior to online analysis (Griffiths *et al*, J.Chrom, in press). The second stage of development of this project, designed to address issues of phosphopeptide retention and fractionation, is now well underway and should deliver novel capabilities to phosphoproteome analysis to PICR groups in 2012.

Biological Resources Unit

The Paterson Institute animal facility has run at full capacity throughout 2011 with a steady throughput for both the transgenic and experimental areas. We currently have over 150 lines in production and these have been maintained under the centralised service project licence with animals transferring to the required scientific project licence for experimental use.

The public consultation on the UK implementation of Directive 2010/63EU closed on the 5th September and this gave us the opportunity to respond; we are now awaiting the summary from the Home Office which will be used to formulate the necessary changes to the UK legislation 1st Jan 2013.

Transgenic Services
This year has seen a slight reduction in demand with five embryonic stem cell (ES cell) microinjections and two constructs for pronuclear microinjections performed. The pronuclear microinjection experiments have produced over 230 implanted embryos for each line with an expression rate of 13-17% transgenic in live young.

Eight new transgenic lines have also been successfully imported via surgical embryo transfer whilst one cryopreserved line has been exported to Amsterdam.

Cryopreservation has continued with approximately 5,000 embryos/ 13 strains being preserved this year and the sperm cryopreservation developments continue to be implemented enhancing sperm motility post thawing.

New developments are also being introduced for *in-vitro* Fertilisation (IVF) where increased rates of fertilisation are beginning to be realised even for the more difficult strains. Continual improvements with the sperm cryopreservation and IVF will ensure that this will offer a viable addition to embryo cryopreservation longer term.

At the end of 2010 we had identified that there was a real need to expand the existing BRU facility at the Institute. Due to the scientific research need, a favourable agreement between the PICR, Manchester Cancer Research Centre and the University of Manchester led to the offer of additional space located at the University giving us the additional space required to provide high quality and high health status transgenic animals.

In early summer, an extensive programme of refurbishment works began at the newly acquired area to accommodate in the region of 5,600 newly purchased, individually ventilated cages for transgenic activity and associated techniques for the microinjection service. In this scheme of building works there was also the need to update the cage wash area with new sterilising equipment to support the day to day husbandry. As the year draws to a close, the area has just completed Home Office trials and will soon be operational.

In early 2012 the existing transgenic lines will move to the new area and over a period of time all lines will be decanted out of the existing Institute facility. We will no longer have the requirement for a quarantine area at the Paterson site as all aspects of this work will be carried out at the University sharing their re-derivation area.

Experimental Services
The experimental area has continued to support all aspects of the *in vivo* requirements for the Institute's twelve scientific project licences; this has involved surgical and non surgical techniques, Home Office record keeping and full daily health and welfare checks.

The blood analyser in the facility had been heavily used for the last twenty years and had reached the end of its economic life so earlier this year a new F-820 Sysmex analyser was purchased, the previous equipment only allowed us to carry out total blood counts whereas the new machine also allows us to complete differential and reticulocyte analysis. This has been a useful tool and since it was installed it has been used to track leukaemia development.

The experimental area of the animal facility will also be expanded over a period of time as the transgenic area becomes vacant, this will allow us to increase the procedural activity that currently has 960 individually ventilated cages but with an acquired 2300 extra cages we will be able to accommodate greater throughput.

Cancer Research UK GeneChip Microarray Service

Stuart Pepper, Gill Newton, Julie Watson, Jodie Whitaker

The microarray service based in the PICR Molecular Biology Core Facility has now been running for a decade, and is still heavily used by groups across the whole of CR-UK. The service has been used by around 200 CR-UK scientists and the total number of projects has now passed 500, with 54 new projects submitted this year.

For several years the facility has been supporting expression profiling of archival paraffin embedded samples. This approach presents problems, both in extracting the RNA for microarray analysis and in analyzing the data as the data quality is generally not as good as obtained with standard RNA extractions. This year, we collaborated with Catharine West's group and demonstrated the power of expression profiling on archival samples by classifying cervical cancers. In this case, exon arrays were used to generate the most detailed expression analysis of archival samples published to date.

The service at PICR is based on the Affymetrix platform, however there are now two other companies that offer a solution for archival profiling. This year we have set up a collaboration with James Hadfield at the CR-UK Cambridge Research Institute in order to carry out a platform comparison between Affymetrix, Illumina and Agilent arrays so that we can be sure that CR-UK scientists will be using the best available platform for this approach.

There has been a new development to the service this year with the addition of microRNA profiling using the Affymetrix platform to provide a simple, cost effective alternative to using RNAseq. Expression profiling of miRNA from archival samples has been described in the literature though as yet this is not a routine application on clonal sequence platforms.

Over the last few years the service has supported numerous projects using the linear amplification system from NuGEN Inc. This has been very successful for samples in the 0.5 to 1.0ng range but has been limited to samples where there is sufficient material to extract and purify the RNA through a column. This year we have used alternate amplification systems that have allowed us to work with pools of twenty cells obtained by FACS and generate good quality expression data. We are currently developing the service to be able to support single cell profiling and hope to have this operational during 2012.

Flow Cytometry Facility

Morgan Blaylock, Michael Hughes, Jeff Barry

The Cytometry Core Facility at the Paterson provides state-of-the-art instrumentation, education and expert technical assistance to investigators for the successful performance of flow cytometry based studies. The goal of the facility is both to support current research applications and to continuously extend the repertoire of flow cytometric methods available, providing the tools to help our researchers understand, treat and prevent cancer in its many forms.

The use of flow cytometry in the Paterson Institute can broadly be divided into two broad categories; analytical cytometry and cell sorting.

Analytical Cytometry

The ability of flow cytometers to evaluate cells at an extremely rapid rate (e.g. up to 20,000 events per second) makes this technology ideally suited for the reliable and accurate quantitative analysis of selected physical properties of cells of interest. The sensitivity of these instruments for detecting the presence of molecules expressed at low levels is impressive; given high quality cell preparations and reagents, as few as 50 molecules per cell may be detected. The Core currently has four bench top cytometers including one plate based bead reader. These are all user-operated systems which we offer basic training in a group setting which is supplemented with one to one training for specific applications.

FACS Calibur - 3 colour single laser (blue)

FACS Calibur - 4 colours dual laser (blue and red)

FACS Array - 4 colours, dual laser (green and red)

LSRII - 17 colours, quadruple laser (UV, violet, blue and red)

Cell sorters

One of the properties of the larger flow cytometers is the ability to electronically deflect cells with preset, defined properties into a separate collection tube. For cell purification, flow cytometry is especially well suited for applications requiring high purity. Because multiple fluorochromes (e.g. up to fourteen distinct fluorescent probes reacting with different cell associated molecules) can be assessed simultaneously, cell sorting by flow cytometry can separate complex mixtures of cells on the basis of multiple marker expression. The sorting suite currently houses three cell sorters which are able to retrieve up to four specifically defined populations so that cells may be recovered for further study including re-culture, RNA or DNA extraction or use in functional cell assays. The cell sorters are operated solely by the Flow Cytometry team on a daily basis:

BD FACS Jazz – 2 way sorting 6 colours, dual laser (blue and red)

BD FACS Aria II u - 4 way sorting 12 colours, triple laser (violet, blue and red)

BD InFlux - 4 way sorting, 14 colours, equipped with 5 lasers (UV, violet, blue, red and orange)

Other services

Our facility offers a full range of educational and cytometric services. We are able to provide advice on a wide variety of cytometry related subjects including experimental design, selection of reagents, data analysis, presentation, interpretation, we also act as a beta test site for novel cytometry equipment and applications, we also advise on data presentation. The latter is becoming more and more important as journals require cytometric data to be more transparent.

Technical developments

This year we were fortunate to be the first in the world to acquire the new and so far unreleased BD FACS Jazz™ sorter as a direct replacement for the BD FACS Vantage. As part of our constant internal annual review of our service we have decided to retire the BD FACS Vantage after 18 years solid service. The Jazz is the first of its kind, a small form sorter capable of two-way sorting of eight parameters and is based on the already proven InFlux platform. The ethos of this sorter is to remove the complexity found in modern day high end sorters and to provide a very basic but pure system capable of being operated by all. The Jazz is a dual laser two-way sorter capable of measuring six specific colours and is a welcome addition to our fleet of sorters. We have been really pleased by the size, accuracy, modularity, design, speed and purity of the system. The stability and simplicity of this system has allowed us to implement this machine as our fluorescent protein sorter freeing time on the high end sorters for our more complex procedures.

In addition to training 47 new flow cytometry users and providing ongoing advice, further training and guidance on experimental design to the 200+ active users, we have presented and demonstrated to more than 200 members of the public on the uses of flow cytometry and its impact on the field of oncology.

Histology

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

The histology unit has seen exceptional demand during 2011 in all areas of the services offered. The recruitment and retention of existing staff has allowed for the continued development of the unit. With the introduction of new equipment all samples are now 2D bar-coded. Generally both frozen and fixed / processed samples of mouse, human and cell lines are routinely prepared for both H&E morphological analysis and for further downstream analysis. In addition, special stains such as Alcian blue, Gordon and Sweets, MSB and Oil Red O have all been used to support current research projects. A more specific example being the use of Pearls and Turnbulls blue special stains to investigate the proposed hypothesis that the inhibition of UROD (uroporphyrinogen decarboxylase), a radio-sensitising target in head and neck cancer results in an increase in ferrous $Fe2^{+}$ iron which upon radiation is converted to $Fe3^{+}$ which subsequently enhances radiation induced cell death.

This year saw the introduction of a Leica Bondmax immunohistochenistry (IHC) platform which together with the existing Biogenix i6000 compliment each other, offering high throughput routine, troubleshooting and antibody validation service availability together with unrivalled standardisation and reproducibility. The majority of the research groups have made use of this service, with numerous antibodies successfully optimised. IHC and its use in the assessment of tissue quality during acquisition and the subsequent stabilisation of tissue are areas we are also currently investigating.

Tissue microarrays enable the high throughput standardised analysis of a large number of tissue samples. Both the ATA27 and MTA1 tissue microarray platforms have been heavily used this year. TMAs from disease groups including breast, melanoma, prostate (cores and chips), bladder, lymphoid, small cell and non small cell lung cancer have all been constructed incorporating well in excess of 2000 donor blocks. In addition, mouse model and cell pellet control microarrays have been constructed. Construction of the prostate core TMA and ensuring true representation of the disease was particularly challenging. The quality of all the TMAs constructed has been exceptional.

The unit also houses the MCRC Biobank which has now been up and running for more than three years and to date, around 2500 tissue samples have been processed for use in subsequent research with 40 projects approved. Blood and bone marrow from haematological malignancy patients is also processed and stored. One of our key aims is to focus on ensuring sample quality. Samples are also being processed and microtomy performed for Cancer Research UK's Stratified Medicine Programme (SMP), a national pilot study involving the collection, processing and genetic testing of tissue from six cancer types. The ultimate aim of the programme is to link genetic data with clinical data and to

provide information at the genetic level which may identify patients at risk of specific disease or most likely to benefit from a particular therapy. The pilot study will test the logistics of the approach and determine whether this is feasible and reproducible on a larger scale at the national level.

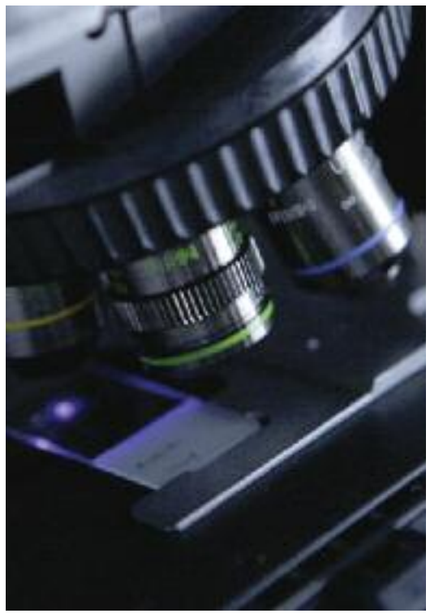
In addition there has been some exciting work in other areas. A technique which allows both the extraction of high quality RNA and protein from the same tissue sample in addition to allowing the morphology of the sample to be reported has been developed. The use of the proximity ligand assay for the detection and quantification of proteins, protein interactions and modifications in fixed cells and tissue samples continues to be evaluated.

In addition, an upgrade to the laser capture microdissection system (LMD6000) with fluorescent capabilities is imminent. This together with our recent work on improving sample acquisition and ultimately quality using the system will prove very useful over the next year.

Laboratory Services

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

Following an internal review during 2011, the department has undergone some changes; we have taken on an additional post and are in the process of adding an additional glass washer and replacing an older autoclave.



The Lab Services department continues to provide three main roles within the Institute:

- Washing and sterilizing of glassware followed by re-stocking of lab supplies
- Production of sterile pipette tips and Eppendorfs
- Production of sterile water and PBS

The department's autoclaves are also available to sterilize material sent up from labs and we supply the Institute with its requirements for liquid media and agar plates. We can produce over 1000 litres of liquid media a month and liaise with labs in producing new media types as required.

Lab Services provides each lab with a Laboratory Aide to perform a range of housekeeping duties tailored to the specific needs of the lab. These duties can also include equipment monitoring such as Safety Cabinet Airflows.

A new role taken on this year is the maintenance of the Institute's two X-ray film processors.

Logistics

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

The Logistics facility provides a comprehensive and vital role in supporting the research carried out at the Institute. Duties include the accurate receipting, checking, booking in and efficient distribution of goods ordered by numerous personnel in the Institute as well as the collection and removal of waste. They are also accountable for the collection and refilling of liquid nitrogen containers and delivery of dry ice. Ordering and distribution of the Central Stores stock via the intranet E-mail "Order Stock Items" function is also our responsibility and it is our duty to ensure adequate stock levels are maintained at all times. Included in this is the media and enzymes stored in the Institute freezers in Phase 5 (Sigma, Invitrogen, Roche, Promega, New England Biolabs, Fisher kits and Qiagen), again the Logistics department is responsible for the ordering, distribution and stock levels of these items.

At the beginning of the year, the Promega freezer went onto a swipe card system, where once user cards had been set up they are able to access the freezer via their swipe card and take the product they require which then is automatically replenished by Promega the following week. The Institutes usage of gas cylinders is looked after by the porters who are in charge of replacement and ordering as and when is necessary. The department works closely with all groups and helps out where necessary, be it tracing and confirming delivery of goods with suppliers, and dealing with missing, damaged or wrong items. We also assist or manage the moving of heavy equipment or furniture, and the setting up of various meeting rooms for numerous events.

Molecular Biology Core Facility

Stuart Pepper, Chris Clark, Yvonne Hey, Rheanna Makorie, Julie Watson

The Molecular Biology Core Facility has a team of six people who between them support a broad range of services and equipment, including the CR-UK microarray service (described separately). The regular sample processing services have all been busy this year with the team processing 13,350 samples for plasmid DNA extraction and over 32,500 samples for DNA sequencing. These services continue to offer a good turnaround such that plasmid DNA can be prepared and then sequenced on the same day.

Over the last two years the facility has also offered a new cell line authentication service based on STR profiling. For this service we have constructed a database of STR profiles for many commonly used cell lines so that for most cell lines we are now able to give a positive identification. Over the last year we have processed 60 cell lines samples spread across several research groups. The STR profiling service is one of the diagnostic services on offer; we also run monthly Mycoplasma screening to check the status of cell lines in use in the building, last year we processed nearly 1400 samples with, thankfully, very few positives being detected.

Alongside the diagnostic services the core facility offers more technical support for applications based on quantitative PCR. We have two ABI 7900 machines and an Eppendorf epMotion robot to allow work in 384 well format. During the last year we have had sixty one people using this machine requiring support that varies from minimal training on the machine to full training on assay design, machine operation and data analysis.

The biggest development area this year has been in our clonal sequencing service. During July and August we had a new AB 5500 system installed and this instrument has already proved to be a major improvement on previous machines. One major application of interest to the Institute is the use of clonal sequencing for expression profiling generally known as RNAseq. This approach relies on generating very large numbers of sequence reads from cDNA libraries and then using the relative frequency of reads that map to each transcript to estimate relative abundance. This application is very demanding in that large numbers of reads are required to give coverage of the whole transcriptome. On the new machine we are able to generate approximately one billion reads per slide, compared to approximately 0.3 billion reads on the older system.

There are two approaches to RNAseq; the most common approach is to use a polyA selection to enrich for RNA molecules that code for proteins. This simplifies the complexity of the sample being analysed but still yields more complete data than microarrays as the entire coding sequence of a transcript is interrogated. Microarray platforms generally have either a single, or a fairly small number of probes to assay each transcript. An alternate approach to RNAseq is to sequence all the RNA from a sample without enrichment. The major problem with this approach is that a large percentage of RNA in most cells is ribosomal RNA, and so >95% of all reads can end up mapping to ribosomal genes which in most cases are not of major biological interest. This problem has to some extent been countered by using techniques to remove ribosomal sequences though it is still the case that 25-40% of samples can end up mapping to ribosomal genes. This year some new products have been released that have significantly improved the removal of ribosomal sequences and we are now seeing less than 5% of reads mapping to ribosomal genes. This improvement, coupled with the increase in the number of reads generated per run, is making total RNA sequencing much more cost effective. Over the last few years it has become clear that the traditional description of RNA into three classes (ribosomal- transfer- and messenger- RNA) has missed many classes of RNA, such as miRNA or snoRNA, which are now being analysed in detail. During the next few years RNAseq will be a valuable technique both to analyse in detail the RNA species that we know about, and possibly discover new categories that have not yet been recognised.

Research publications

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

Refereed Research Papers

Bitton, D. A., Wood, V., Scutt, P. J., Grallert, A., Yates, T., Smith, D. L., Hagan, I. M. and Miller, C. J. (2011)
Augmented annotation of the Schizosaccharomyces pombe genome reveals additional genes required for growth and viability. *Genetics*, **187**, 1207-17.

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Geoff Margison (page 16)
Carcinogenesis Group

Refereed Research Papers

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Zhang, H., Xie, C., Spencer, H. J., Zuo, C., Higuchi, M., Ranganathan, G., Kern, P. A., Chou, M. W., Huang, Q., Szczesny, B., Mitra, S., Watson, A. J., Margison G. P. and Fan, C. Y. (2011)
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Sabharwal, A., Waters, R., Danson, S., Clamp, A., Lorigan, P., Thatcher, N., Margison, G.P. and Middleton, M. R. (2011)
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Karim Labib (page 18)
Cell Cycle Group

Other Publications

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Iain Hagan (page 20)
Cell Division Group

Refereed Research Papers

Bitton, D. A., Wood, V., Scutt, P. J., Grallert, A., Yates, T., Smith, D. L., Hagan, I. M. and Miller, C. J. (2011)
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Nic Jones (page 22)
Cell Regulation Group

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Angeliki Malliri (page 24)
Cell Signalling Group

Refereed Research Papers

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Caroline Dive (page 26)
Clinical and Experimental Pharmacology

Refereed Research Papers

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Ivan Ahel (page 28)

DNA Damage Response Group

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Donald Ogilvie (page 30)

Drug Discovery Group

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The image shows a word cloud taken from all the abstracts of the Institute's research output for 2011. The 150 words that appeared most often are indicated, with font size being proportional to frequency. The text has been filtered to remove the more common words.

Peter Stern (page 32)
Immunology Group

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Nullin Divecha (page 34)
Inositide Laboratory

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Tim Somervaille (page 36)
Leukaemia Biology Group

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John Brognard (page 38)
Signalling Networks Group

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Georges Lacaud (page 40)
Stem Cell Biology Group

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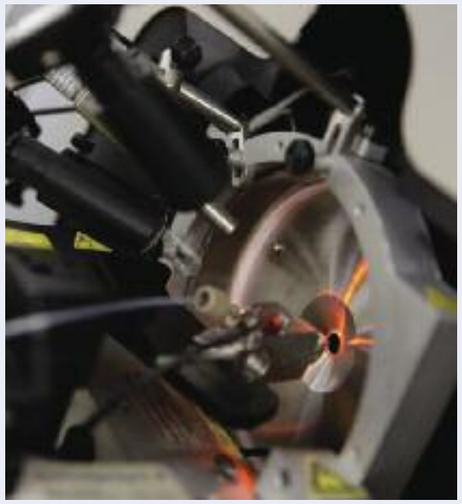

Valerie Kouskoff (page 42)
Stem Cell and Haematopoiesis Group

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Akira Orimo (page 44)
Stromal-Tumour Interaction Group

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Vaskar Saha (page 48)
Children's Cancer Group

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Robert Hawkins (page 50)
Medical Oncology: Clinical and Experimental Immunotherapy Group

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Gordon Jayson (page 52)
Medical Oncology: Translational Anti-Angiogenesis Group

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Tim Illidge (page 54)
Targeted Therapy Group

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Catharine West (page 56)
Translational Radiobiology Group

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Donaldson, S.B., Betts, G., Bonington, S.C., Homer, J.J., Slevin, N.J., Kershaw, L.E., Valentine, H., West, C.M., and Buckley, D.L. (2011)
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Development and validation of a nomogram for prediction of survival and local control in laryngeal carcinoma patients treated with radiotherapy alone: a cohort study based on 994 patients. *Radiother Oncol*, **100**, 108-115.

Gee, H.E., Buffa, F.M., Camps, C., Ramachandran, A., Leek, R., Taylor, M., Patil, M., Sheldon, H., Betts, G., Homer, J., West, C., Ragoussis, J., and Harris, A.L. (2011)
The small-nucleolar RNAs commonly used for microRNA normalisation correlate with tumour pathology and prognosis. *Br J Cancer*, **104**, 1168-1177.

Hall, J.S., Leong, H.S., Armenoult, L.S., Newton, G.E., Valentine, H.R., Irlam, J.J., Moller-Levet, C., Sikand, K.A., Pepper, S.D., Miller, C.J., and West, C.M. (2011)
Exon-array profiling unlocks clinically and biologically relevant gene signatures from formalin-fixed paraffin-embedded tumour samples. *Br J Cancer*, **104**, 971-981.

Lyons, A.J., West, C.M., Risk, J.M., Slevin, N.J., Chan, C., Crichton, S., Rinck, G., Howell, D., and Shaw, R.J. (2011)
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Other Publications

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West, C (2011)
Molecular targeting for improving the outcome of radiotherapy. *In Recent Advances Research Updates: Medical Physics and Radiobiology.* Eds LG Marcu & E Bezak. Researchman Publishers PVT Ltd (Kerala, India) **pp 81-90**.

Active Patents

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

Additional Publications

Meyer, S., Bristow, C., Wappett, M., Pepper, S., Whetton, A.D., Hanenberg, H., Neitzel, H., Wlodarski, M.W., Ebell, W., and Tonnies, H. (2011)
Fanconi anemia (FA)-associated 3q gains in leukemic transformation consistently target EVI1, but do not affect low TERC expression in FA. *Blood*, **117**, 6047-6050.

External Seminar Speakers 2011

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. 2011 was another successful year for scientific interaction with an excellent set of internationally renowned speakers visiting the Institute. In its third 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.

Asifa Akhtar
Max Planck Institute of Immunobiology and Epigenetics, Germany

Rene Bernards
The Netherlands Cancer Institute, Amsterdam

Cédric Blanpain
Free University of Brussels (ULB)

Vania Braga
Imperial College London

Tony Carr
Centre for Genome Damage and Stability, University of Sussex

John Diffley
London Research Institute

Ivan Dikic
Institute of Biochemistry II, Goethe University, Frankfurt, Germany

Jesus Gil
Imperial College London

Eyal Gottlieb
The Beatson Institute, Glasgow

Tessa Holyoake
The Beatson Institute, Glasgow

Cristina Lo Celso
Imperial College London

Pentao Lui
Wellcome Trust Sanger Institute, Cambridge

Florian Markowetz
Cambridge Research Institute

Chris Marshall
Institute of Cancer Research, London

Katrin Ottersbach
Cambridge Institute for Medical Research

Mark Petronczki
London Research Institute

Jonathon Pines
The Gurdon Institute, Cambridge

John Rouse
MRC Protein Phosphorylation Unit, Dundee

Elmar Schiebel
Center for Molecular Biology, University of Heidelberg, Germany

Almut Schulz
London Research Institute

Chris Smith
Department of Biochemistry, University of Cambridge

Bas Van Steensel
The Netherlands Cancer Institute, Amsterdam

Daniel St Johnson
The Gurdon Institute, Cambridge

David Taussig
Barts Cancer Institute, Queen Mary University of London

David Tuveson
Cambridge Research Institute

Frank Uhlmann
London Research Institute

Henning Walczak
Imperial College London

Breakthrough Breast Cancer Research Unit Seminar Series 2011

Mohamed Bentires-Alj
Friedrich Miescher Institute for Biomedical Research, Switzerland

Marina Glukhova
Curie Institute, France

Nancy Hynes
Friedrich Miescher Institute for Biomedical Research, Switzerland

Clare Isacke
Breakthrough Breast Cancer Research Centre, London

Christoph Klein
Department of Pathology, University of Regensburg, Germany

Seth Schor
Unit of Cell and Molecular Biology, School of Dentistry, University of Dundee

Andy Sims
Breakthrough Breast Cancer Research Unit, Edinburgh

Mathijs Voorhoeve
Department of Biochemistry, Duke - National University of Singapore

Postgraduate Education

http://www.paterson.man.ac.uk/education

Postgraduate Education Manager
Julie Edwards

Postgraduate Tutor
Crispin Miller

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 labs they are working in. In 2011, we welcomed another eight graduate students from around the world to join our PhD programme, working in fields as diverse as yeast genetics, stem cells and clinical research. It was also particularly gratifying to see that over the course of the year, eight students (including three who completed in 2010 along with five current students) published first author articles in journals as diverse as the Journal of Clinical Oncology and Molecular Systems Biology. During the course of the year, a total of eleven PhD students and five Clinical Fellows were awarded their PhDs.

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 talks, 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). Each student is assigned an advisor (similar to a personal tutor on an undergraduate programme) whose role is to provide impartial support and advice, while further support is also available from the postgraduate tutor and a student welfare group.

The Paterson 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 which the students attend, and they have the opportunity to present their own work in lab meetings within the institute.

The annual Paterson Colloquium, held in September, is an excellent opportunity for our new intake of students to meet other established PhD students, members of the Institute, including Group Leaders, postdoctoral fellows, and scientific officers. This forum provides up to date science presentations both orally and posters given by Group Leaders, 2nd year PhD students and Postdoctoral scientists.

PhD studentships

All our CR-UK funded studentships are of four years 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 giving the students exposure to different disciplines. Recruitment is highly competitive, with hundreds of applications competing for around 8-10 places each year. Interviews are typically conducted over a two-day period in early 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, in 2007 the Paterson Institute, in collaboration with the MCRC and AstraZeneca, established a fellowship scheme in Clinical Pharmacology Research. 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/Paterson, 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 2011

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 things are progressing as they should, 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 from April replacing Jenny Varley)
Fiona Blackhall
Richard Cowan
Julie Edwards
Dave Gilham
Ian Hampson
Karim Labib
Angeliki Malliri
Crispin Miller
Donald Ogilvie
Vaskar Saha
Tim Somervaille
Catharine West
Caroline Wilkinson

Student Representatives

Tim Maculins (until December)
Emily Holmes
Hadir Marei (from December)

Operations

Director of Operations
Pippa McNichol

During the course of 2011, the Operations department underwent significant restructuring resulting in the creation of two new positions, namely the Scientific Operations Manager and Head of Research Services, which have been taken up by Caroline Wilkinson and Stuart Pepper respectively. Stuart has the additional responsibility of overseeing IT services while the Logistics and Estates departments now fall under the leadership of the Finance and Purchasing Manager, Margaret Lowe. In September, Ruth Perkins joined us as the Administration Services Coordinator, replacing Becky Allen. During these changes, everyone in the Operations department has worked hard to ensure that we have maintained the high standards of service that underpin the smooth running of the Institute.

Administration Department

Amy Weatheritt, Becky Allen, Ruth Perkins, Steven Morgan

The administration department has been working hard to provide a consistent level of support to the Institute during a dramatic period of change. The department will be reviewed and possibly restructured once the new Director is in place in 2012 in order for it to best serve the Directorate and Institute as efficiently as possible.

Over the year, and in conjunction with staff at CR-UK, the department has helped to facilitate several high profile visits and has provided administration support to the Manchester Cancer Research Centre (MCRC) in the early stages of the new MCRC building planning. The department has assisted in the management of several conferences and events over the course of the year, including the Paterson Colloquium at its new location in Lancaster.

The 2011 seminar series has been a great success and the 2012 series 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. A list of speakers for 2011 can be found at www.paterson.man.ac.uk/seminars

Director's Office

EA to the Director

Amy Weatheritt

In February 2011 Nic Jones was appointed as Chief Scientist for Cancer Research UK, stepping down as Director of the Paterson Institute. The office has been working hard to support Nic in this new role alongside his continuing position as Director of the MCRC. Prior to the appointment of Richard Marais as Director of The Paterson Institute, the department has provided assistance and support to the Paterson Senior Management Team who have been leading the Institute in the interim period. The office has also stepped in to provide support to the Manchester Breast Centre and Breakthrough Unit until a full time administrator is appointed.

The Director's Office looks forward to the challenges 2012 will bring. Despite inevitable changes following the appointment of a new Director, the Administration Department and Director's Office will continue to provide a high level of support to the Institute. Siana Peters will join 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.

Estates

Steve Alcock, Graham Hooley, Tony Woollam

This year has been particularly challenging for the Estates team with a significant amount of capital work carried out in the Institute. The main scheme implemented was the upgrade to the main electrical supply and its associated main distribution switchgear; it had been identified that the existing transformer rating was exceeded during the summer months and the main switchgear was obsolete causing it to fail on more than one occasion.

To carry out the physical work in a safe manner was logistically challenging; in order to keep the disruption to the Institute's work to a minimum, the project was carried out over three weekends in May accounting for several hundred man hours. As a result of this work, the Institute now has a more secure electrical supply. Other small schemes carried out to improve facilities for the research groups included the refurbishment of a small lab which specifically houses work with radio-isotopes.

The Estates team also concentrates on sustainability and reducing the Institute's carbon footprint as much as possible. Whenever a scheme is required, energy saving devices are utilised and more efficient heating/cooling and ventilation systems are installed. We are in the process of putting together a report on one area which has intensive energy usage, (IT main server room). Once completed, it will identify different ways to reduce the energy this room consumes, with varying investment required.

The Estates team has been proactive throughout the year, attending to many legislative requirements including Legionella best practices and fire alarm testing. In addition, team members have attended relevant courses to help improve their skills and keep their knowledge up to date with current working practices and changing legislation.

Finance & Purchasing

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

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. Early in 2011, the University introduced E-Marketplace which is an enhancement tool with allows the comparison of products across suppliers. The catalogues on E-Marketplace include up-to-date and accurate product and pricing details in line with agreed contracts. Easy price comparisons give an instant cost saving as well as greater use of preferred suppliers resulting in a longer term saving on contract negotiations. The Central Procurement team is continually adding to the list of suppliers available on the system. In addition to this, they advise on capital purchases working with the researchers to ensure legal legislation is adhered to and that the best possible price is obtained for the equipment.

It is crucial that the management information provided is current and accurate at all times to assist Group Leaders in managing their budgets and for the Director to have a full overview of the Institute's finances. We also assist the Group Leaders with costing grant applications and provide full post-award administration support.

Health & Safety

Colin Gleeson

The second phase of the Institute-wide Risk Management Survey was carried out. This

comprised a check made by management, that safety related tasks in the areas for which they are responsible, had been completed. The Risk Management exercise allows Institute managers to demonstrate that they have allocated resources to health and safety related tasks and have made appropriate management checks that those tasks have been completed.

A heavily revised Institute health and safety policy document was developed. The document contains the Institute's health and safety policy statement, the organisational responsibilities for health and safety and an arrangements section. The latter details how the Institute achieves its health and safety policy aims. A new Institute code of practice for working with biological agents was also developed. The development of both documents involved consultation with staff to ensure that they were user friendly.

Safety inspections of Institute laboratories were undertaken, with focus on the use of human blood and tissues and/or the use of sharps. We also undertook an audit of those laboratories that use radio-chemicals to ensure they were compliant with the legislation and local rules. Surveys were also undertaken of the acquisition and/or use of certain controlled chemicals, pathogens and toxins. This was to ensure that the Institute has the necessary permissions and registrations required under the law for these activities. There was also an Institute-wide portable appliance testing programme encompassing some 9000 pieces of plug-in electrical equipment!

Induction training for new starters was carried out and supplemented with biological agent and hazardous chemical training presentations. Additionally a lentivirus workshop was held due to the expanding number of people in the Institute using this technology. A series of fire extinguisher training sessions, with hands-on experience of using extinguishers was arranged. This proved popular with some seventy people trained in fire extinguisher use.

We have also made considerable progress in the development of an intranet based genetic modification risk management system. This comprises bespoke genetic modification risk assessment forms with an integral document management system. We anticipate that this will be rolled-out in early 2012.

Human Resources

Rachel Powell, Laura Humes, Julie Jarratt

Over the past year the HR Department has continued to successfully develop and deliver a high quality professional HR service to the Institute, providing advice to managers and staff on all employment-related matters such as policy guidance, legislation and best practice. This year we have successfully recruited 34 highly skilled individuals in order to compliment and further enhance the work of the Institute.

It has been a busy and challenging year with the main focus being on the implementation of a new online absence management system. The new system allows for employees and managers to easily request and record leave and sickness. This enables more accurate and useful information to be recorded. The benefits of effective absence recording to the Institute will be a reduction in costs, the ability to manage trends in absences and to rehabilitate staff back into the workplace.

Joint partnership working with the unions has continued throughout the year which has resulted in the agreement of several new policies including the Recruitment and Selection Policy and the Presence of Children and Young Persons policy. Moving forward the main focus for the next year will be the successful implementation of the online probationary process system and a review of the current policies and procedures.

Information Technology

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

This year the IT service has balanced the demands of increasing the breadth of support offered to scientists at the Institute, whilst maintaining existing services at the same high standards that users have come to rely on. The IT service has maintained an excellent service desk which offers rapid response rates to ensure that problems are rectified as soon as possible. The team has also maintained the continuous improvement philosophy that is required to maintain a modern IT infrastructure. The success of such a strategy is illustrated by the stability of the Institute's systems which are rarely unavailable.
In terms of development there have been a number of new solutions implemented which are aimed at either improving support for high performance computing or allowing more flexible work patterns. We introduced a new remote access system which has provided a straightforward interface that will allow all staff to access information from anywhere in the world. Support for tablet devices such as the Apple iPad, and greater use of the in house WiFi network are allowing staff to access scientific publications and personal data in increasingly flexible ways.

In collaboration with the Applied Computational Biology and Bioinformatics Group (ACBB), a new Lustre system has been added to the existing High Performance cluster. This has provided immediate benefits including increased processing capacity and productivity, greatly improving the ability of ACBB to handle large data sets such as those generated by Next Generation Sequencing. The public face of the Institute, in the form of our Internet pages, has also been revamped this year to help reflect the ever evolving work of the Institute.

Some aspects of the infrastructure are now nearing the end of their useful life and to ensure we meet the needs of the next generation of research requirements we will need to commission new elements to our service. As 2011 draws to a close, plans are under way to purchase a new storage solution that will provide sufficient capacity for the Institute's varied data storage needs. As always, creative solutions will be developed to ensure that we maintain the high standards of IT that users have become accustomed to whilst continuing to work in a highly cost effective manner.

Scientific Operations Manager

Caroline Wilkinson

This new post provides support to the Director in order to facilitate the day-to-day running of the Institute. Over the last year, this has meant working closely with the Senior Management Team and then towards the end of 2011, with the new Director Richard Marais to help facilitate his transition to the Institute.

The Scientific Operations Manager provides reports for a number of external sources, including Cancer Research UK and the University of Manchester, as well as editing publications such as the Annual Scientific Report, writing updates for the intranet and external website and producing content for the Institute newsletter. 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. In 2011, this event moved to its new venue at the University of Lancaster. As ever, the colloquium provided an ideal platform for interactions and discussions between the Institute's scientists and we look forward to returning to Lancaster in 2012.

All grant submissions submitted by our scientists are screened for the appropriate ethical approvals as well as the ability of the Institute to accommodate the proposed programme of work. Along with Stuart Pepper, the Head of Research Services, the SOM manages all aspects of space usage in the Institute as well as overseeing the equipment budget. This year, we managed to secure the purchase of an ex-demo macro-confocal microscope which resulted in a significant reduction in cost and is proving to be an excellent addition to our Imaging Facility. Finally, we also carried out a detailed space audit of the Institute that will help in planning research activities over the next couple of years.

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 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 organization. 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 to beat cancer in the years ahead.

Cancer Research UK's Local Engagement and Development

LEAD Manager
James Dunphy

2011 has been a fantastic year of local engagement and development activity in Manchester. Researchers have been involved in over 50 events, with 25,000 Cancer Research UK supporters being reached, and a further 600 people having the opportunity to visit the Institute.

In addition to the monthly lab tour programme, schools' day and open day, an extra event was held to commemorate the five year anniversary of Manchester Cancer Research Centre. Key people from the founding partner organisations attended along with approximately 75 fundraisers from Cancer Research UK, Breakthrough Breast Cancer and The Christie. The event was a great success and attendees enjoyed hearing about advancements that had been made over the last five years as well as plans for the future.

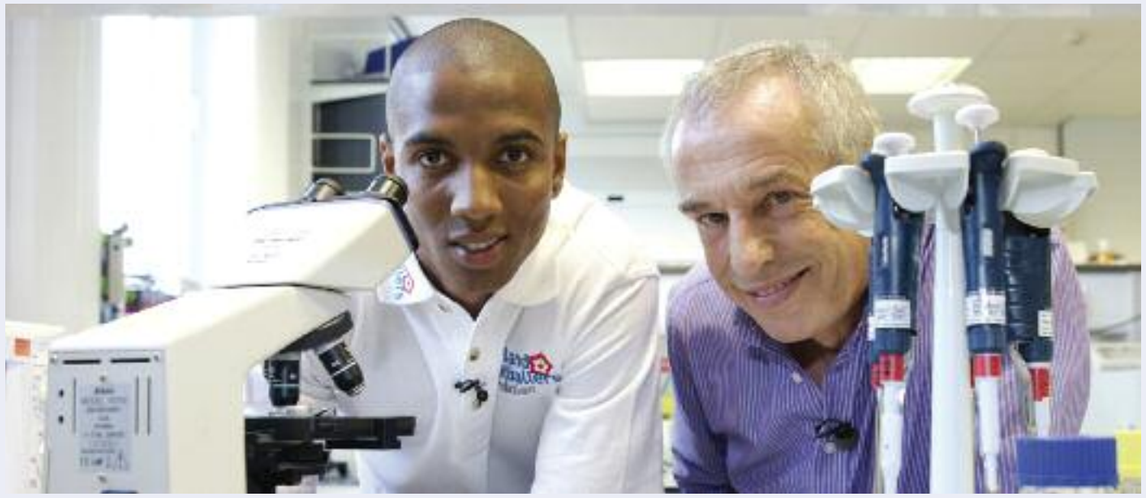
The Paterson has also welcomed a variety of high profile visitors over the last year. Mark Hunter MP, Sajjad Karim MEP and Tony Lloyd MP visited in the summer to learn about the research taking place in the Institute. They met with Professor Caroline Dive to see some research in action and found out more about the future policy priorities for Cancer Research UK. It was a great opportunity for them to see some of the world class research that is being carried out in Manchester and highlighted why it is so important to support the vital research which could make a significant difference to the 36,100 people diagnosed with cancer in the North West every year.

Speaking after the visit, Sajjad Karim MEP said: "Each year, there are an estimated 2.45 million new cancer cases and 1.23 million deaths from cancer in the EU. The Paterson Institute and other cancer research centres in the UK currently receive a large proportion of their funding from charitable sources, with very little funding coming from the European Union. Therefore as a Member of the European Parliament I will be pushing the European Commission for this to be increased in the future and for a greater availability of grant funding for scientists and researchers working in this important area."

In the autumn, a Manchester United and England footballer swapped his team strip for a lab coat when he enjoyed a visit. Ashley Young visited the Paterson Institute, thanks to Cancer Research UK's relationship with the England Footballers Foundation. Cancer Research UK has been chosen as an official charity partner of the Foundation for the next four years. Ashley met Cancer Research UK's chief scientist, Professor Nic Jones, before taking a tour of the labs.

The 26-year-old winger said: "Everyone knows someone who has been affected by cancer in some way. Visiting the Paterson Institute for Cancer Research has been a real privilege. I hadn't realised that such amazing research work, saving so many lives, is taking place in Manchester – right on my door step."

Representatives of the Paterson Institute have continued to support their local fundraisers with inspirational speeches and voluntary help at Race for Life, Relay for Life and Shine events, throughout the year. The Paterson was utilised as a pit stop for the second successful Shine event, with researchers manning the teas and coffees throughout the night. This year, around 3500 people took part, in the night-time half or full marathon and it is hoped this event will raise over £1million for the charity. In addition to this support, the Institute has once again fundraised for the charity with the Keswick to Barrow and Relay for Life teams raising a combined total of over £3000.

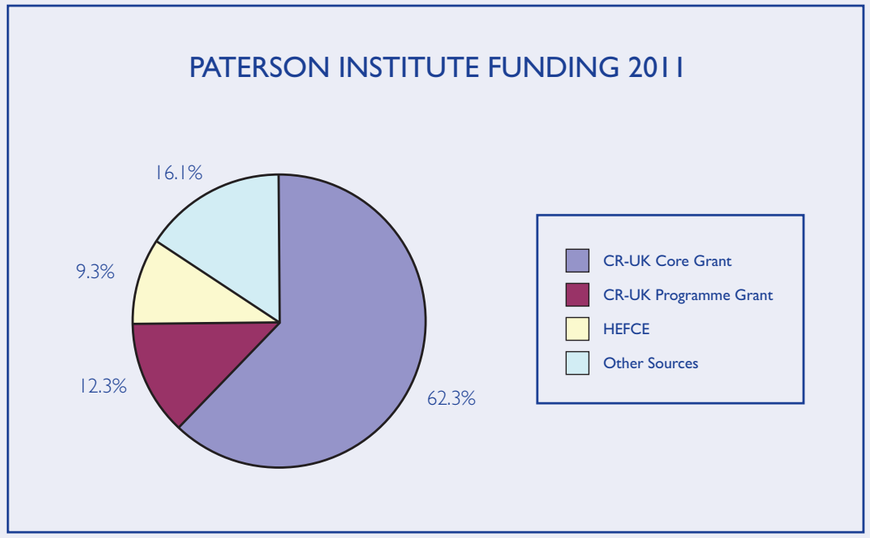


Paterson scientists with MPs Tony Lloyd and Mark Hunter

Footballer Ashley Young with Paterson Institute Group Leader and Chief Scientist of Cancer Research UK, Professor Nic Jones

Acknowledgement for Funding of the Paterson Institute

The total funding of the Paterson Institute for 2011 was £17.6m. The major source of this funding (62.3%) was through a core grant from Cancer Research UK (CR-UK). The actual value of this award in 2011 was £10.96m. This is divided between the various scientific groups and service units within the Institute to enable them to carry out their research. In addition to this the CR-UK awarded us £2.16m to run the Drug Discovery unit (12.3%).



The infrastructure of the Paterson Institute is funded by HEFCE generated income at a cost of £1.6m (9.3%)

The final 16.1% 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:

- AstraZeneca
- Roche
- European Commission
- ECMC
- BBSRC
- Leukaemia & Lymphoma Research Fund
- Novartis
- Qiagen
- Chugai
- Abbott Laboratories
- Cambridge University
- GlaxoSmithKline
- Christie Hospital NHS Foundation Trust

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 with partners including the Paterson Institute, The Christie Hospital NHS Foundation Trust, The University of Manchester 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. First year students will complement their laboratory skills by attending a small number of specialised postgraduate taught and training courses allowing them to gain a sound knowledge base of the latest developments in cancer treatment and research. 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 at five 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/index.asp), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

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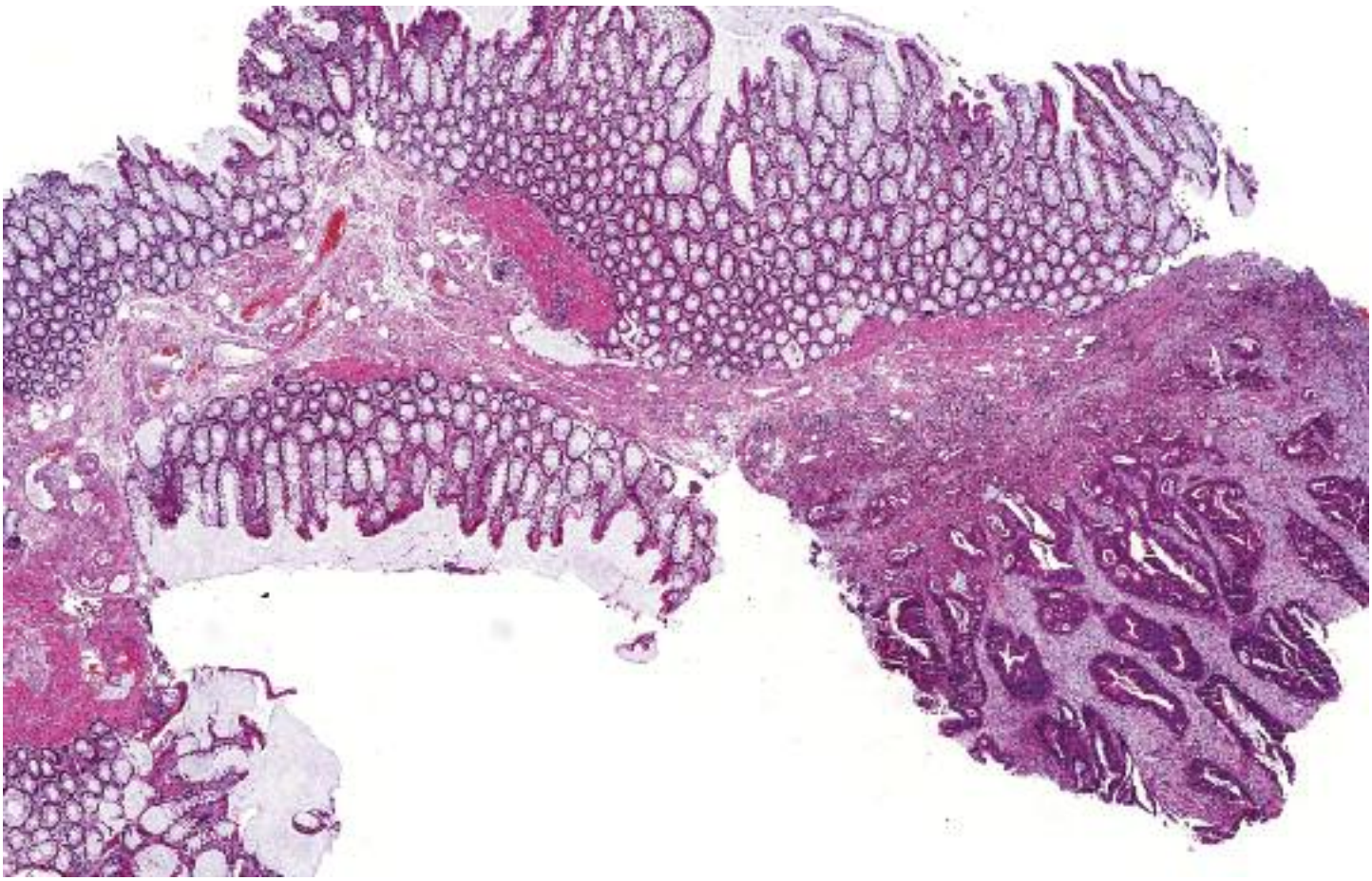

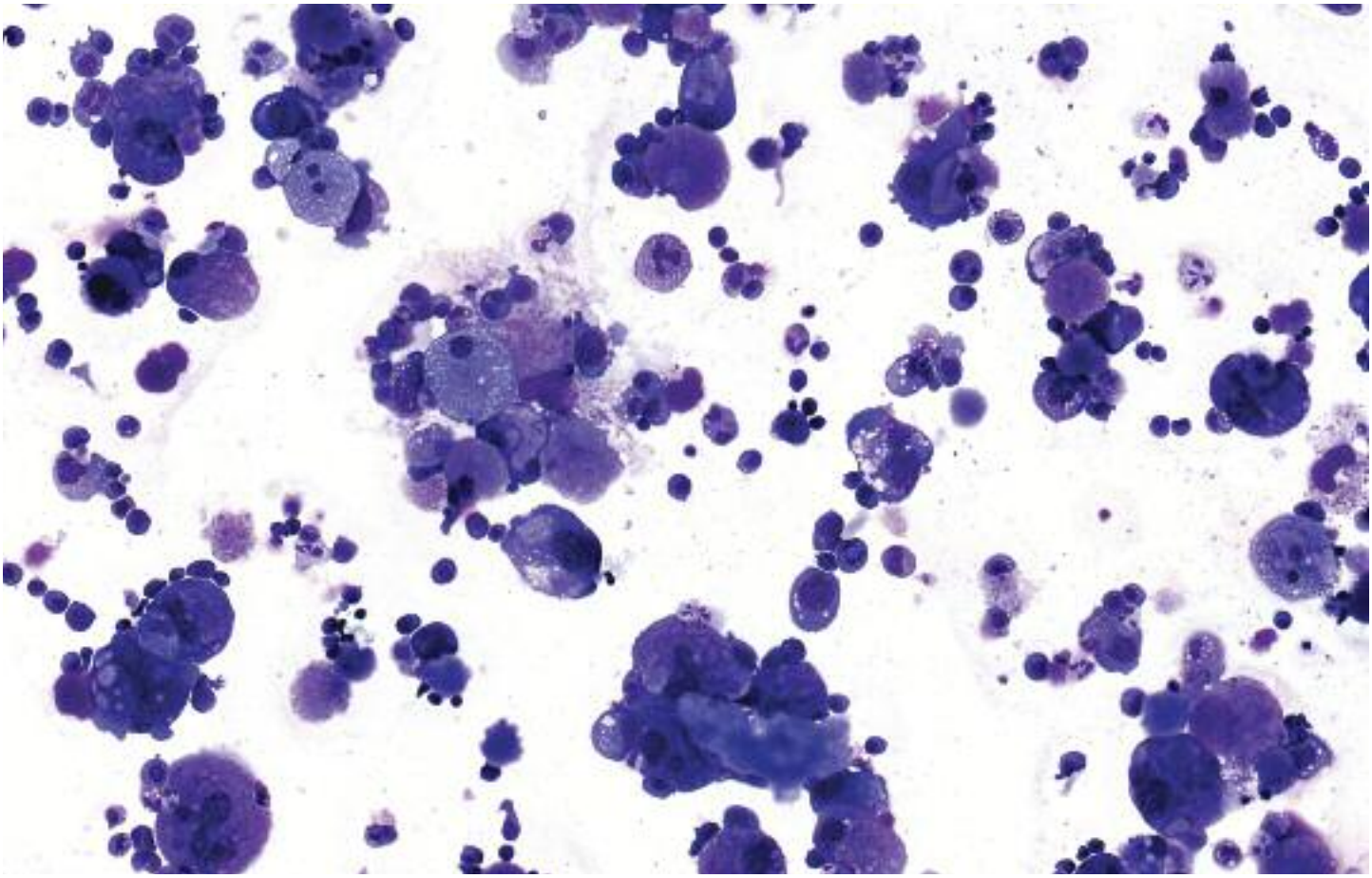

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