

Paterson Institute
for Cancer
Research

Scientific
Report
2007

Together we will beat cancer

CANCER RESEARCH UK 

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Main Image supplied by Terry Allen, Christophe Lancrin, Georges Lacaud and Valerie Kouskoff.

The image shows a scanning electron micrograph of cell-cell interaction during haemangioblast differentiation in vitro.

Additional images:

Front cover. Bovine pulmonary artery endothelial cells labelled with quantum dots. In the image, actin filaments (green) are labelled with Qdot 525 ITK amino (PEG) quantum dot phalloidin conjugate and Golgi complex (red) with rabbit anti-giantin and Qdot 585 anti-rabbit conjugate. Magnification 1000x. Image courtesy of Steve Bagley (Advanced Imaging Facility)

Rear cover. Immuno-fluorescent detection of cell surface 5T4 molecules (green) and actin cytoskeleton (red) in mouse embryo fibroblasts using widefield restorative microscopy. Total magnification x1000. Image courtesy of Tom Southgate and Peter Stern (Immunology Group)

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Paterson Institute for Cancer Research

Scientific Report 2007



Paterson
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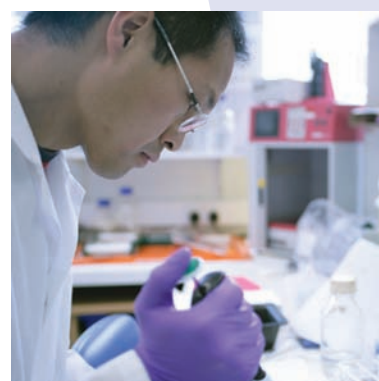
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Director's Introduction

2007 was a busy year for the Institute particularly with respect to recruitment. We welcomed three new Group

Leaders and a new Service Head providing a major boost to our research efforts.

The lifeblood of the Institute is its research staff and especially the Group Leaders who develop their independent research programmes. The success of these programmes determines the overall success and recognition of the Institute and therefore it is crucial that we recruit well and provide the best supporting environment for the research programmes to flourish. During the last year, three new Group Leaders were recruited. Nallin Divecha joined us from the National Cancer Institute in Amsterdam where for a number of years he has led a very successful and productive research group focussed on defining the roles of phosphoinositides, a family of phospholipid second messengers, in the regulation of cellular stress pathways and the process for tumourigenesis (see page 26). Significant evidence now exists that these second messengers play an important role in human cancer. His expertise will greatly strengthen the cellular signalling area of the Institute's research. Akira Orimo joined us from the Whitehead Institute in Boston where he was a postdoctoral fellow in the laboratory of Bob Weinberg. Akira's research has focussed on the role of the tumour microenvironment in human mammary carcinomas and in particular the role of the myofibroblasts (activated fibroblasts), a major component of the tumour stroma. These fibroblasts provide signals which support carcinoma cell growth. His research programme in the Paterson will extend these important initial findings, characterising the signalling pathways involved in more detail and examining the role of the myofibroblasts in tumour invasion and metastasis (see page 36). Tim Somervaille

moved from Stanford University in California where he was a postdoctoral fellow in Michael Cleary's laboratory carrying out research on leukaemic stem cells. He developed a mouse model of human acute myeloid leukaemia that has allowed him to study the molecular characteristics of this critical cell population. He will continue this work in the Paterson and through these studies better understand the regulation of leukaemic stem cell proliferation and differentiation and how they may be eliminated or controlled therapeutically (see page 28). We hope to recruit additional Group Leaders over the coming year to further bolster key areas of our research activities.

The work of one of our existing Group Leaders, Crispin Miller, was deservedly recognised by promotion to Senior Staff Scientist. Crispin leads an internationally competitive bioinformatics research group that is developing new, innovative and cutting-edge bioinformatics tools in addition to providing an invaluable bioinformatics service to the rest of the Institute and the wider scientific community. His research has been particularly focussed on the analysis of microarray data and his interaction with the CR-UK wide microarray facility in the Institute has been invaluable in its development and reputation. His attention is now turning to proteomics data and the challenge of integrating protein and mRNA expression patterns. It is always a difficult balancing act to accomplish very high standards in both research and service but Crispin does so with great success and his promotion signifies our long-term commitment to support his research programmes.

This year also brought with it the departure of Terry Allen, a Group Leader who has been associated with the Institute for 37 years. During this time he has contributed enormously to the Institute in a number of different ways. Terry was widely recognised as a world-class electron microscopist, pioneering new techniques and approaches especially involving the scanning electron microscope. His own research area involved

the characterisation of the nuclear pore complex, a structure critical for nuclear transport. His images of these complexes were often breathtaking and provided great insights into their structure and assembly. However, his expertise facilitated the work of many other groups in the Institute and world-wide. We wish him well in his retirement.

Equally important to the Institute are the research services which provide access to state-of-the-art technologies and expertise for our research groups. It is essential that these services are reviewed periodically to ensure continued and appropriate development and during the year we reviewed the Flow Cytometry Service with the help of outside experts. As a result of the review we recruited a new Head of the Service, Morgan Blaylock who joined us from the Institute of Medical Sciences at Aberdeen University. Morgan will oversee a refurbishment of the facility and an expansion in the capacity and capability of the service. Expansion in the Histology Service is also underway to provide facilities for tissue processing associated with a new centralised tumour tissue Biobank that will be located in the Paterson and developed by the Manchester Cancer Research Centre (MCRC).

As described in last year's report, the Institute is a major partner in the MCRC which was established to integrate cancer research activities within The University of Manchester in partnership with the Christie Hospital and Cancer Research UK. Development of the MCRC is crucially important for the Institute since it provides the means by which it can facilitate translational and clinical research in addition to its major focus on fundamental research. This last year has seen major advancements important for the MCRC. One important area is in early phase clinical trials which is already a major strength and the dedicated trials unit at the Christie is already one of the top units in Europe. However, there is considerable scope for further expansion and development. During the last year, the Christie Trust Board approved plans for a major new Treatment Centre part of which will be dedicated to early phase clinical trials. This will result in one of the biggest such facilities in the world. The unit works closely with the Clinical and Experimental Pharmacology laboratory in the Paterson (see page 22) which provides associated translational

research assaying samples from trial patients to maximise information gained and identify biomarkers with potential predictive value. This development therefore will be a major boost for MCRC's efforts. Another important component of the MCRC's strategy is to strengthen tumour-specific research programmes and breast cancer research was given a major boost by the decision of the Breakthrough Breast Cancer Charity to site a new research unit within the Paterson Laboratories. Funding will support the research of three new groups and recruitment into these positions is underway. We anticipate many interactions between researchers in the unit and the Institute. Finally, the development of research infrastructure is crucial if the MCRC is to achieve its goals particularly in translational research. Plans have been developed and are being implemented to initiate a major new initiative in tissue biobanking; this has involved the citing of tissue collectors in five different hospitals in the Manchester conurbation where samples will be collected and sent to the Paterson for placement in a central bank. This resource will be invaluable to a wide range of cancer researchers including those within the Institute.

We are greatly indebted once again to the PACCAR Foundation who provided additional generous support for the Clinical and Experimental Pharmacology laboratories. In recognition of their continued support for this work the laboratories have been named the 'PACCAR Therapeutics Centre'. We are also grateful for numerous other private donations that help our research endeavours (see page 79).

The coming year will provide many new challenges and opportunities for the Institute. Additional Group Leaders will be recruited and a new Drug Discovery Centre developed through additional funding from CR-UK. In order to realise ambitions to expand further both Institute and MCRC-based research the development of new laboratory facilities will be essential. Plans for such a development will be advanced during the coming year.

Nic Jones
Director

Research Highlights

In this section we feature a number of research publications from 2007 which report significant advances in specific areas. These publications demonstrate the breadth of research being undertaken at the Paterson Institute from basic studies through to reports describing improved therapy.

Okoniewski, M.J., Yates, T., Dibben, S. and Miller, C.J. An annotation infrastructure for the analysis and interpretation of Affymetrix exon array data. *Genome Biol* 2007; 8: R79

Okoniewski, M.J., Hey, Y., Pepper, S.D. and Miller, C.J. High correspondence between Affymetrix exon and standard expression arrays. *Biotechniques* 2007; 42: 181-185

Affymetrix recently introduced a new generation of microarrays that contain probesets designed to target every known and predicted exon in the human genome. These arrays offer the potential to explore, globally, changes in gene expression across the length of a gene, and therefore to identify novel events such as changes in splicing patterns between cells. Not surprisingly, given the complexity of the arrays, they present a number of significant challenges for data analysis, and when the arrays were released, little software was available that supported their comprehensive analysis.

In the first of the two papers above Okoniewski *et al.* describe the software approaches that have been developed to support these arrays. These include a novel genome browser (xmap.picr.man.ac.uk) and the first Bioconductor package designed specifically for Affymetrix exon arrays. The latter has allowed the Applied Computational Biology and Bioinformatics Group to develop a set of standard workflows for their analysis and for us to routinely support them within the Paterson Institute. The second paper is the first to use the technology and describes a validation study performed in collaboration with the Cancer Research UK Affymetrix Service. This work helped the Affymetrix Service to make a very early decision to switch to using exon arrays for expression analysis; we now have a number of projects

underway that use them and already developed a significant amount of expertise in exon array data.

Alvarez-Tabares, I., Grallert, A., Ortiz, J.M. and Hagan, I.M. Schizosaccharomyces pombe protein phosphatase I in mitosis, endocytosis and a partnership with Wsh3/Tea4 to control polarised growth. *J Cell Sci* 2007; 120: 3589-3601

Rapid and reversible protein phosphorylation is a commonly employed way to flip a protein between states to control cell fate. The phosphatases that remove phosphates are invariably formed by the association of a ubiquitous catalytic sub-unit with a range of regulatory subunits that confer substrate specificity to the complex. As human protein phosphatase I (PPI) interacts with at least 50 distinct regulatory sub-units genetically malleable model systems offer excellent opportunities to address PPI function. This study charted the distribution of fission yeast PPI catalytic sub-units. PPI localized to nuclei, centromeres, endocytic vesicles and the growing tips of these rod shaped cells. Recruitment to cell tips relied upon docking to a PPI binding motif in a polarity protein called Wsh3/Tea4. When Wsh3/Tea4 was mutated to block PPI recruitment to tips, cell polarity was perturbed, in



particular during recovery from stress. As polarized cell migration into stressful environments is a feature of metastatic tumour cells these findings identify areas in which to probe for the function of human PPI.

Hodgson, B., Calzada, A. and Labib, K. MrcI and TofI regulate DNA replication forks in different ways during normal S phase. *Mol Biol Cell* 2007; 18: 3894-3902

The progression of DNA replication forks is very carefully controlled in eukaryotic cells in order to maintain genome stability. The MrcI and TofI proteins are conserved in all eukaryotes and in budding yeast have been shown to associate with the MCM helicase and play a key role in controlling fork progression. We compared control cells with cells lacking either MrcI or TofI, and measured the rate of fork progression throughout two chromosomal replicons of 70kb. We found in the absence of MrcI that forks progress at about 40% of the wild type rate, whereas fork progression is only mildly defective in cells lacking TofI. We then examined the ability of replication forks to pause at a diverse range of chromosomal loci at which non-nucleosomal proteins bind very tightly to DNA. Pausing at all these sites required TofI but not MrcI. Our findings indicate that MrcI and TofI play distinct roles in the regulation of fork progression during chromosome replication.

Spencer, H.L., Eastham, A.M., Merry, C.L., Southgate, T.D., Perez-Campo, F., Soncin, F., Ritson, S., Kemler, R., Stern, P.L. and Ward, C.M. E-cadherin inhibits cell surface localization of the pro-migratory 5T4 oncofetal antigen in mouse embryonic stem cells. *Mol Biol Cell* 2007; 18: 2838-2851.

This publication establishes that early differentiation of pluripotent mouse ES cells involves upregulation of 5T4 oncofoetal molecules as a part of an epithelial mesenchymal transition (EMT) process which is known to be important in development and cancer. We have also shown this for human ES differentiation (Eastham *et al.*, *Cancer Res* 2007; 67: 11254). EMT is characterized by E-cadherin down-regulation involving the transcription factors Slug and Snail, upregulation of N-cadherin, matrix metalloproteinases, increased motility and altered morphology. We showed that surface 5T4 expression can be induced when cell surface E-cadherin molecules are modulated by E-cad specific antibody treatment. Such abrogation of E-cadherin mediated cell-cell contact in undifferentiated ES cells results in a reversible mesenchymal phenotype in the absence of EMT-associated transcript expression. The loss of cell surface E-cadherin leads to translocation of 5T4 molecules from the cytoplasm to the cell surface in an energy-dependent manner. We



hypothesize that E-cadherin stabilizes the cortical actin cytoskeletal arrangement of ES cells, preventing cell surface localization of the 5T4 molecules. Previous work has established that over-expression of 5T4 in epithelial cells can down-regulate E-cadherin expression effecting changes in adhesion, motility and morphology. The association of 5T4 expression with poorer clinical outcome in many different human cancers is likely to derive from the functional influence of 5T4 expression, integrated within the other EMT associated changes, on ability to metastasize.

Thistlethwaite, F.C., Elkord, E., Griffiths, R.W., Burt, D.J., Shablak, A.M., Campbell, J.D., Gilham, D.E., Austin, E.B., Stern, P.L. and Hawkins, R.E. Adoptive transfer of T(reg) depleted autologous T cells in advanced renal cell carcinoma. *Cancer Immunol Immunother* 2007; epub Sept 27.

There is increasing evidence that raised levels of systemic and/or local T regulatory cells in various cancers can be associated with poor clinical outcome and we have now shown this in untreated renal cell carcinoma (RCC) patients (Griffiths *et al.*, *Cancer Immunol Immunotherapy* 2007; 56: 1743). Naturally occurring and inducible T regulatory cell mechanisms exist to control potential autoimmunity but can also limit anti-tumour responses. Our further work has shown that increased anti-5T4 oncofetal antigen CD4 T cell responses are measurable *in vitro* if T regulatory cells are depleted prior to specific immune stimulation (Elkord *et al.*, *Cancer Immunol Immunother*. 2007; epub Nov 15). These observations led to a clinical trial in RCC patients who were treated to deplete T regulatory cells *in vivo*. This was achieved

by adoptive transfer of a Treg-depleted autologous leukapheresis harvest after conditioning chemotherapy. Interestingly, evidence of altered anti-5T4 tumour immunity was seen to be coincident with the nadir of a transient decrease in Treg levels in the peripheral blood of one of the six patients. These studies support the further development of T regulatory cell modulation strategies as a therapeutic approach in some cancers.

Breitwieser, W., Lyons, S., Flenniken, A.M., Ashton, G., Bruder, G., Willington, M., Lacaud, G., Kouskoff, V. and Jones, N. Feedback regulation of p38 activity via ATF2 is essential for survival of embryonic liver cells. *Genes Dev* 2007; 21: 2069-2082.

ATF2 and ATF7 are highly homologous components of AP-1 transcription factors. Their main regulation is via phosphorylation by various MAP kinases, most notably JNK and p38, and their activities involve transcriptional regulation in response to cellular stress and inflammatory signals as well as cytokines and growth factors. We sought to analyse ATF2 and ATF7 functions *in vivo* using various mouse knockout approaches. Firstly, we showed that MAP kinase activation of ATF2 is vital in animals, because a phosphorylation mutant knock-in leads to postnatal lethality of homozygous mutant mice. Furthermore, germ line loss of both ATF2 and ATF7 functions results in strong developmental defects, including severe anaemia and abrogated heart and liver development. The mutant foetal liver is characterised by high levels of



apoptosis in developing hepatocytes and haematopoietic cells. Furthermore, we observe a significant increase in active p38 due to loss of a negative feedback loop involving the ATF2/ATF7-dependent transcriptional activation of MAPK phosphatases. We showed that this increase drives apoptosis since it can be suppressed by chemical inhibition of p38. Our findings demonstrate the importance of finely regulating the activities of MAP kinases during development.

Winter, S.C., Buffa, F.M., Silva, P., Miller, C., Valentine, H.R., Turley, H., Shah, K.A., Cox, G.J., Corbridge, R.J., Homer, J.J., Musgrove, B., Slevin, N., Sloan, P., Price, P., West, C.M. (joint senior author) and Harris, A.L. Relation of a hypoxia metagene derived from head and neck cancer to prognosis of multiple cancers. *Cancer Res* 2007; 67: 3441-3449.

April saw the publication of this paper describing the microarray analysis of a series of head and neck cancers and derivation of a hypoxia-associated gene signature. The paper was the culmination of a study set up three years earlier by Dr Catharine West in collaboration with Professor Adrian Harris in Oxford. The microarray data were obtained from the Cancer Research UK GeneChip Microarray Service at the Paterson. Data analyses were carried out by Dr Francesca Buffa in Oxford in collaboration with Dr Crispin Miller. A gene signature was obtained by microarray profiling 59 tumours and clustering around the *in vivo* RNA expression known hypoxia-related genes. The 'knowledge-based' approach was used to derive a 99-gene signature. High RNA expression of genes in the signature was associated with an adverse prognosis not only in an independent series of head and neck cancers but also in a well known breast cancer microarray dataset. Prognostic significance was also independent of known clinicopathological variables and intrinsic trained profiles. The novel method used to derive the signature highlighted the validity and potential of using microarray data from analysis of *in vitro* stress pathways for deriving biologically relevant gene signatures in human cancers *in vivo*.



Du, Y., Honeychurch, J., Glennie, M., Johnson, P. and Illidge, T. Microscopic intratumoral dosimetry of radiolabeled antibodies is a critical determinant of successful radioimmunotherapy in B-cell lymphoma. *Cancer Res* 2007; 67: 1335-1343.

Morschhauser, F., Illidge, T. (joint first author), Huglo, D., Martinelli, G., Paganelli, G., Zinzani, P.L., Rule, S., Liberati, A.M., Milpied, N., Hess, G., Stein, H., Kalmus, J. and Marcus, R. Efficacy and safety of yttrium-90 ibritumomab tiuxetan in patients with relapsed or refractory diffuse large B-cell lymphoma not appropriate for autologous stem-cell transplantation. *Blood* 2007; 110: 54-58.

The first publication built on previous work which enhances our understanding of the mechanisms of action of radioimmunotherapy (RIT) *in vivo*. We had previously shown that antibody induced intracellular signaling works in combination with radiation to eradicate lymphoma in radioimmunotherapy (Du *et al.*, Blood 2004; 103: 1485). This more recent work focused on the importance of the micro-distribution of radiolabelled mAb to targeting radiation effectively to tumour. We found important therapeutic differences between targeting different tumour antigens and their ability to deliver tumouricidal doses of targeted radiation at the microscopic level. In these complex studies we specifically investigated how the microdistribution of the targeted radiotherapy component affects the long-term clearance of lymphoma. The profound differences in therapeutic outcome observed were independent of the levels of antibody tumour-binding or antibody-dependent cytotoxicity. Instead, the microscopic intratumoural dosimetry seemed to be critical with the more therapeutically effective ¹³¹I-labelled antibodies delivering



more concentrated and therefore substantially higher radiation dose to tumour cells. We believe that these new insights will influence the selection of new antigen targets and the design of dosimetric methods in RIT of lymphoma.

The second publication was the first study to demonstrate the efficacy of Yttrium-90 Ibritumomab Tiuxetan (Zevalin) in diffuse large B cell lymphoma and Illidge was the first investigator in Europe to use the drug. The study demonstrated high efficacy and responses even in patients who were refractory to chemotherapy. The work represents potentially a land mark development in using Ibritumomab Tiuxetan (Zevalin) in aggressive lymphoma and has led to further studies investigating the use of the drug in this disease



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Bioinformatics exists to help manage and manipulate the complex datasets generated by research in the biosciences. Computational Biology involves building mathematical models to help interpret these datasets and make predictions using them. In the Applied Computational Biology and Bioinformatics group we are interested in the development and application of software tools and analytical strategies for the analysis of cancer related datasets. We collaborate closely with other groups working on both the clinical- and the molecular-biology aspects of cancer research.

Exon arrays and alternative splicing

Recent advances in technology have made it possible to produce microarrays with a probeset targeting every known and predicted exon in the entire human genome. With over 6.5 million probes (corresponding to about 1.4 million probesets), Affymetrix Exon arrays provide many challenges to data analysis and interpretation. A major focus of our group has been the development of novel software tools to support these arrays. We have been developing tools to represent the complex relationship between the probes and probesets on the array and the exons, transcripts and genes that they target, and to bring this information into an environment that allows it to be placed alongside the statistical methods necessary to analyse the expression data generated using this latest generation of microarrays.

X:Map: a database and genome browser

X:Map is a database designed specifically to support exon arrays (Okoniewski *et al.*, 2007b). Since as much as 90% of the genome is predicted to be transcribed, X:Map is built by searching each of the 6.5 million probes on the array against the genome, and storing every location where they match. This allows them to be mapped to the genes, transcripts and exons that occur at the same locations. By using tools such as exonmap (see below) it is possible to use the data in X:Map to associate changes in expression with the genomic features they correspond to.

X:Map also supports a web-based genome browser that uses the Google Maps API (usually used to present street maps and satellite images) to present a fully dynamic view of the genome that can be scrolled and manipulated in real time. X:Map is the first fully functioning genome browser to do this.

X:Map is freely available on the web at
<http://xmap.picr.man.ac.uk>

Exonmap

Bioconductor is an open source, open development environment for analysing biological data. It consists of a set of software packages written in the R programming language. One advantage of R is that it has been used extensively for statistical analysis and offers a comprehensive statistical toolbox to support analyses. Bioconductor adds the ability to import and analyse biological data within this rich statistical environment.

We have developed a number of Bioconductor packages, including exonmap, which is designed specifically for exon arrays (Okoniewski *et al.*, 2007b). Exonmap makes use of the data in X:Map to support analytical workflows that allow not only individual genes of interest to be explored in detail, but also systematic searches for all genes of interest in an experiment. Exonmap was one of the first software tools

designed specifically for exon arrays and is entirely free and open source.

Platform validation

The first study we performed using exon arrays was in collaboration with the Cancer Research UK Affymetrix Service (page 54). Exon arrays were compared with the previous generation of Affymetrix HGU133plus2 arrays, and were found to produce high quality data that could be compared with those arising from the earlier chips. This led to one of the first publications using these chips (Okoniewski *et al.*, 2007a), and helped us to make a decision to start using them by default for all our new projects; we now have a number of ongoing collaborations based on the technology. In other work with the Affymetrix Service we have considered differences between expression summary algorithms and their influence on the data returned by microarray experiments (Pepper *et al.*, 2007). This has allowed us to identify (and validate by real time PCR) groups of differentially expressed transcripts that are not found using standard workflows.

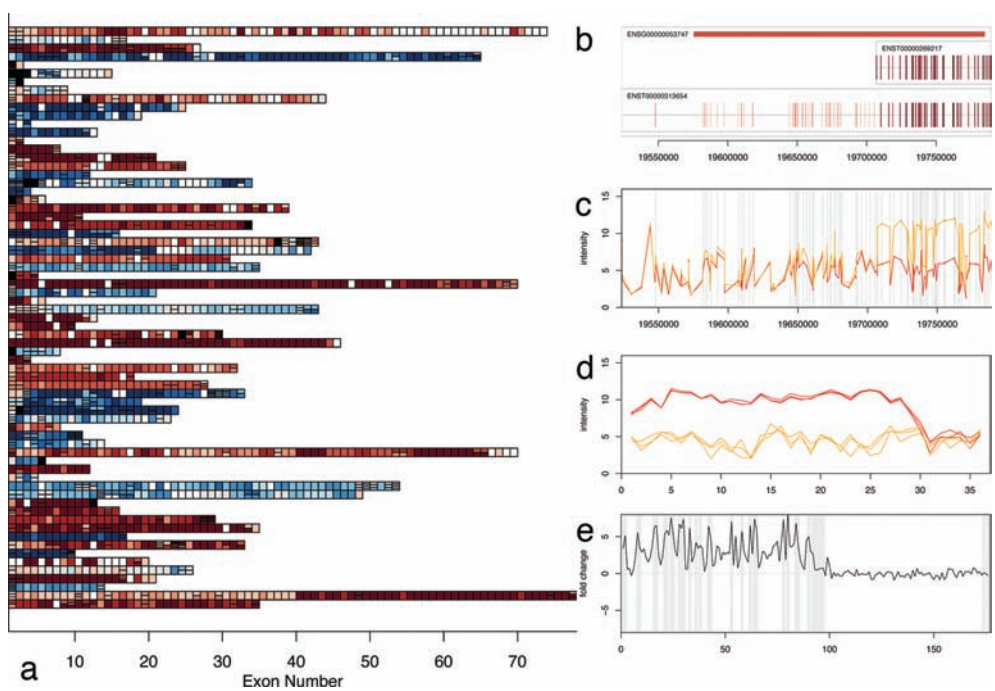


Figure 1. Some of the visualizations available in exonmap for exploring exon array data. (a) Overview of many genes. Each row corresponds to a gene, each rectangle an exon. The plot is coloured by fold change between two cell lines, MCF7 and MCF10A. (b-e) four alternative views of the same gene. (b) exons coloured by fold change, (c) mean raw intensities for each replicate group against probeset position (d) raw intensities for each sample, exon targeting probesets, only (e) fold change against probesets., ordered by position, grey bars, exonic probesets, white bars, intronic probesets.

The complexities of biological data

One of the challenges in gene expression analysis is to determine whether an observed difference between samples is due to real a biological effect or chance fluctuation. Current approaches rely on dividing samples into groups (control vs. treatment, for example) and using statistical models to assess the significance of the differences between them. The complexities of biological data mean that there is often a substantial amount of variability within each replicate group, much of it due to real biological differences. While current methods attempt to account for this variability, we have developed statistical techniques that aim to exploit it (figure 2). The approach, developed in collaboration with Catharine West (page 38), leads to improved methods for finding correlation between probesets (and thus the transcripts the target) and is particularly applicable to clinical data (Möller Levett *et al.*, 2007).

Exploration of proteomics data

Advances in proteomics technology are making it possible to generate quantitative proteomics data for large numbers of proteins in a single experiment. We are continuing to collaborate with Tony Whetton and the Stem Cell and Leukaemia Proteomics Laboratory (SCALPL) at The University of Manchester to explore proteomics data and we also have projects within the group looking at developing novel techniques for proteomics data analysis.

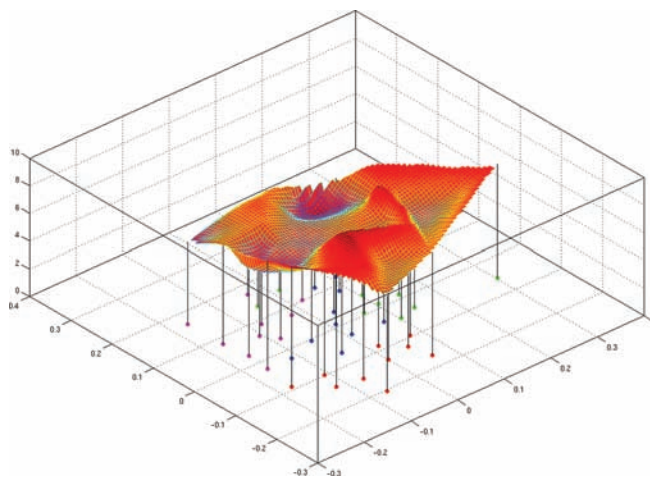


Figure 2. An enriched expression profile. Samples are first ordered in two dimensions according to similarity in expression profiles, and then expression data fitted for each gene. A comparison between the resultant surfaces yields a novel measure of similarity.

Publications listed on page 60



Carcinogenesis Group

<http://www.paterson.man.ac.uk/carcinogenesis>

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A variety of cellular “genome maintenance” networks exist that remove endogenously generated, potentially lethal damage from DNA. Many types of cancer treatment exploit the cell killing effects of therapeutic agents that can generate the same types of damage in DNA. Understanding how DNA damage leads to cell death, and how these repair systems process the damage, may provide opportunities to improve the effectiveness of existing cancer therapies, and develop new agents. Our main focus is on DNA damage and the ensuing DNA repair processes that follow exposure to certain types of alkylating agents, one example of which is the CR-UK drug, Temozolomide. We recently discovered, in fission yeast, a new mechanism for the processing of this damage, and much of our effort has been directed to the characterisation of the proteins involved.

Background

Alkylating agents are a group of chemicals that generate varying amounts of a dozen different types of lesions in DNA. Agents of this class are used in cancer chemotherapy

and there is increased understanding of the mechanisms by which some of the lesions they generate result in cell killing. Methylating agents such as Dacarbazine and Temozolomide produce, amongst other lesions, O^6 -methylguanine in DNA and this kills cells via the action of the post replication mismatch repair (MMR) system. Cell killing can be prevented by the action of the damage reversal protein, O^6 -alkylguanine-DNA alkyltransferase (the human version of which is MGMT). This protein likely evolved to attenuate the toxic and possibly mutagenic effects of endogenously or environmentally generated lesions in DNA. However, it can also reduce the therapeutic effects of chemotherapies and hence there is increasing interest in attenuating the expression of such pathways in tumours in order to increase the differential killing effects and thus to improve clinical outcome. One of the strategies we have pursued has involved the use of a low molecular weight inhibitor of MGMT. This drug, now called Lomeguatrib, is just completing phase II clinical trials in combination with Temozolomide in malignant melanoma.

MGMT removes alkyl groups from the O^6 -position of guanine by stoichiometric transfer to a cysteine residue in its active site, a process that results in its irreversible inactivation. We recently discovered, by *in silico* analysis, a family of proteins that have extensive amino acid sequence homology to MGMT, but without this critical cysteine residue. These proteins are present in a number of organisms, including *E. coli* and *S. pombe*. We have named this family the alkyltransferase-like (ATL) proteins, and we are now trying to establish their mechanism of action.

Clinical trials of Lomeguatrib

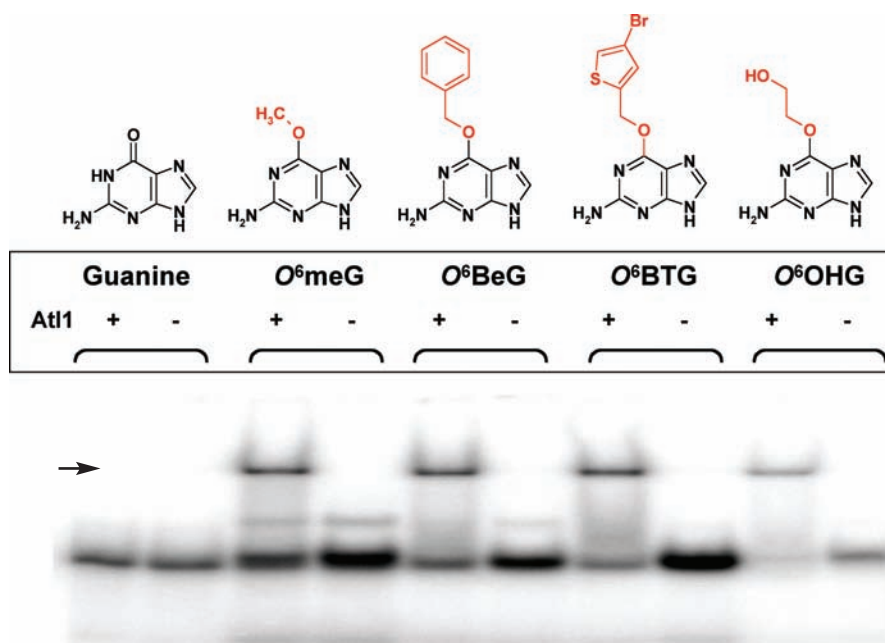
Lomeguatrib effectively inactivates MGMT and sensitises human tumour xenografts to the killing effect of Temozolomide. Phase I clinical trial of this drug started here at Christie Hospital in 2000, and established a dose combination of Lomeguatrib and Temozolomide for use in

Phase II trials. These trials, which are close to completion, have been carried out under the auspices of KuDOS to whom Lomeguatrib is licensed, and patients have been recruited from several centres in the UK, USA and Australia. In addition, another trial sponsored by CR-UK addressed the effectiveness of Lomeguatrib in inactivating MGMT in a number of tumour types in order to establish the doses required for complete inactivation. These trials have required us to develop and validate, to Good Clinical Laboratory Practice standards, quantitative assays for both functionally active and total MGMT protein. Dose escalation studies have now established the effective dose of Lomeguatrib for brain, prostate and colorectal tumours. These results should lead to Phase II trials in these tumour types in combination with Temozolomide. This work would not have been possible without the longstanding collaboration with Prof Brian McMurphy and the late Dr Stanley McElhinney (and their group at the Chemistry Department, Trinity College, Dublin), and the support of CR-UK Drug Development and Formulation Units and also Cancer Research Technology. We are also investigating the role of MGMT promoter methylation ("epigenetic silencing") in regulating MGMT expression and influencing response in glioma.

Another aspect of these clinical trials involved the determination of the major DNA methylation product, 7-methylguanine, in DNA by means of a sensitive immunoslot-blot assay. We have now shown that the unexpected variation of this product in lymphocyte DNA of Temozolomide-treated patients correlates with the levels of expression of the DNA repair enzyme, alkyladenine-DNA glycosylase. The implications of this, if any, for Temozolomide therapy and toxicity have yet to be established.

Alkyltransferase-like (ATL) proteins

The ATL-encoding genes from *E. coli* and *S. pombe* have been cloned and we have named the latter AtlI. In order to establish if AtlI has any role in the sensitivity of *S. pombe* to alkylating agents, the gene was inactivated by PCR-based gene disruption. The resulting deletants had similar growth characteristics to the wild type strain, but had substantially increased sensitivity to the growth inhibitory effects of a number of alkylating agents: there was no detectable effect on sensitivity to other classes of genotoxic agents. AtlI is therefore an important protein in the protection of *S. pombe* against the toxic effects of these alkylating agents. Exactly how it achieves such protection has yet to be established.



Structures of guanine and the O⁶-alkyl modified guanine residues incorporated into the oligonucleotide sequence 5'-AACAGCCATATXGCCC (where X is the modified base). Oligos were 5'-³²P-labelled, incubated with purified recombinant AtlI, subjected to non-denaturing polyacrylamide gel electrophoresis and phosphorimaged. The arrow indicates the position of the shifted band. O⁶meG=O⁶-methylguanine; O⁶BeG=O⁶-benzylguanine; O⁶BTG=O⁶-(4-bromophenyl)guanine; O⁶OHG=O⁶-hydroxyethylguanine

We have shown that, *in vitro*, AtlI binds to methylated DNA and by doing so it can inhibit the action of MGMT. In collaboration with David Williams (Sheffield) we have shown using gel shift assays that a purified fusion protein of AtlI with maltose binding protein (see figure) and the ATL from *E. coli* bind to short single- or double-stranded oligonucleotides containing a number of O⁶-alkyl-substituted guanines including methyl, benzyl, hydroxyethyl and 4-bromophenyl (i.e. Lomeguatrib embedded into an oligonucleotide, which is the most potent MGMT inactivating agent so far described). However, whilst binding to these potentially toxic lesions can be demonstrated, there is no evidence of an MGMT-like alkyl transfer; nor of glycosylase or endonuclease activity, so the protein seems likely to be a specific DNA damage sensing protein that signals to other DNA repair networks. Indeed, crossing the atlI deletant with deletants in other DNA repair pathways (epistasis analysis) has suggested that AtlI is part of, or signals to, the nucleotide excision repair pathway and not the base excision or recombination repair pathways. Further analyses of this type may confirm this.

One approach to establishing the mechanism of action of AtlI is by identifying interacting proteins, and several parallel co-precipitation strategies are now being employed to pursue this. A comprehensive assessment of the interaction of AtlI with the substrate molecules that it recognises is also being undertaken.

Publications listed on page 60



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Our group studies chromosome replication and cytokinesis in eukaryotic cells. During 2007 we showed that the MrcI and TofI proteins control the progression of DNA replication forks in different ways during chromosome replication. Both MrcI and TofI are associated with the DNA helicase responsible for unwinding the DNA duplex at replication forks, as part of "Replisome Progression Complexes". We showed that MrcI is important to maintain the normal rate of progression of replication forks, whereas TofI allows forks to pause at specific sites in chromosomes where non-nucleosomal proteins are bound very tightly to DNA. In parallel with this work, we have identified a novel protein, InnI, which is required for cytokinesis. During 2007 we showed that this protein contains two key features: a carboxy terminal portion that is required to recruit InnI to the actomyosin ring, and a C2-domain that is required at the actomyosin ring for ingression of the plasma membrane.

Regulation of the progression of DNA replication forks

We previously showed that a set of regulatory proteins is

recruited to the MCM helicase at nascent DNA replication forks in budding yeast to form large "Replisome Progression Complexes" (RPCs; Gambus *et al.*, Nat Cell Biol 2006; 8: 358). Each of the components of these complexes has a single orthologue in all eukaryotic species, and it seems very likely that the progression of DNA replication forks will be controlled in a very similar manner in yeast and human cells.

RPCs contain what is probably the active form of the MCM DNA helicase, which is essential for the progression of DNA replication forks. Some of the other components of RPCs are not essential for the progression of DNA replication forks, but are still important for the maintenance of genome stability, and serve to control aspects of fork progression. Amongst these, the MrcI and TofI proteins were previously shown to restrain the progression of DNA replication forks that stall in response to the depletion of nucleotides by the drug hydroxyurea. We have studied the roles of these proteins during the normal process of chromosome replication, and have found that they are required for different aspects of the progression of DNA replication forks.

We previously found that TofI is required for DNA replication forks to pause at a "Replication Fork Barrier" that is formed by the very tight binding of the FobI protein to a site in the ribosomal DNA repeats (Calzada *et al.*, Genes Dev 2005; 19: 1905). In contrast, MrcI is not required for forks to pause at the FobI replication fork barrier. In collaboration with Arturo Calzada at the University of Salamanca in Spain, we have now shown that TofI is also required for forks to pause at other naturally occurring replication fork barriers that are formed by tight binding of other non-nucleosomal proteins to specific loci, such as kinetochore complexes at centromeres, or where proteins bind very tightly to the promoters of tRNAs (Hodgson *et al.*, 2007). Once again, MrcI is not required for forks to pause at any of these sites, indicating that MrcI normally plays a distinct role to TofI during chromosome replication.

Oscar Aparicio's group used two-dimensional DNA gels to study DNA replication intermediates and found that MrcI is important for the normal rate of progression of DNA replication forks (Szyjka *et al.*, Mol Cell 2005; 19: 691). We

made similar observations with the same technique, but did not observe such a defect in cells lacking Tof1, consistent with the idea that Mrc1 and Tof1 work in different ways at replication forks (Calzada *et al.*, *Genes Dev* 2005; 19: 1905). However, Philippe Pasero's group then showed that both Tof1 and Mrc1 are important to maintain the normal rate of progression of DNA replication forks during an apparently normal round of DNA replication, if cells were labelled with the nucleotide analogue Bromodeoxyuridine (BrdU; Tourriere *et al.*, *Mol Cell* 2005; 19: 699). As cells are not able to grow following incorporation of BrdU, we wanted to address the role of Mrc1 and Tof1 during normal S-phase in unperturbed cells, without using BrdU.

Ben Hodgson used dense isotope substitution to measure the rate of fork progression in a quantitative manner in control cells or in cells lacking either Mrc1 or Tof1. This technique involves the use of growth medium that contains the stable isotopes of carbon and nitrogen ^{13}C and ^{15}N that occur at a low level in nature and support normal cell growth. We found that the rate of fork progression in cells lacking Mrc1 is about 40% of the equivalent rate in control cells. In contrast, forks are only slightly slower than normal in the absence of Tof1 (Hodgson *et al.*, 2007). These findings indicate that Mrc1 and Tof1 do indeed regulate fork progression in different ways, and the challenge is now to understand in molecular detail how these proteins act.

Inn1 couples contraction of the actomyosin ring to ingression of the plasma membrane during cytokinesis

In our systematic screen for new cell cycle proteins Alberto Sanchez-Diaz identified a protein that we call Inn1, which is essential for cell division. We have found that Inn1 associates with the contractile actomyosin ring at the end of mitosis. Inn1 is not required, however, for the ring to form or contract, and instead is essential for contraction of the ring to be coupled to ingression of the plasma membrane. We have shown that amino acids 1-134 of Inn1 form a C2-domain (these are usually membrane-targeting domains) that is essential for membrane ingression. The remainder of the protein is essential to target Inn1 to the actomyosin ring, by fusing it to other ring proteins, and found that this restored cell viability and membrane ingression to cells that lacked the endogenous INN1 gene. These data indicate that key to the function of Inn1 is that amino acids 135-409 are required to take the protein to the actomyosin ring, where the C2 domain then plays an essential role during ingression of the plasma membrane. We are now trying to understand how the C2 domain and the remainder of Inn1 perform these functions during cytokinesis.

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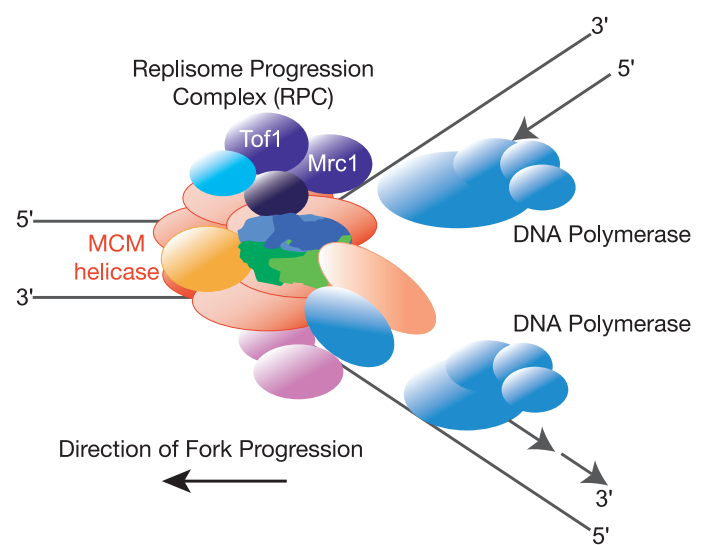


Figure 1

Mrc1 and Tof1 form part of Replisome Progression Complexes and control the progression of DNA replication forks.

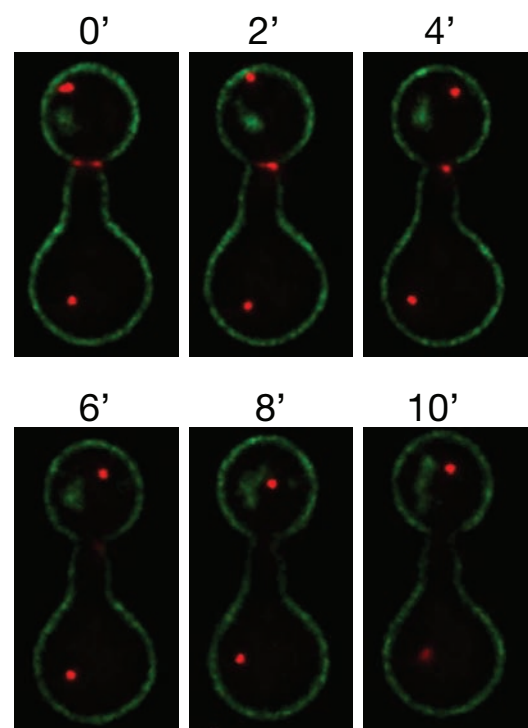


Figure 2

Inn1 is required for ingression of the plasma membrane during cytokinesis in budding yeast. The picture shows time-lapse imaging of a live cell that has just completed mitosis in the absence of Inn1 (the red dots at the top and bottom of the cell correspond to the spindle poles). The red signal in the middle of the cell shows the contractile actomyosin ring, and the green signal corresponds to a protein that associates with the plasma membrane.



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Life is dynamic; cells respond to cues to grow, move, divide and die. The inappropriate cell proliferation that underlies cancer arises from alterations in any or all of these processes. The dynamic changes that accompany and drive changes in these processes are governed by signal transduction cascades. Protein modification by reversible phosphorylation lies at the heart of these transduction networks as the addition or removal from key residues can flip a molecule from one state of activity into another, thereby turning on or off a particular aspect of cell behaviour. Because many of these regulatory networks are highly conserved across species, studying the basic principles of signal transduction in the relatively simple and highly malleable yeast can greatly accelerate the analysis of the more complex networks in man.

Protein Phosphorylation

The conjugation of phosphate to serine, threonine or tyrosine residues of target molecules in signal transduction is mediated by protein kinases. Kinases are generally of two types: those in which a catalytic kinase domain is fused to a second domain that targets this catalytic activity to particular targets

and those in which the enzyme is essentially a catalytic domain alone and it is the association with particular regulatory subunits that confers substrate specificity (figure 1). More kinases fall into the first category than the second. A key facet of phosphorylation is its reversibility as this enables the cell to rapidly switch back and forth between two states. The reversibility stems from the activity of a second class of enzymes, protein phosphatases, that remove the phosphate conjugated to target molecules by the kinases. Unlike protein kinases, the association of a universal catalytic subunit with multiple regulatory subunits is far more prevalent than the stand-alone fusion of catalytic and docking motifs within the same molecule. There are therefore far fewer protein phosphatases than kinases in all eukaryotes.

Protein Phosphatase I

We have characterised the function of fission yeast *Schizosaccharomyces* protein phosphatase I (PPI). PPI is a highly conserved phosphatase whose association with over 50 regulatory sub-units controls diverse processes ranging from metabolism, motility, through chromosome segregation to the control of cell division. While either one of the fission yeast genes encoding PPI catalytic sub-units can be deleted, deletion of both is lethal (Ohkura *et al.*, Cell 1990; 63: 405), underscoring the importance of PPI function in basic cellular processes.

We inserted sequences encoding the green fluorescent protein (GFP) from *A. victoria* into the native loci of genes encoding the catalytic sub-units of PPI, *dis2*⁺ and *sds21*⁺. The fluorescence of the resultant fusion proteins enabled us to determine the distribution of the fusion proteins. Even though the primary amino acid sequences of these two proteins share a striking 79% identity, their localisations were significantly different. Dis2.GFP was found in far more locations than Sds21.GFP; the growing tips of the cell, endocytic vesicles, centromeric sequences and a general nuclear stain. Deletion of *dis2*⁺ led to an increase in the levels of Sds21 and its recruitment all locations that would otherwise be occupied by Dis2.

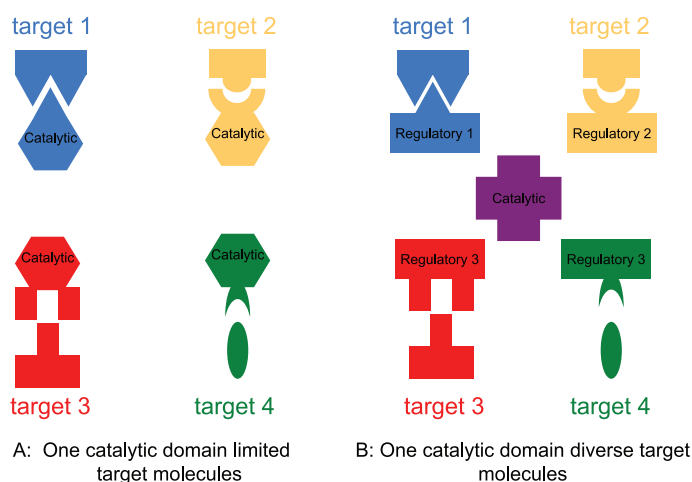


Figure 1
Kinases and phosphatases are composed either of catalytic domains fused to a targeting domain that directs the enzyme to a limited number of target molecules, or are catalytic domains that associate with a broad number of targeting sub-units that direct the enzyme to particular substrates.

Cell polarity in fission yeast

Cell growth in fission yeast mirrors the interplay between the actin and microtubule cytoskeletons that underlies growth, migration and signalling in human cells. We therefore focused upon the recruitment of Dis2 to sites of cell growth at the tips of these rod-shaped cells. Microtubules, but not actin, were required for recruitment of Dis2.GFP to cell tips, suggesting that PPI maybe playing a key role in microtubule-mediated processes at sites of cell growth. The polarity factor Tea1 is delivered to cell tips on the ends of polymerising microtubules (figure 2). There it leaves the microtubules and associates with a membrane anchor protein called Mod5. A second Tea1 binding protein, Tea4/Wsh3 is transported to cell tips in a complex with Tea1 where Tea1 anchors it to cell ends to interact with molecules that promote actin polymerisation (For3 and Bud6). Thus the delivery of Tea1 by microtubules to cell tips directs actin polymerisation to orientate cell growth. Consequently, cells lacking Tea1 often bend or branch.

PPI recruitment to cell tips by Tea4/Wsh3 controls polarity

Dis2 recruitment to cell tips was mediated by a docking motif in Tea4/Wsh3. Mutating this motif to block PPI recruitment to cell tips led to the same cell polarity defects as arose from loss of Tea1 function. Thus, reversible protein phosphorylation plays a critical role in the interplay between the actin and microtubule cytoskeletons that determines cell polarity (figure 3). Tea4/Wsh3 also associates with components of stress response pathway, a signalling transduction cascade that alters cell homeostasis in response to a variety of stresses. This association is assumed to be important for maintaining polarised growth following stress as cells lacking Tea4/Wsh3 can not maintain polar growth following stress. Cells in which Tea4/Wsh3 could no longer recruit PPI to cell tips were severely compromised in their ability to maintain normal growth patterns following stress. Thus, we have established that PPI plays a critical role in

controlling cell polarity in normal growth and response to stress.

Lessons from yeast

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

The realisation that PPI controls cell morphogenesis identifies novel opportunities in the analysis of cell migration in human cells. As cell migration into stressful, inappropriate environments is the hallmark of metastatic cancer and most tumour cells are growing in sub-optimal environments that impose a range of stresses, extending our understanding of polarity by analysis in yeast will provide clues for new therapeutic avenues in cancer treatment.

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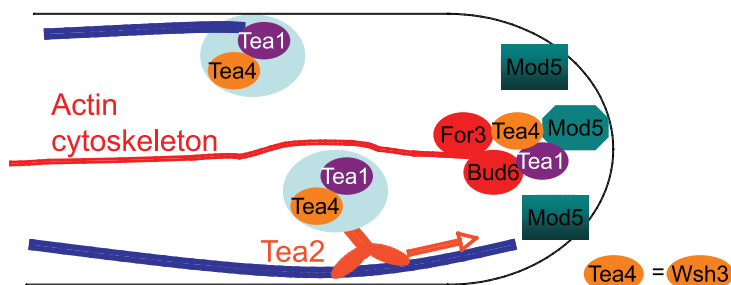


Figure 2
Microtubules and polarity in fission yeast. Complexes containing Tea1-Wsh3/Tea4 are delivered to cell tips via an association with microtubule plus ends or their association with a microtubule motor protein called Tea2 that move the complex to cell tips. The association of Tea1-Tea4/Wsh3 with Mod5 at tips stimulates For3 and Bud6 to polarise the actin cytoskeleton and hence, cell polarity.

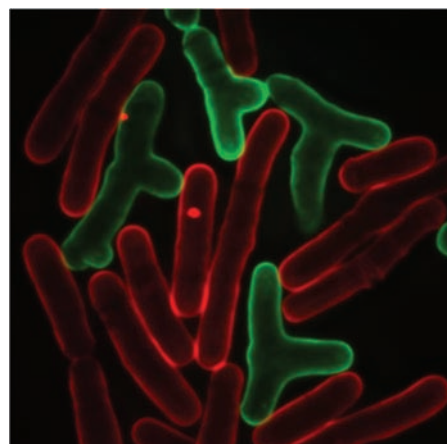


Figure 3
Recruitment of PPI to cell tips is essential to maintain cell polarity. Mutant cells in which Tea4/Wsh3 is unable to recruit Dis2 (PPI) because the docking site on Tea4/Wsh3 has been mutated were stained with green lectin and imaged alongside strains of an identical genetic background grown in identical conditions that have been stained with a red lectin. The mutant (green) cells are unable to maintain the linear cell extension characteristic of wild type (red) cells.



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Cells commonly respond to extracellular signals by modulating the activity of specific transcription factors and subsequently the expression of many target genes. Response to cytotoxic and genotoxic stress results in the mobilisation of a battery of protective and repair mechanisms or the induction of apoptosis. Failure to respond appropriately can result in cellular damage and thereby drive tumourigenesis.

The AP-1 transcription factor plays a key role in the response of cells to extracellular signals. In mammalian cells it is regulated by a plethora of physiological and pathological stimuli including mitogens, hormones, genotoxic agents, stress signals, viral infections and cytokines. Not surprisingly therefore, it has been linked to many cellular events including cell proliferation, differentiation and apoptosis. AP-1 is implicated in tumourigenesis and downstream targets include genes encoding cell cycle regulators.

The factor is complex since it consists of a mixture of dimeric complexes composed of members of the Jun, Fos, ATF and MAF protein families. Different dimeric combinations can be regulated by distinct signalling pathways. Of particular importance are the mitogen-activated protein kinase (MAPK) signalling pathways: the ERKs, JNKs and p38 kinases. They are activated by many of the stimuli that regulate AP-1 and directly phosphorylate members of the AP-1 complex.

Homologues of AP-1 family proteins are found in all eukaryotic organisms and their involvement in stress responses is highly conserved. In fission yeast the major transcriptional responses to stress conditions are coordinated

by the transcription factors Atf1 and Pap1, which are related to mammalian ATF and Jun proteins respectively. In addition, the activity of Atf1 is regulated by the Sty1 MAP kinase, a homologue of the mammalian p38 kinase. Thus fission yeast serves as a useful model for understanding the role and regulation of AP-1 proteins in mediating stress responses.

Functional characterisation of ATF2

ATF2 is a member of the AP-1 family and can bind to DNA either as a homodimer or as a heterodimer with other AP-1 family members, most prominently c-Jun. ATF2 is activated by the p38 or JNK kinases through phosphorylation of two N-terminal threonine residues, T69 and T71. To better understand the biological importance of ATF2 we have generated a number of genetically modified mice where ATF2 activity is compromised.

Characterisation of these mice has uncovered a number of essential developmental functions of ATF2. The ATF2 germ line knockout embryos develop to term but die shortly after birth due to respiratory distress. A similar phenotype is observed in a neuronal cell specific knockout of ATF2 and histological analysis of mutant embryos at neonatal and late embryonic stages revealed a series of defects in brain architecture, most prominently in the cerebellum and the hindbrain. In the latter region, specific somatic neuron nuclei are under-developed or absent in ATF2 mutants suggesting a role in inducing neuronal subtype identity.

Embryonic lethality was observed when the ATF2 knockout was combined with the knockout of its closest homologue, ATF7. The mutant embryos do not develop further than midgestation and are characterised by severe heart and liver defects. We found that ATF2/7 are important regulators of liver cell survival, both in the haematopoietic and the hepatocyte lineages (figure 1). *In vivo*, excessive liver cell apoptosis in the double mutants correlates with the hyperactivity of p38 and chemical p38 inhibitors slowed or reversed the apoptotic fate of the mutant cells. ATF2 functions in a negative feedback loop that controls the

magnitude and duration of p38 activation through transcriptional activation of several members of the DUSP family of MAPK phosphatases which, in the developing liver, have a crucial regulatory effect on p38. ATF2 also regulates the expression of DUSPs in mouse embryonic fibroblasts; disruption leads to loss of density dependent growth arrest and attenuated growth arrest upon serum withdrawal.

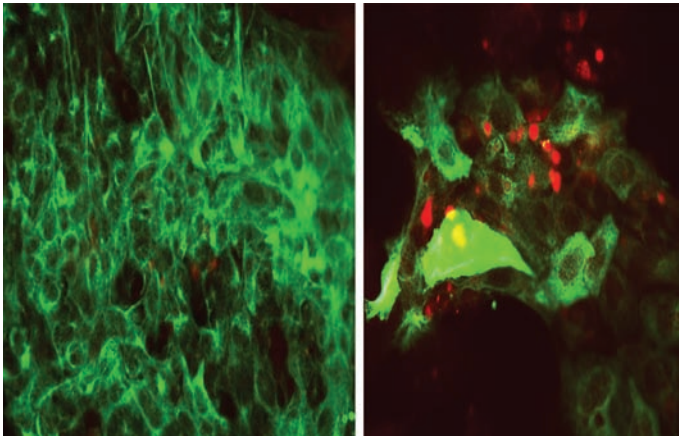


Figure 1
Apoptotic defects in ATF2/7 mutant embryonic hepatocytes. Cytokeratin 18 (green) staining shows that mutant cells (right panel) are proliferating poorly, and are highly apoptotic (activated caspase 3, red).

Characterisation of mouse models has also revealed a potential tumour suppressor function of ATF2. Mice with conditional knockout of ATF2 in T-cells are significantly more sensitive to the development of lymphomas following gamma-irradiation. In addition, collaborative studies with Ze'ev Ronai's group (Burnham Institute, San Diego) have shown that keratinocyte-specific knockout mice, when subjected to the classical DMBA/TPA skin tumourigenesis protocol, develop papillomas more rapidly and with an enhanced frequency than wild-type mice. These studies are continuing so as to define the mechanism of ATF2 function in tumour initiation, progression and in response to therapeutic intervention.

Stress response in fission yeast

We use fission yeast as a model system for studying stress responses since there appears to be remarkable conservation involving similar signalling pathways and the mobilisation of closely related transcription factors.

Both the StyI kinase and the Atf1 transcription factor are crucial for fission yeast to respond normally to a range of different stress conditions by orchestrating the expression of a common set of environmental stress response genes encoding numerous defence and repair proteins. In collaboration with Jürg Bählers group (Sanger Institute), the response to oxidative stress has been examined in greater detail. There is considerable complexity in the response with overlapping but different patterns of gene regulation depending upon the type and dose of oxidant. No single oxidant can be considered representative of oxidative stress as not only the gene expression signatures, but also cell cycle

arrest phenotypes and requirements for regulatory factors differ according to the stress applied. The transcriptional response to various oxidants is dependent upon the function of multiple factors: StyI, Atf1, Pap1, the Prr1 transcription factor and a newly identified zinc-finger protein Hsr1. The latter protein is induced specifically upon H₂O₂ stress and is required for full transcriptional induction of oxidative stress genes. We postulate that Hsr1 functions in a positively-acting and H₂O₂-specific feedback loop.

Atf1 exists as a heterodimer with another b-ZIP transcription factor, Pcr1. However, loss of Atf1 results in a more severe phenotype than the deletion of *pcr1*. An explanation has emerged from comparing microarray analysis of stress genes in *atf1Δ* and *pcr1Δ* mutants (figure 2). Some genes clearly require both factors for activation but a significant number of genes are still induced in an Atf1-dependent but Pcr1-independent manner. We suggest that in *pcr1Δ*, other Atf1-containing heterodimers can form and bind to at least some stress-regulated genes.

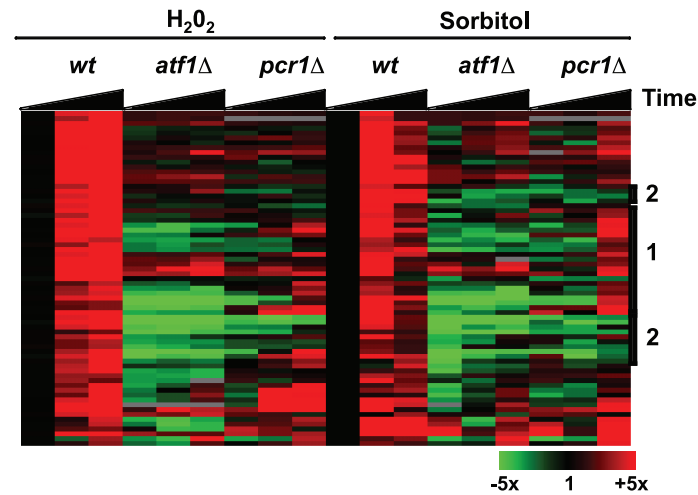
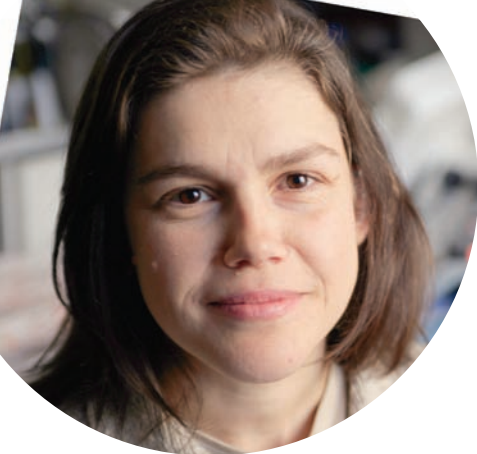


Figure 2
The expression of Atf1-dependent genes in *atf1Δ* and *pcr1Δ* mutants. Genes still induced in *pcr1Δ* are indicated (1) whereas those dependent upon both factors are also shown (2).

Atf1 is directly phosphorylated and regulated by the StyI MAP kinase. Surprisingly, phosphorylation is not required for the activation of Atf1 *per se* but instead regulates Atf1 stability. Interaction of the crucial E3 ligase with Atf1 is stress-sensitive and we propose that phosphorylation determines the extent to which Atf1 is able to interact with the ubiquitination machinery. How StyI regulates Atf1 function was analysed using ChIP assays which showed that StyI is directly targeted to the promoters of stress response genes in an Atf1-dependent manner. Furthermore, this targeting requires StyI kinase activity but is not dependent upon phosphorylation of Atf1. These findings suggest that phosphorylation of a StyI target other than Atf1 is necessary for optimal recruitment. We hypothesise that the presence of StyI at the promoter is crucial for gene activation.

Publications listed on page 61



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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 and 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 have emerged to directly implicate Rho proteins in tumour initiation and progression *in vivo*. Our group investigates how various oncoproteins, such as Ras and Src, target regulators of the Rho protein Rac. We are also identifying signalling events downstream of Rac that modulate tumour susceptibility and disease progression.

Rho proteins, such as Rac1, RhoA and Cdc42, are guanine nucleotide binding proteins that cycle between an inactive GDP-bound state and an active GTP-bound state. In the active state, Rho proteins bind and stimulate effector molecules that in turn govern cell morphology, adhesion, motility, as well as cell cycle progression and cell survival. Of relevance to cancer, Rho proteins are transforming *in vitro*

assays, particularly when expressed in combination with Ras effectors, and they are required for Ras-induced transformation. The activity of Rho proteins is controlled by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs activate small GTPases by promoting the exchange of GDP for GTP, whereas GAPs enhance the intrinsic rate of hydrolysis of bound GTP for GDP, leading to inactivation. Tiam1 (for T-lymphoma invasion and metastasis protein) belongs to the GEF family of proteins and selectively activates Rac in response to growth factors and cell-substrate interactions. Precisely how these upstream events engage the Tiam1-Rac signalling module is unclear. One possible mechanism is suggested by the observed association of Tiam1 with the second messenger Ras through a Ras-binding domain (RBD). Activated Ras and Tiam1 then cooperate to activate Rac (Lambert *et al.*, Nature Cell Biol 2002; 4: 621). Significantly, Tiam1-deficient cells are resistant to Ras-induced cellular transformation (Malliri *et al.*, Nature 2002; 417: 867), implying that this interaction is important for tumorigenesis.

Tiam1/Rac signalling and tumorigenesis *in vivo*

Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by application of a two-stage chemical carcinogenesis protocol and consequent oncogenic activation of the *c-Ha-Ras* gene (Malliri *et al.*, Nature 2002; 417: 867). Tiam1-deficient tumours were not only fewer but also smaller than wild-type tumours and this correlated with increased apoptosis and reduced proliferation in carcinogen-exposed skin of Tiam1-deficient mice. Tiam1 is also a potent modifier of intestinal tumorigenesis (Malliri *et al.*, J Biol Chem 2006; 281: 543). The majority of intestinal tumours are caused by mutations in the canonical Wnt signaling pathway, leading to aberrant expression of Wnt-responsive genes. *Tiam1* is a Wnt-responsive gene, and is expressed in the proliferative compartments (crypts) of the adult mammalian intestine where the Wnt pathway is normally active. It is also up-regulated in adenomas from patients with either sporadic colorectal polyps or familial adenomatous polyposis (FAP), as well as in adenomatous polyps in Min (multiple intestinal

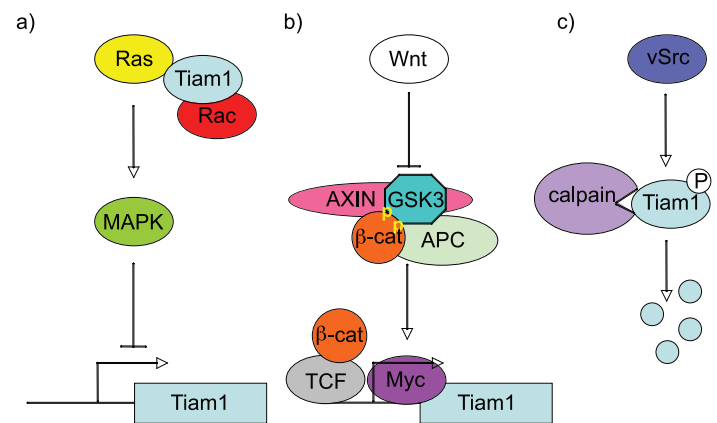
neoplasia) mice. In each instance, the Wnt pathway is hyperactivated due to a mutation in the Apc tumour suppressor. Further, by comparing tumour development in Min mice expressing or lacking Tiam I, it was found that Tiam I deficiency significantly reduces the formation as well as growth of polyps *in vivo* (Malliri *et al.*, J Biol Chem 2006; 281: 543).

These two studies on tumorigenesis *in vivo* demonstrate that two independent oncogenic signalling pathways of major clinical significance (Ras and Wnt) recruit the Tiam I-Rac signalling pathway by specific, albeit distinct mechanisms. In the context of oncogenesis, activation of this signaling module promotes tumour initiation and growth. Moreover, this role is specific to Tiam I since its loss cannot be compensated for by other Rho GEFs.

Tiam I/Rac signalling and malignant progression

The skin carcinogenesis model revealed an additional role for Tiam I in tumorigenesis. The few skin tumours arising in Tiam I-deficient mice progressed more frequently to malignancy than those in wild-type mice, suggesting that Tiam I deficiency promotes malignant conversion (Malliri *et al.*, Nature 2002; 417: 867). Analysis of Tiam I expression in skin tumours of wild-type mice revealed that benign papillomas maintained high levels of Tiam I expression, whereas expression was reduced in squamous cell carcinomas and was completely lost in highly invasive spindle cell carcinomas. Paradoxically, the increased Ras signalling associated with advanced skin malignancies (resulting from amplification of the mutated Ras allele) seems to be responsible for the reduction or loss of Tiam I expression in the later stages of tumour progression, as demonstrated *in vitro* for Ras-transformed MDCK cells (Zondag *et al.*, J Cell Biol 2000; 149: 775). Thus, while Tiam I/Rac co-operate with Ras in establishing tumours, they antagonize Ras during tumour invasion. Similarly in intestinal tumours, lack of Tiam I increased the invasiveness of malignant cells (Malliri *et al.*, J Biol Chem 2006; 281: 543).

One probable mechanism by which Tiam I and Rac suppress malignant progression is through their ability to stimulate cell-cell adhesion. *In vitro* studies have shown that over-expression of activated Rac or Tiam I can promote the formation of adherens junctions 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, using both RNA interference and cells derived from Tiam I-deficient mice, it has been shown that endogenous Tiam I 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 frequently hyperactivated in carcinomas, is well known for its ability to antagonize intercellular adhesion, resulting in increased cancer cell invasion and metastasis. Recently, we have found that Src's ability to convert epithelial cells to mesenchymal-like cells



Targeting of Tiam I by different oncogenic pathways. a) Ras interacts directly with Tiam I to activate Rac. This interaction is probably required for Ras initiation of tumours and promotion. However, increased levels of Ras, mediated through MAPK, results in suppression of Tiam I expression (see text for references). b) Wnt signals, through inhibiting the destruction complex comprising axin, Apc, and the serine/threonine kinase glycogen synthase kinase-3 β (GSK3 β) activate Tcf/ β -catenin transcription factors, which directly or indirectly through another Wnt-responsive gene, c-Myc, induce expression of Tiam I (see text for references and Sansom *et al.*, Nature 2007; 446: 676). c) Direct phosphorylation by Src primes Tiam I at cell-cell adhesions for degradation by calpain proteases.

(EMT) requires direct tyrosine phosphorylation of Tiam I. This occurs specifically at adherens junctions, priming that fraction of Tiam I responsible for mediating intercellular adhesion for degradation by calpain proteases. Abrogating Tiam I degradation suppresses Src-induced EMT. Interestingly, it also affects the regulation of cell-substrate adhesions by Src, suggesting a previously un-described cross-talk between cell-cell and cell-substrate adhesions. These data establish a new paradigm for regulating local concentrations of Rho-GEFs, as well as linking Tiam I-Rac signalling with a further oncoprotein.

It is increasingly apparent that Rho GEFs do more than simply activate Rho molecules; several studies now point to their role in influencing the choice of biological response elicited by a given Rho protein. GEFs have been shown to bind to effectors directly or to scaffold proteins that complex with components of effector pathways. Tiam I contributes to the signalling specificity downstream of Rac via associating with IB2/JIP2, a scaffold that promotes Rac activation of p38 kinase cascade over JNK MAP kinase cascade (Buchsbbaum *et al.*, Mol Cell Biol 2002; 22: 4073). Tiam I can also influence Rac signalling specificity through its interaction with spinophilin, a scaffold that binds to p70 S6K, another kinase regulated by Rac. Spinophilin binding suppresses the ability of Tiam I to activate Pak I, a different Rac effector (Buchsbbaum *et al.*, J Biol Chem 2003; 278: 18833). In our lab, we are using biochemical approaches to identify Rac and Rac GEF interacting proteins involved in different aspects of transformation including malignant progression (acquisition of invasiveness).



Clinical and Experimental Pharmacology Group

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CEP incorporates pre-clinical drug target validation, drug combination studies, hypoxia impact on drug efficacy and biomarker discovery. Within the PACCAR Good Clinical Laboratory Practice (GCLP) laboratory, CEP also validates and implements pharmacokinetic (PK) and pharmacodynamic (PD) assays for early clinical trials. We focus on novel agents targeted to apoptosis and angiogenesis. Technology development initiatives include preclinical model generation with inducible expression of drug targets, serum proteomics, enumeration of circulating tumour cells, Q-Dots to enhance immunohistochemistry and multiplex biomarker assays. Disease orientated translational research focus is placed on lung, GI and paediatric cancers.

Clinical trials at the Derek Crowther Unit (DCU).

In 2006/07 DCU supported >100 trials and 10,000 patient visits. It contains a dedicated clinical sample processing and

storage laboratory with close onsite access to CEP biomarker research laboratories. Plans are being pursued to expand DCU, making it one of the largest Trials Units in the world. One example of CEP/DCU collaboration is the CR-UK Phase I trial of Aegera Therapeutics' AEG35156 (antisense XIAP). An initial 7 day infusion study was extended to include a 3 day infusion protocol and both closed in 2007. Data from both studies are currently being compiled and reported. Results from these studies were sufficiently encouraging to prompt a 2h infusion protocol that is ongoing.

Training the next generation of early clinical trialists

Training of translational scientists and academic pharmacologists (a national deficit) is achieved through our Clinical Pharmacology Fellowship Scheme that is funded equally by CR-UK and AstraZeneca leading to a PhD in Clinical and Experimental Pharmacology. With three fellows in post (and one starting in early 2008), their projects are biomarker-based involving collaborations with clinical colleagues within the MCRC. Fellows undertake their laboratory-based research in CEP, elsewhere within MCRC and at AstraZeneca and attend one clinic per week in DCU.

Challenges of the GCLP environment in academia being met in CEP

Scientists conducting biomarker studies on clinical trials must comply with European Directive (2001/20/EC). This year CEP placed emphasis on patient sample tracking and biomarker method validation, followed by implementation of procedures for data handling, archiving and reporting. New developments included switching to templates to aid construction of analytical plans, final study report writing and pro-forma laboratory notebooks custom designed for individual trials or assays. We are pursuing a 'fast-track', yet scientifically rigorous, approach to method validation. There is also ongoing development of electronic resources for sample

tracking and data analysis. To comply with FDA regulations on electronic records, we have integrated electronic signatures, user logs and access control into our system. Our versatile QA system responds to new developments in translational academic research such as high throughput analysis, while always striving towards increased compliance. The CEP GCLP laboratory was audited by CR-UK and the HTA in 2007, no critical findings were revealed.

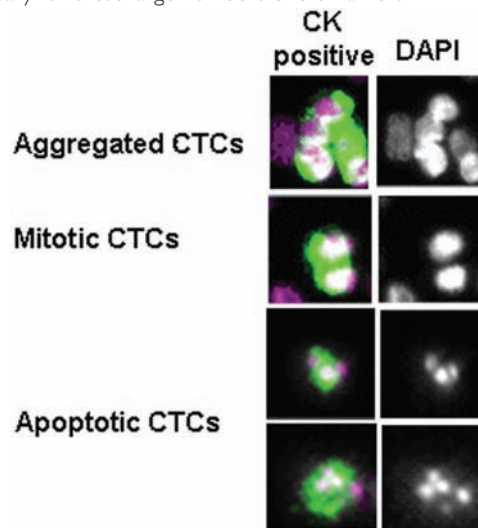
Serological biomarkers of cell death, an example of biomarker research in CEP.

The availability of tumour biopsies pre- and serially post-drug treatment is often unpredictable, particularly in early clinical trials. CEP therefore has placed considerable effort investigating the utility of potential PD biomarkers in readily available clinical samples such as whole blood, serum and plasma. As the desired endpoint of many drug treatments is tumour cell death, we especially focussed on serological biomarkers of apoptosis and necrosis. A large number of clinical studies on conventional and novel cancer therapeutics are underway evaluating the M65™ and M30 apoptosome™ ELISA assays that detect full length and caspase cleaved cytokeratin 18 (CK18) respectively and are proposed as serological biomarkers of cell death. The study in Small Cell Lung Cancer (SCLC) described below serves as a representative example of our approach. SCLC is initially chemosensitive, but relapse with resistant disease is common. Baseline plasma samples from patients with SCLC and healthy controls were analysed and serial samples were taken from drug responsive patients during the first cycle of platinum based chemotherapy. There was considerable heterogeneity in CK18 levels between patients. The assay was initially shown to be non-linear outside a discrete dynamic range. Dilution in pig plasma rectified this problem. Baseline M30 levels were similar for SCLC patients and controls, M65 baseline levels were higher in patients. These studies demonstrated these assays fit for purpose for clinical trials. Ongoing studies will evaluate the prognostic significance of M65. M30 and M65 signals rose during the first 8 days of treatment then fell to below baseline. The gradual increase in M30 post treatment suggests an apoptotic response to therapy. High pre-treatment levels of M65 may be explained by rapid tumour proliferation and associated necrosis. Correlation of M30/M65 levels with duration of response and overall survival is ongoing to determine their utility as surrogate biomarkers of relevant clinical endpoints for new drug development.

Proof of concept PD biomarkers of angiogenesis

Assays that inform on tumour neo-vasculature are essential for development of novel drugs targeting angiogenesis. Non-invasive imaging modalities are often used in clinical trials to assess vascular permeability and where serial biopsies are available, immunohistochemistry detects endothelial cells (CD31, CD34, CD105). A plethora of molecules measured in serum have been recently reported to have potential clinical utility as PD biomarkers of angiogenesis. Two classes of molecules were studied; angiogenic factors and molecules released from neo-vasculature during angiogenesis or after therapeutic intervention. We are developing biomarker

panels whose signatures have potential as surrogate clinical endpoints in evaluation of anti-angiogenesis therapy, considered alongside imaging data. The assays are 'fast track' validated as singleplex ELISAs. We are currently exploring the validation of multiplex technology to reduce blood volumes necessary for these large numbers of biomarkers.



Representative images of Circulating Tumour Cells (CTCs) from an SCLC patient. Cells are stained for Cytokeratin (CK) and do not stain for CD45 (a marker of white blood cells). DAPI stained nuclear morphology allows an assessment of mitosis and apoptosis, and clusters of CTCs can also be identified.

Circulating tumour cells (CTCs)

With CR-UK funding for the 'Immunicon Cell Search System' we are enumerating and evaluating CTCs. In SCLC, we are asking whether CTC numbers predict disease relapse and whether, if sufficiently pure CTC populations can be isolated, their biology can inform on optimal drug treatment strategies. With the approaching clinical evaluation of Bcl-2 inhibitors at DCU, we are examining levels of molecular determinants of drug resistance to such agents in CTCs. So far our data show that CTCs are detectable in the majority of patients with SCLC and in greater numbers than reported for other cancer types. These early data demonstrate a prognostic role for CTC number and the feasibility of utilising these biomarkers in clinical trials of novel agents for this disease (see figure). This technology will also be used to enumerate circulating endothelial cells (CECs) on trials of angiogenesis-targeted drugs.

Key MCRC Collaborators

Medical Oncologists: Fiona Blackhall (lung cancer), Gordon Jayson (angiogenesis, gynaecological tumours), Guy Makin (paediatric oncology) and John Radford (haematological cancer). Ian Stratford, Kaye Williams (Experimental Chemo/Radiotherapy), Richard Byers (Molecular Pathologist), Andrew Hughes (Translational Oncology), Tim Illidge (Targeted Radioimmunotherapy), Crispin Miller (Bioinformatics), Andrew Renehan (GI Academic Surgeon) and Tony Whetton (Clinical Proteomics).

Publications listed on page 61



Immunology Group

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Mariam Al-Muftah (with
David Gilham)
Andrzej Rutkowski (with
Crispin Miller)

The continuing theme of Immunology has been the investigation of shared properties of developmental tissues and cancer cells with a view to identifying new targets for diagnosis, prognosis or therapy. This focus and application at the translational interface has enabled our ideas to transfer successfully from the bench to the clinic. In the past year, we have further characterised immune responses to 5T4 oncofoetal antigen facilitating the evaluation of ongoing trials of both TroVax® (MVA based 5T4 vaccine), Anyara® (5T4 antibody based superantigen therapy) and other clinical studies. We have also studied the biological functions of the 5T4 molecules exploiting ES cells.

Human 5T4 CD4 T cell responses

The human 5T4 (h5T4) oncofoetal antigen is expressed by a wide variety of human carcinomas including colorectal, ovarian, gastric and renal, but rarely on normal tissues. Its restricted expression on tumour tissues as well as its association with tumour progression and poor prognosis has driven the development of TroVax® which has been tested in several early phase clinical trials and these studies have led to the start of a phase III trial in renal cell carcinoma patients. We have previously shown that CD8⁺ T cells recognising

h5T4 can be generated in the absence of CD4⁺ T cells from peripheral blood lymphocytes of human healthy individuals. We have now demonstrated the existence and expansion of human CD4⁺ T cells against h5T4 by stimulation with autologous monocyte-derived dendritic cells infected with a replication defective adenovirus encoding the h5T4 cDNA (Adh5T4). The h5T4-specific T-cell responses in normal individuals are enhanced by initial depletion of CD25⁺ cells (potential T regulatory cells) prior to the *in vitro* stimulation. We have identified a novel h5T4-derived 15-mer peptide recognised by CD4⁺ T cells in HLA-DR4 positive healthy individuals. CD4⁺ T cells spontaneously recognising a different 5T4 epitope restricted by HLA-DR were also identified in tumour-infiltrating lymphocytes (TIL) from a regressing renal cell carcinoma (RCC) lung metastasis (see figure). Such h5T4-specific CD4⁺ T cells boosted or induced by vaccination could act to modulate both cell or antibody mediated anti-tumour responses (Elkord *et al.*, 2007). Importantly, these cells secreted both interferon (IFN)- γ and IL-10 which could indicate a T cell regulatory phenotype. Approaches to modulating T regulatory activity and studying 5T4 immunity in patients in clinical trials are reported in the Biological, Immune and Gene Therapy (BIGT) report.

5T4 Knockout (KO) mouse immunological studies

5T4 KO mice in the C57BL/6 background are viable but exhibit a high frequency of hydrocephaly. The 5T4 KO mice show no obvious changes in T cell, B cell and/or monocyte populations. When WT and KO mice were challenged with B16m5T4 tumour cells, both developed tumours at a similar rate. However, the KO mice were able to survive significantly longer in both protection and active therapy models when immunised with Adm5T4 a week before or after challenge respectively with B16m5T4 cells. We have shown that the KO mice generate strong m5T4 specific CTL and antibody responses compared to WT mice. We are particularly interested in whether the repertoire and affinity of m5T4 specific T cells may be regulated in some way by T regulatory

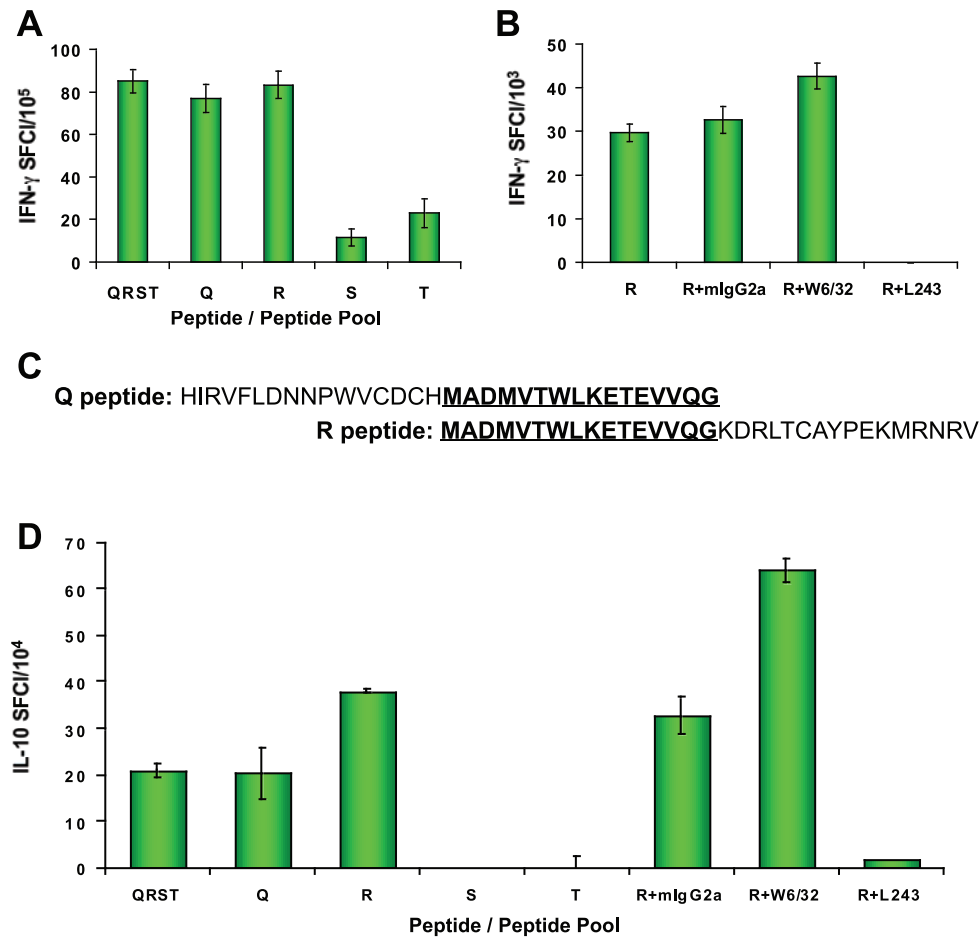
cells (natural and/or induced). Recent studies have demonstrated that the expression of self-antigen in the thymus is essential for the induction and maintenance of self-tolerance due to mechanisms of negative deletion of the antigen specific autoreactive T cells and the differentiation of FOXP3 regulatory T cells through the antigen expression and presentation by Aire (Autoimmune regulator gene) positive medullary thymic epithelial cells. We have shown that 5T4 is expressed in murine thymus and thus might influence the repertoire and/or induction of specific T regulatory cells leading to control of natural or vaccine induced immunity. Interestingly, adoptive transfer of spleen cells from Adm5T4 immunised KO mice to KO mice but not WT mice was able to prevent tumour growth. We are studying the mechanisms by which endogenous expression of 5T4 influences T cell immunity and tolerance, such as the generation of T effector and T regulatory cells *in vivo* and how the T cell repertoire/function balance is affected by vaccine strategies and their consequence for autoimmunity.

5T4 expression in embryonic stem (ES) cells: relationship to epithelial mesenchymal transition (EMT).

EMT occurs during embryonic development and is important for the metastatic spread of epithelial tumours. During EMT, E-cadherin is down-regulated and this correlates with increased motility and invasion of cells. In collaboration with Chris Ward's group at the University of Manchester we have shown that early differentiation of human ES cells is an EMT like event (Eastham *et al.*, 2007) as established for murine ES cells (Spencer *et al.*, 2007). We have shown that differentiation of human ES cells in monolayer culture is associated with an E- to N-cadherin switch, increased vimentin expression, upregulation of E-cadherin repressor molecules (Snail and Slug proteins) and increased gelatinase (MMP-2 and MMP-9) activity and motility, all characteristic EMT events. The 5T4 oncofoetal antigen, previously shown to be associated with early human ES cell differentiation (Ward *et al.*, Exp Cell Res 2006;

312: 1713), is also shown to be part of this process. Abrogation of E-cadherin mediated cell-cell contact in undifferentiated ES cells using a neutralising antibody (nAb) resulted in increased motility, altered actin cytoskeleton arrangement and a mesenchymal phenotype which was concomitant with presentation of the 5T4 antigen at the cell surface. nAb-treated hES cells remained in an undifferentiated state, as assessed by OCT-4 protein analysis, and loss of E-cadherin alone was insufficient to induce EMT-associated transcript expression. Removal of nAb from hES cells resulted in restoration of cell-cell contact, absence of cell surface 5T4, a decreased mesenchymal cellular morphology and motility and enabled differentiation of the cells to the three germ layers upon their removal from the fibroblast feeder layer. We conclude that E-cadherin functions in hES cells to stabilise cortical actin cytoskeletal arrangement and this prevents cell surface localisation of the 5T4 antigen. We are exploiting 5T4 KO ES and embryo fibroblast cells to further investigate the function of 5T4 molecules in cancer and development.

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IFN- γ ELISPOT response to 5T4 peptides of TILs from a regressing RCC lung metastasis (A) Epitope mapped to Q or R 32-mer peptides; (B) Blocking by anti-HLA-DR (L243) but not anti-HLA-ABC (W6/32) or control mIgG2a; (C) Q and R peptides overlap by 16 amino acids; (D) TILs also secrete IL-10 (HLA-DR restricted).



Paterson Institute for Cancer Research

Inositide Laboratory (Since October 2007)

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Phosphoinositides are a family of lipid second messengers interlinked by the activities of an extensive and highly regulated network of kinases and phosphatases that modulate phosphoinositide levels in response to environmental changes. Alterations in phosphoinositide levels can regulate many different cancer-relevant cellular pathways including survival, proliferation, migration, cell substratum interactions and transcription. $\text{PtdIns}(4,5)\text{P}_2$ is at the heart of phosphoinositide signalling, being the substrate for both phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PIC) (see figure).

The PI3K /PTEN pathway is often deregulated in tumours promoting cell survival and proliferation through the activation of PKB and flux through the PIC pathway is often up-regulated in human tumours. Furthermore $\text{PtdIns}(4,5)\text{P}_2$ is itself a regulator of cytoskeletal dynamics and cell survival pathways. Loss of $\text{PtdIns}(4,5)\text{P}_2$ signalling may constitute a tumour suppressive mechanism as attenuation of matrix/integrin interactions, which occurs during processes such as metastasis, leads to a depletion of $\text{PtdIns}(4,5)\text{P}_2$ levels, loss of growth factor signalling and the induction of anoikis/apoptosis (cell death).

In October 2007 our laboratory moved from the Netherlands Cancer Institute, a multidisciplinary research and

hospital facility in Amsterdam, to a newly refurbished laboratory at the Paterson Institute for Cancer Research. The laboratory is now functional and contains state of the art equipment for cell biology - HPLC, FPLC and surface Plasmon resonance analysis - aimed at studying various aspects of phosphoinositide signalling.

Understanding how $\text{PtdIns}(4,5)\text{P}_2$ levels are regulated: PIP5Ks and PIP4Ks.

$\text{PtdIns}(4,5)\text{P}_2$ is present in the plasma membrane where its levels can be regulated in response to receptor activation and is also synthesised within the nucleus where its levels can be regulated distinctly from the plasma membrane pool. Understanding how these two pools of lipids are maintained and which cellular pathways they regulate represents a major goal of the laboratory. $\text{PtdIns}(4,5)\text{P}_2$ can be synthesised by the action of two distinct but related kinases (collectively termed PIP kinases). PIP5K phosphorylates $\text{PtdIns}4\text{P}$ on the 5' position, while PIP4K phosphorylates $\text{PtdIns}5\text{P}$ on the 4' position. It is likely that PIP5Ks are the major producers of $\text{PtdIns}(4,5)\text{P}_2$ while the role of PIP4K may be to regulate a distinct minor pool of $\text{PtdIns}(4,5)\text{P}_2$ and/or the levels of $\text{PtdIns}5\text{P}$. Of direct relevance to cancer research is the observation that in nearly all cancer cell lines tested one of the isoforms of PIP5K is up-regulated. Furthermore forced up-regulation of PIP5K can induce dramatic changes in cell morphology, increase migratory capacity and attenuate apoptosis in response to cellular stressors. PIP4Ks on the other hand appear to regulate the levels of nuclear $\text{PtdIns}5\text{P}$ which can impinge on the activity of the tumour suppressor protein p53 and a number of other transcriptional regulators that are implicated in the development of cancer.

One approach to understanding how PIP kinases are regulated is to elucidate how they may be post-translationally modified. Post translational modifications such as phosphorylation, acetylation, methylation or ubiquitination can induce changes in enzymatic activity, localisation, interaction partners and stability. Using mass spectrometry we have

identified 12 different sites of phosphorylation on PIP5K and 3 sites of phosphorylation and 8 sites of acetylation on PIP4Kbeta. We have generated modification-specific antibodies and are using these to elucidate how and when the sites of modification are regulated and what the impact of modification is on the subcellular localisation and activity of the enzymes.

PIP5Ks and their role in regulating cytoskeletal dynamics.

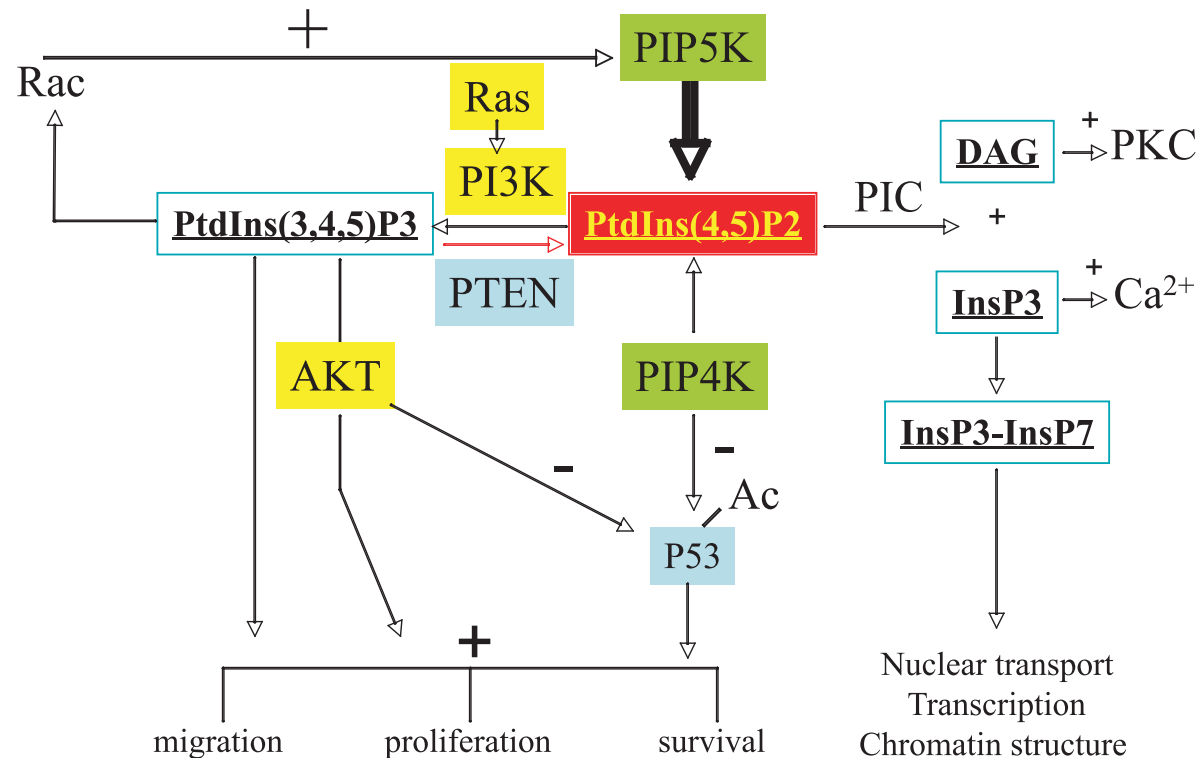
The ability to migrate from their natural environment and to invade other tissues is a hallmark of metastatic cancer cells and its onset often defines a poor prognosis for the patient. A key regulator of the migratory capacity of cells is the small molecular weight G protein Rac. Rac is also a potent regulator of PIP5K and PtdIns(4,5)P₂ levels. We have recently defined specific regions of PIP5K-alpha that interact with Rac and have established a mutant of PIP5K that no longer interacts with Rac (PIP5K-RacM). Interestingly this mutant no longer localizes to the membrane and is compromised in its ability to both synthesise phosphoinositides *in vivo* and to regulate focal adhesion dissolution required during cell contraction and migration. Using this mutant we aim to define the role of Rac in the regulation of PIP5K activity both

in vitro and *in vivo* and on the process of tumourigenesis. We hope that modulation of the interaction between PIP5K may yield a novel target for cancer therapy.

PIP5K and the development of small molecular weight inhibitors.

Our and others previous research has gone some way to identifying PIP5K as a potential drug target for therapies against cancer. In collaboration with Cancer Research Technology (CRT) we have screened and identified small molecular weight compounds that are able to inhibit PIP5K activity. Chemical modification and structure function studies have identified important chemical moieties on these small molecules which have led to the development of more potent PIP5K inhibitors. Future experiments will concentrate on identifying novel pathways regulated by PIP5Ks and whether these inhibitors can be used either by themselves or in combination with conventional therapies to combat tumour cell growth.

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A PtdIns(4,5)P₂-centric view of oncogenes and tumour suppressors. PtdIns(4,5)P₂ can be synthesized by two distinct PIP kinases (green blocks) and can be used by both the PI-3-kinase (PI3K) and phospholipase C (PIC) signaling pathways. PtdIns(3,4,5)P₃ regulates the activation of the oncogene AKT as well as other pathways which culminate in inhibiting cell death and activating proliferation and migration. PIC-mediated cleavage of PtdIns(4,5)P₂ leads to the generation of Diacylglycerol (DAG) which regulates PKC activity and InsP3 which can modulate intracellular calcium levels. InsP3 can be further phosphorylated to generate numerous higher phosphorylated inositols (InsP3-InsP7) some of which have been shown to have a role in regulating nuclear transport, transcription and the structure of chromatin. PIP4K regulates the levels of PtdIns5P which in turn regulates the acetylation and activity of the human tumour suppressor p53. Yellow boxes highlight known oncogenes, while the light blue boxes denote tumour suppressors.



Paterson Institute for Cancer Research

Leukaemia Biology Group (Since September 2007)

<http://www.paterson.man.ac.uk/leukaemia>

Group Leader **Tim Somervaille**

Human malignancies are extremely heterogeneous in their cellular origins and compositions, but are generally considered to consist of at least two functionally distinct cell types. So-called cancer stem cells comprise the self-renewing fraction and possess extensive proliferative potential, with the capacity to initiate, sustain and expand the disease. Conversely, their derivative non-self-renewing progeny cells make up the bulk of the tumour and account for much of the disease symptomatology. In order for malignancies to be cured, it may be necessary and sufficient to exclusively eliminate the cancer stem cells.

Cancer stem cells were first formally described in acute myeloid leukaemia (AML), thanks to the pioneering work of John Dick and others in the 1990s. A minority of cells within the malignant clone, at the apex of a cellular hierarchy, are leukaemia stem cells (LSCs) and possess the capacity to self-renew extensively. By contrast, most cells are downstream non-self-renewing progeny that make up the bulk of the disease but do not contribute to its sustenance (figure 1). Thus, human Acute Myeloid Leukaemia (AML) can be considered as a caricature of normal haematopoiesis. Within the hierarchy LSCs are the critical cellular component that must be eliminated in order to cure the patient because they are specifically responsible for disease relapse following

treatment failure. There is consequently considerable current interest in identifying the genes and pathways that differentially regulate LSCs, both by comparison with non-self-renewing downstream progeny cells within the malignant clone but also by comparison with normal haematopoietic stem cells (HSCs) (figure 2).

There are approximately 2,500 new cases of AML each year in the United Kingdom, but despite significant advances in knowledge regarding its molecular genetics, the mechanisms that specifically define and maintain malignant cells within the self-renewing leukaemia stem cell (LSC) sub-fraction of the disease remain poorly understood. Although there have been some recent advances in therapy, the mean five-year survival for patients with the disease is 20-25%, rising to 40-50% in patients under 50 years of age. Scientific approaches which define and evaluate the properties of the LSC in AML represent one important strategy for identifying novel therapeutic targets in the disease. Some progress has been made: functional roles for constitutive activation of specific molecules such as nuclear factor κ B or phosphatidylinositol-3-kinase in LSCs have been suggested, as has aberrant accumulation of nuclear beta-catenin in myeloid blast transformation of chronic myeloid leukaemia. However, it is unlikely that these various factors contribute to all cases of human AML, which is highly heterogeneous in its molecular pathology.

One gene which is frequently mutated in human AML is the *Mixed Lineage Leukaemia (MLL)* gene. Working in Mike Cleary's laboratory at Stanford University in the United States, I utilised a mouse model of MLL fusion oncogene AML, which faithfully recapitulates many of the pathological features of the human disease, to further understand the mechanisms by which LSCs undergo self-renewal. The work has resulted in a number of important observations. First, the frequency of LSCs in these experimentally-induced myeloid leukaemias varied significantly according to the MLL

oncogenic fusion protein used to initiate the disease. Thus, even where leukaemia is initiated by similar oncogenes, the cellular architecture of the resulting leukaemia, including the frequency of the self-renewing LSC, can be quite different. Furthermore, in the case of MLL oncogenic fusion proteins, a critical function of the MLL fusion partner protein is to specify the probability of self-renewal versus differentiation at the level of the LSC.

A related observation is that within an individual murine MLL fusion oncogene leukaemia, LSCs are highly enriched among cells with a c-kit^{hi} immunophenotype, and rare among cells with a c-kit^{lo} immunophenotype. These findings enabled us, using microarray data from prospectively sorted populations of AML cells, to generate a transcriptional profile that selectively and significantly correlates with the LSC phenotype, and which is highly informative in describing the biology of the LSC in this model. The profile suggests that the LSC is a metabolically active, proliferating myeloid cell occupying a position that is downstream of normal stem and progenitor cells in the haematopoietic hierarchy. This is in marked contrast to the prevailing paradigm for AML stem cells, which are considered to be quiescent with an immunophenotype most similar to normal haematopoietic stem cells.

A further surprising finding from this work is that MLL LSCs can differentiate into non-self-renewing progeny despite continued high-level expression of a Hox/Meis1 subprogram that is directly subordinate to the initiating MLL oncoprotein, and which is essential for leukaemia initiation. Thus, the genetic programs responsible for LSC maintenance may not necessarily be shared with HSCs or exclusively correspond to the initiating oncogenetic program.

Now that I have moved to the Paterson Institute to start my own research group, I plan to further define and investigate the LSC compartment in both human and experimentally-induced murine haematological malignancies, with the longer term goal of identifying novel therapeutic targets for human disease.

Publications listed on page 63

MALIGNANT LEUKAEMIA CLONE

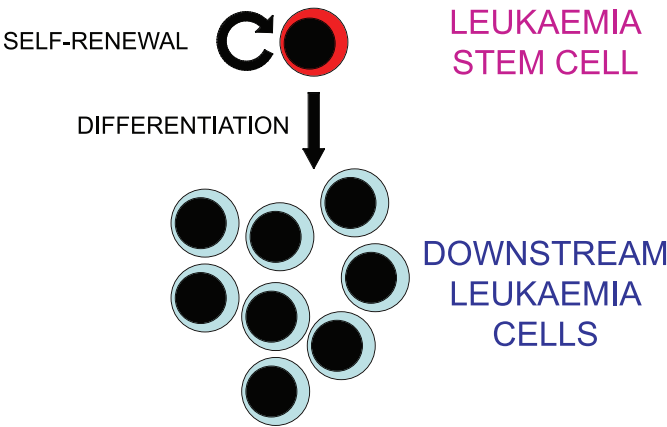


Figure 1 Schematic representation of leukaemic haematopoiesis

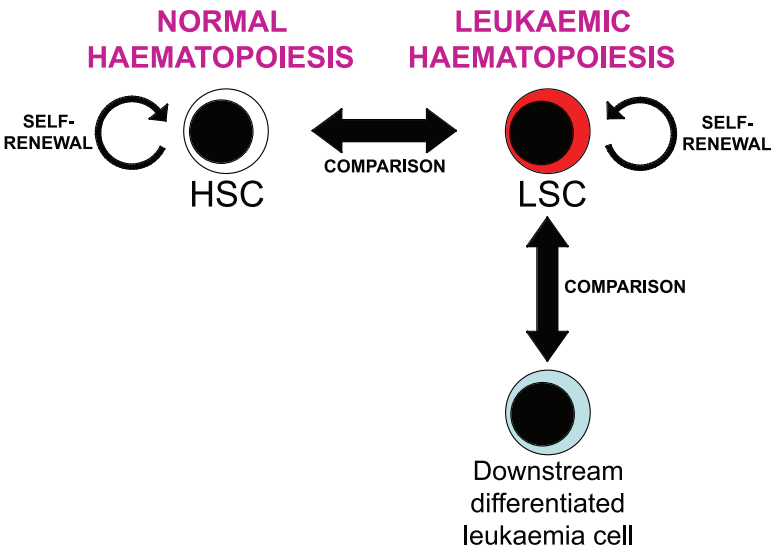


Figure 2 Comparing the LSC with other cell populations



Paterson Institute for Cancer Research

Radiochemical Targeting and Imaging Group

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Nigel Smith

Over the last year, the research in the RTI group has concentrated on the development of radio-quantum dots for multi-modality imaging, allowing both radionuclide and fluorescent signals to be detected using the same probe. The radio-quantum dot $^{109}\text{Cd}^{72}\text{Se}(\text{ZnS})$ probe, capable of detecting a combined fluorescence and radionuclide signal, was developed and evaluated in *in vitro* and *in vivo* studies. Last year, we reported on the bio-distribution and autoradiography profile of intravenously administered probe.

Significant accumulation in the liver and spleen was demonstrated over the first 2 hours, followed by metabolism and significant clearance over 24 hours. Over the last year, we demonstrated biological targeting of the radio-quantum dot when conjugated to annexin V (AV) and evaluated the conjugated protein binding to phosphatidylserine (PS). Binding of quantum dot-conjugated AV to PS was retained indicating that the probe has not significantly altered the biological activity of the AV/PS interaction (figure 1). We have also addressed the toxicity issue associated with conventional fluorescence-only quantum dots. To this end, we investigated the synthesis of no-carrier added tracer level preparations and evaluated their toxicity profile in various cell lines, as well as their ability to retain fluorescence emissions.

We have established a model system, based on $^{109}\text{Cd}^{72}\text{Se}$, for the development and validation of the radio-quantum dot approach to multi-modality imaging. We have synthesised radiotracer quantum dots using two radionuclides, ^{109}Cd and ^{72}Se , produced at the Clatterbridge Cyclotron. Such probes emit both gamma-ray and fluorescence signal, enabling thereby simultaneous detection of both radioactivity and optical emissions. Such probes were found to be non-toxic at tracer concentrations in various cell lines (figure 2) and that the toxicity is directly related to the concentration of the probe. Radiotracer level synthesis showed no growth inhibition of various tumour cells lines compared to carrier added preparations where cell viability was not retained due to the chemical toxicity of the probe (figure 2).

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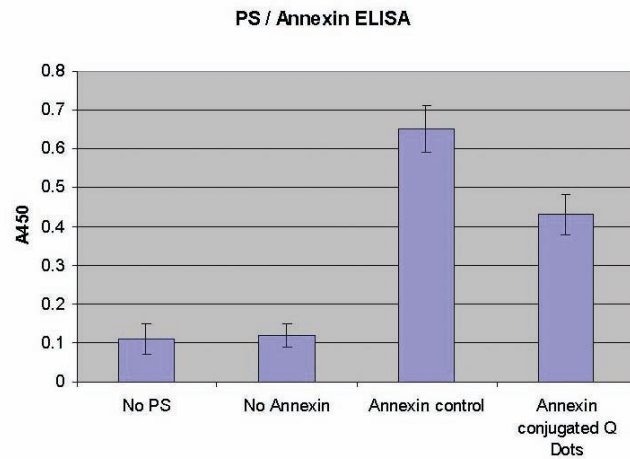


Figure 1. Binding of radio-quantum dot conjugated annexin V to its target Phosphatidylserine using A Radio-ELISA assay.

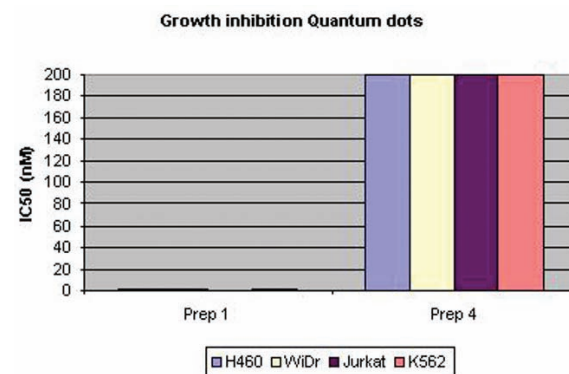
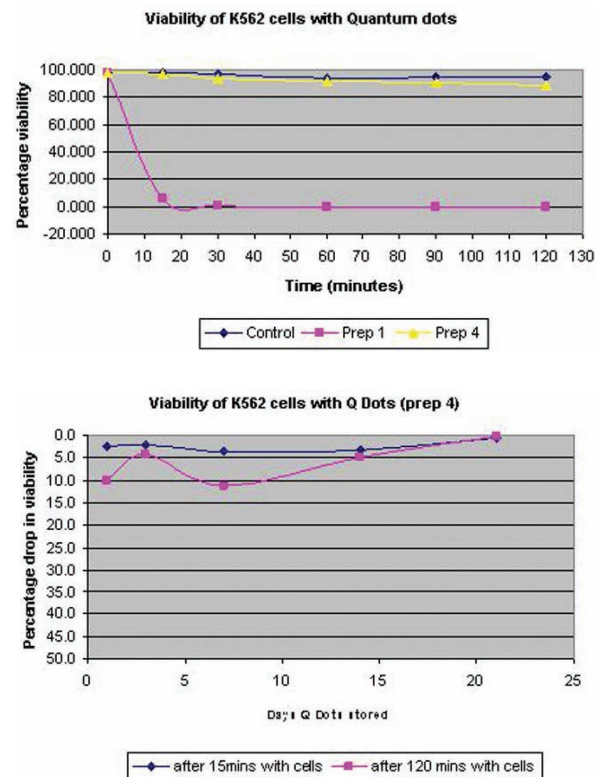


Figure 2. Toxicity, cell viability and stability of water soluble quantum dots in tumour cell lines (A and B). IC₅₀ growth inhibition of radiotracer quantum dots in different cell lines (C).



Stem Cell Biology Group

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Our group studies the molecular and cellular mechanisms of haematopoietic development using both *in vitro* differentiation systems based on mouse embryonic stem (ES) cells and *in vivo* mouse models. Our research program focuses on two genes implicated in the development of leukemia, the transcription factor Runx1/AML1 and the transcriptional co-activator MOZ. Our goals are to further define the role of Runx1 and MOZ in early haematopoietic development to better understand how alterations of their function lead to leukaemogenesis.

Early haematopoietic development: haemangioblast and haemogenic endothelium

The earliest site of blood cells development in the mouse embryo is the yolk sac where blood islands, consisting of primitive erythroid cells surrounded by a layer of angioblasts, develop at approximately day 7.5 of gestation. The parallel development of these 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 instead 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 and haematopoietic. The BL-CFC that

generates these colonies, represent a transient population that appears in the embryoid bodies (EBs) prior to the emergence of any other haematopoietic lineage precursors. The characteristics of the BL-CFC suggest that it represents the *in vitro* equivalent of the haemangioblast and as such the earliest stage of haematopoietic development described to date. Although recent studies have now provided evidence for the presence of this bipotential precursor *in vivo*, the precise mechanism of generation of haematopoietic cells from the haemangioblast still remains completely unknown.

During 2007, we performed a series of studies to determine the cellular and molecular events leading to the generation of blast colony from BL-CFC. Our data demonstrate 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 generation of haematopoietic cells from this haemogenic endothelium. These results demonstrate that the two *a priori* conflicting theories on the origin of haematopoietic development, haemangioblast and haemogenic endothelium, can be merged into a single linear developmental process leading to the formation of the first committed haematopoietic precursors.

Transcriptional targets of Runx1/AML1

The AML1/Runx1 transcription factor is a frequent target of gene rearrangements and mutations in human acute myelogenous leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its initial implication in leukaemias, Runx1 has been shown to be critical for normal haematopoietic development. Our previous analysis of early EBs revealed a profound defect in the potential of the Runx1^{-/-} ES cells to generate blast colonies and therefore indicated that Runx1 is a master regulator of blood

development. Runx1 is therefore likely to regulate the expression of an important set of genes at this stage of development.

To identify these genes, we compared the patterns of gene expression of haemangioblast-enriched cell populations or haemangioblast-derived cell populations from either *Runx1* deficient or *Runx1* competent ES cells. We validated the differential expression of candidates on samples generated from the ES/EB system and further documented the regulation by Runx1 of the transcription of several of these genes by promoter assays or chromatin immunoprecipitation. We are currently evaluating the specific function of some of these genes at the onset of haematopoietic development and testing their potential to rescue haematopoietic development in absence of Runx1. Selecting some previously uncharacterized transcriptional target genes of Runx1, we have initiated a series of experiments, such as conditional knock-out and knock-in, to determine the pattern of expression and function of these new genes.

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. One intriguing hypothesis is that the different isoforms of this transcription factor could fulfill distinct functions at the different stages of the establishment of the haematopoietic system.

To address this question, we have generated ES cells containing a reporter gene knock-in in the different isoforms and produced knock-outs altering the specific expression of these isoforms. We have now demonstrated that the expression of these isoforms is differentially regulated during early haematopoietic development both *in vitro* and *in vivo* and that their expression defines specific stages of haematopoietic development. We are currently investigating the biological potential of cells expressing the respective isoforms and the function of each isoform.

Critical requirement for the HAT activity of MOZ in self-renewal of haematopoietic progenitors.
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*. This mutation renders the protein catalytically dead whereas the rest of the multi-domain protein remains intact. 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. Furthermore many fewer foetal liver haematopoietic precursors are detected in the mutant animals. These *in vivo* results were confirmed with ES cells mutated for the HAT activity of *MOZ* as again less haematopoietic precursors are generated with the mutated ES cells. Further analysis demonstrated that homozygous haematopoietic precursors are unable to steadily expand *in vitro*. Altogether these results demonstrate the critical role of *MOZ* driven acetylation in the balance between proliferation and differentiation during haematopoiesis. We are currently investigating the precise molecular and cellular mechanisms affected in absence of the HAT activity of *MOZ*.

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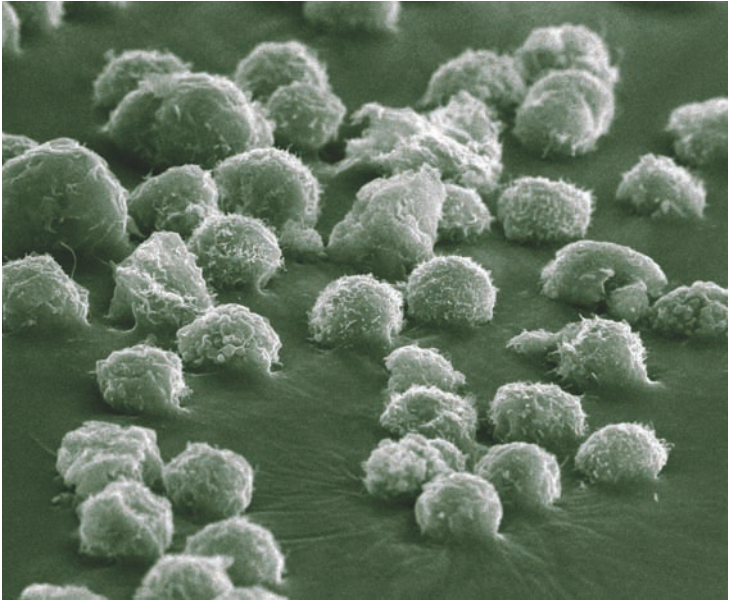


Figure 1: Haematopoietic cells generated upon *in vitro* differentiation of mouse ES cells visualized by scanning electron microscopy (SEM).

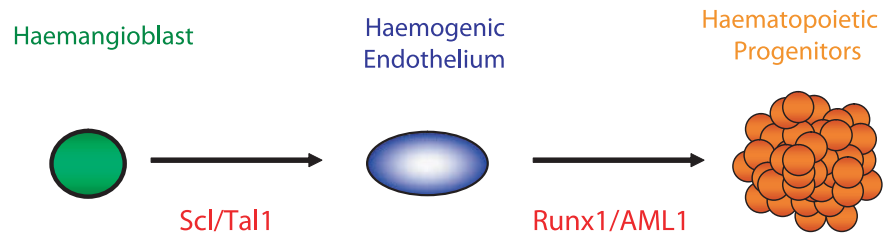


Figure 2: Model of haematopoietic development



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Blood cells are generated from mesodermal precursors at specific times and locations during embryogenesis, first in the yolk sac soon after gastrulation and a few days later in the aorta-gonadomesonephros region of the embryo proper. Using various *in vivo* and *in vitro* systems, we are now starting to understand the cascade of molecular events leading to the commitment of mesoderm and the formation of the first blood precursors, the haemangioblasts. These mesodermal precursors were first identified *in vitro* upon the differentiation of embryonic stem (ES) cells and later *in vivo* in gastrulating embryos. Upon maturation, they generate haematopoietic progenitors, endothelium and smooth muscle lineages.

Differentiation of embryonic stem cells, a model system to study haematopoietic specification

Upon differentiation, mouse embryonic stem (ES) cells can give rise to primitive and definitive haematopoietic precursors, an *in vitro* process that was shown to accurately recapitulate the *in vivo* development of yolk sac haematopoiesis. This progressive differentiation can be monitored by measurement of gene expression and

quantitative analysis of biological potential. We have shown previously that differentiation to the haematopoietic lineage can be driven efficiently by only four factors in the absence of serum and that each step of this differentiation can be controlled by only one or two factors. The removal of leukaemia inhibitor factor (LIF) and feeder cells, which together maintain the pluripotency of ES cells, is sufficient to trigger the progression from ES cell to an epiblast-like cell stage. These data suggest that no added factors are necessary for the transition from ES cells to epiblast-like cells. The transition from epiblast-like cells to mesodermal precursors requires the addition of BMP4 in a dose dependent manner. The specification of haemangioblast from the mesodermal precursors is induced very efficiently and rapidly upon stimulation with FGF and Activin A. The last step of differentiation, allowing the formation of committed blood precursors from haemangioblast, is triggered upon the addition of VEGF to the culture already stimulated by the sequential addition of BMP4, Activin A and FGF. To dissect the molecular mechanisms implicated in haemangioblast commitment, we performed a screen for genes implicated in this process using Affymetrix microarray analysis. A comprehensive analysis of expression profiles between pre- and post-haemangioblast subpopulations allowed us to identify several new genes whose expression was specifically either up- or down-regulated upon stimulation of mesodermal precursors by Activin A and FGF.

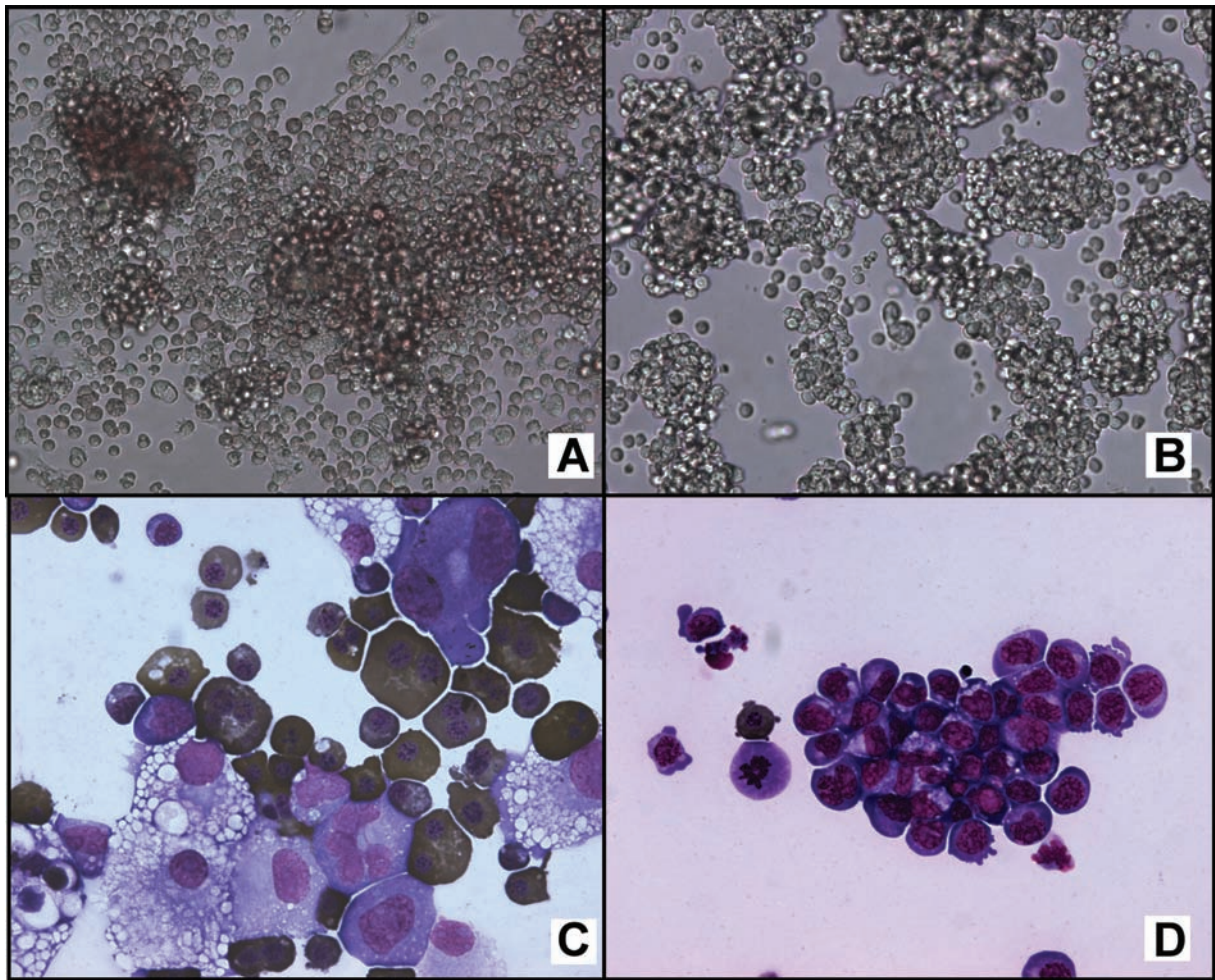
Control of proliferation and differentiation by Sox7

One of our newly identified candidates (Sox 7) belongs to the Sox family members of which have been implicated in many developmental processes ranging from ES cell self-renewal for Sox 2 to sex determination for Sry, the founding member of this family of transcription factors containing twenty genes in mammals. Sox genes belong to the HMG (High Mobility Group) super-family and are highly conserved throughout evolution. Sox genes are subdivided into seven groups according to the respective degree of homology in

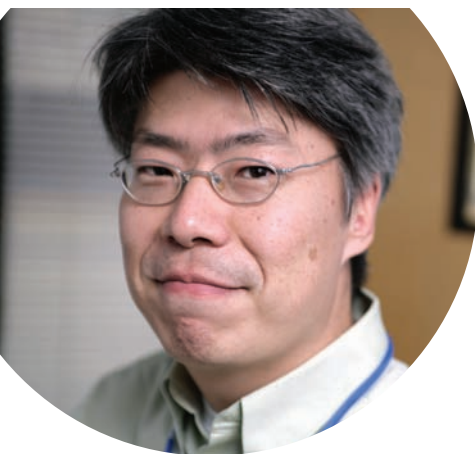
both their HMG boxes and trans-activation domains. Sox7 is a member of the F group along with Sox17 and Sox18. Sox7 has been implicated in the formation of parietal endoderm during embryonic development and in cardiac development in *Xenopus*. However, no role for Sox7 has been described to date in haematopoiesis. Our gene expression analysis revealed that Sox7 expression was sharply up-regulated at the onset of haematopoietic development. We observed a concomitant up-regulated expression for Sox7 and Flk1, the receptor 2 for VEGF, which is expressed by haemangioblast precursors. Interestingly, when these precursors further differentiated to generate fully committed blood cells and endothelium, Sox7 expression was down-regulated. To address the significance of this transient expression at the onset of haematopoietic specification, we assessed the effect of sustained Sox7 expression during haematopoietic differentiation using a doxycycline-inducible ES cell system. Enforced expression of Sox7 in a progenitor replating assay resulted in the generation of colonies blast-like in appearance while the number of mature myeloid or erythroid colonies was strongly decreased. Further analysis revealed that enforced Sox7 expression promoted a dramatic increase in cell proliferation coupled with an arrest in differentiation toward mature blood cells. Highly proliferating cells could be

maintained in culture for many passages in the presence of doxycycline and these cells retained an immature phenotype as assessed by morphology (figure) and expression of cell surface markers. Doxycycline removal led to the down-regulation of Sox7 expression, resulting in a progressive reduction of cell proliferation and promoting the maturation toward all myeloid and erythroid lineages. These findings were extended to an *in vivo* mouse model generated using the inducible Sox7 ES line. Enforced expression of Sox7 in cells derived from gastrulating embryos resulted in the survival and expansion of early haematopoietic precursors that could be maintained for several passages in culture, retaining an immature phenotype. Release from the forced Sox7 block, allowed the generation of all mature myeloid and erythroid lineages. Altogether, these data suggest that expression level of Sox7 is critical for the balance between proliferation and differentiation of progenitors at the onset of embryonic haematopoiesis. Interestingly, the outcome of Sox7 mis-regulation is highly reminiscent of the leukaemogenesis process in which early blood progenitors lose their differentiation potential while acquiring an uncontrolled proliferative capacity.

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Enforced Sox7 expression promotes blocks haematopoietic proliferation:
Early haematopoietic progenitors derived from sox7 inducible ES cells and cultured in the absence (A, C) or presence of doxycycline (B, D). Bright field photos 20x magnification of liquid culture (A, B). Cells cyto-spinned and stained with o-dianidisine (stains hemoglobin in green) followed by May Grunwald Giemsa staining.



Stromal-Tumour Interaction Group

(Since September 2007)

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

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Visiting Student

Ashleigh Hanson

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 and bone marrow-derived cells. The resulting stromal cells reciprocate by fostering carcinoma cell growth and survival during the course of tumour progression.

Studying the heterotypic interactions between the neoplastic cells and the supporting stroma is believed to be essential for understanding nature of a bulk of carcinoma mass. However, such research fails to include and address another variable: that the stroma is itself altered and might co-evolve with the tumour cells during the course of tumour progression.

Tumour-promoting roles of carcinoma-associated fibroblasts (CAFs)

Neoplastic epithelial cells coexist in carcinomas with a biologically complex stroma composed of various types of stromal cells as well as extracellular matrix (ECM), both of which create the complexity of the tumour microenvironment. The significant contribution of stroma to the development of a wide variety of tumours has been

supported by extensive clinical evidence; this contribution is highlighted by the higher incidence of tumour formation in tissues exhibiting a chronically inflamed stroma as well as those undergoing various types of wound healing, in which the stroma plays a central role. Use of mouse models of tumourigenesis also reveals that stromal cells, notably inflammatory cells, vascular cells, and fibroblasts, actively support tumour growth.

Large numbers of myofibroblasts, which are characterized 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 (figure 1). However, the specific contributions of these cells to tumour progression are poorly defined. Myofibroblasts also exist in areas of wound healing and chronic inflammation and are often portrayed as “activated fibroblasts” that play crucial roles in wound repair; myofibroblasts possess greatly increased contractile ability, promote angiogenesis, and stimulate epithelial cell growth through the production of ECM and the secretion of growth factor and cytokines. The striking histological resemblance of tumour stroma and the stroma present in sites of wound healing, both containing large numbers of myofibroblasts, raises the following questions: (i) Do myofibroblasts play essential roles in tumour angiogenesis and can they directly stimulate the growth of epithelial carcinoma cells? (ii) Are myofibroblasts present in tumour biologically indeed equivalent to those observed in wound healing? (iii) Or alternatively, do tumour-associated myofibroblasts acquire “cancer-specific alterations” that distinguish them from those present in wounds.

We sought to elucidate the properties of stromal fibroblasts, termed carcinoma-associated fibroblasts (CAFs), isolated from invasive human mammary carcinomas. Our work has demonstrated that fibroblasts present in the invasive human mammary carcinoma mass are biologically very different from their counterparts located outside tumour masses and from mammary stromal fibroblasts prepared from reduction mammoplasties in several important functional respects; (i) CAFs extracted from invasive human breast carcinomas are more competent than normal fibroblasts in enhancing tumour growth by co-mingled breast cancer cells. (ii) CAFs include larger populations of myofibroblasts, which exhibit high levels of α -SMA expression and increased collagen contractility.

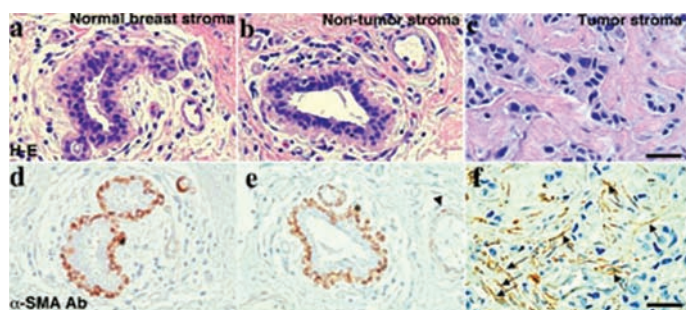


Figure 1. Stromal fibroblasts predominantly produce α -smooth muscle actin (α -SMA). Immunostaining of human breast tissues by an anti- α -SMA antibody (d, e, f) and also staining with H&E (a, b, c). The tumour region (c, f) and non-tumour region (b, e) dissected from the breast tissue of the human breast cancer patient, and the normal breast region (a, d) isolated from the healthy patient are shown. Myofibroblasts (indicated by an arrow) in the tumour region (f) and myoepithelial cells (indicated by asterisks) in non-tumour (e) and normal breast (d) regions are strongly positive for α -SMA. A representative weak signal in pericytes surrounding vasculature is also indicated by an arrowhead (e). Scale bar, 75 μ m. (Orimo et al., Cell 2005; 121:335).

(iii) When co-mingled with a line of human breast cancer cells, CAFs give rise to highly vascularized tumours in contrast to the poorly vascularized tumours generated by admixed normal stromal fibroblasts.

(iv) CAFs release increased levels of SDF-1 (stromal cell-derived factor-1) which are responsible for recruiting endothelial progenitor cells (EPCs) into a tumour mass, thereby boosting tumour angiogenesis. In addition, the SDF-1 secreted from CAFs enhances tumour growth by direct paracrine stimulation via the CXCR4 receptor displayed by human breast carcinoma cells, thereby revealing a second role for stromal SDF-1 in promoting tumour progression *in vivo* (figure 2).

(v) Both the tumour-enhancing and myofibroblastic properties of CAFs are stably retained by these cells in the absence of ongoing contact with breast carcinoma cells.

Evolution of CAFs

CAFs retain their myofibroblastic properties and tumour-promoting phenotypes, even after they have been passaged for ten population doublings (PDs) *in vitro* without ongoing contact with carcinoma cells. Accordingly, even though the CAFs appear to have initially acquired a myofibroblastic phenotype under the influence of carcinoma cells, once it is acquired, they display this trait in the absence of further signalling from the carcinoma cells. Unanswered by these observations are the following questions: (i) How do CAFs acquire and maintain their activated, tumour-enhancing phenotypes? (ii) Might CAFs harbour genetic and/or epigenetic alterations that act to confer their unique phenotypes?

Some reports indicate that stromal regions microdissected from human breast cancers exhibit a high frequency of genetic alterations, such as chromosomal regions of loss of heterozygosity (LOH) and somatic mutations. A recent report also suggests that stromal fibroblasts that have undergone p53 loss are clonally selected during tumour progression, yielding a highly proliferative stroma. However, another report indicates that myofibroblasts isolated from

human mammary breast carcinomas exhibit no detectable genetic alterations, as gauged by array CGH and SNP array analyses; this suggests that any stably maintained phenotype may depend on epigenetic modifications of the genome, such as DNA methylation.

Alternatively, the stabilization of their phenotype may depend on some type of positive-feedback signalling of the sort created by autocrine signalling loops. We note that our CAFs show no detectable aneuploidy as determined by karyotype analysis, no anchorage-independent growth in culture, and no tumorigenicity *in vivo*. Moreover, some of the CAFs begin to senesce after 15 population doublings in culture, similar to the behaviour of normal human stromal fibroblasts.

We propose three alternative models for the evolution of the stromal fibroblasts present within invasive human carcinomas: (1) acquisition of genetic alterations (e.g., p53 loss) may allow clonal selection of a small population of fibroblasts or progenitors that have undergone such alterations; (2) populations of normal stromal fibroblasts recruited into a tumour may trans-differentiate into CAFs without acquiring any genetic alterations. Such a process would mimic that occurring during wound healing, suggesting the possibility that CAFs are essentially equivalent to the myofibroblasts present in sites of wound healing and chronic inflammation. If this were so, populations of CAFs might well be polyclonal, and examinations of their clonality may be helpful in discriminating between these alternative mechanisms; and (3) stromal myofibroblasts are recruited from specialized circulating progenitor cell types.

The nature of the molecules that convey heterotypic signals between the stromal and epithelial compartments are largely obscure. However, ultimately, understanding the complex molecular networks among various types of stromal cells and tumour cells in the carcinoma mass is likely to provide highly useful information for the therapeutic targeting of human carcinomas.

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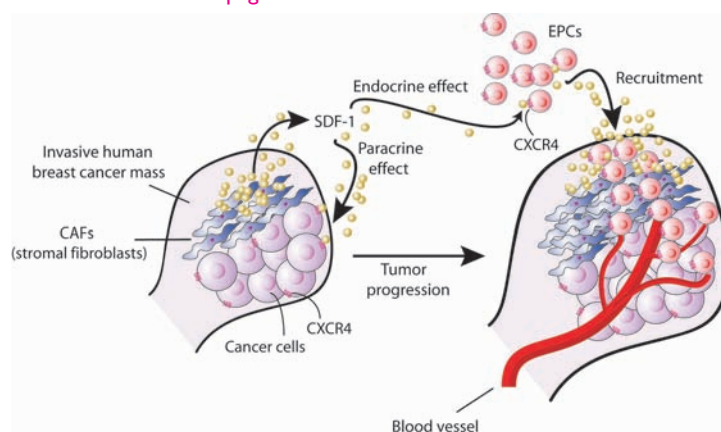


Figure 2. Schematic representation of tumour-promoting effects provoked by stromal fibroblasts within invasive human mammary carcinomas. Stromal fibroblast-derived SDF-1 enhances tumour growth not only by stimulating angiogenesis through recruiting circulating EPCs into the tumour mass but also by direct paracrine stimulation of tumour cells through the CXCR4 receptor expressed by carcinoma cells (Orimo and Weinberg, Cell Cycle 2006; 5: 1597).



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Kathryn Fellows

Each year in the UK there are ~300,000 new cancer patients and ~50% will undergo radiotherapy at some point in their treatment. Of all cancer survivors ~40% received radiotherapy. A key challenge for researchers is to find ways of giving as much radiation as possible to kill cancer cells while minimising dose to surrounding healthy tissue. The Translational Radiobiology Group works to develop methods for the individualisation of radiotherapy. With the advent of high-throughput techniques, the group is interested in characterising molecular profiles that reflect relevant biological phenotypes and predict tumour and normal tissue response to radiation.

Validation of a hypoxia-associated gene signature derived in head and neck cancer

Radiotherapy plays an important role in the management of head and neck cancer; but there is strong evidence that the presence of hypoxia limits our ability to cure the disease. Last year we described our collaborative work with Prof Adrian Harris (Oxford) in microarray profiling 59 head and neck cancers to derive a hypoxia-associated gene signature. The median expression of the 99 genes in the signature was an independent prognostic factor for treatment outcome in independent data sets. Surprisingly, only 27% of the genes

were known to be hypoxia associated. Over the past year Sara Bhana helped by Gemma Foley and Carla Moller investigated nine of the genes not previously linked to hypoxia in order to validate the approach used to derive the signature. The nine genes selected covered a range of cell functions and were ranked both high (strongest Pearson correlation) and low in the gene signature. Five human tumour cell lines were used: two head and neck, one breast, and one colorectal line with its dominant negative HIF-1 α knockout derivative. Cells were exposed to hypoxia (1%) and reoxygenation. RNA expression was analysed using qRT-PCR.

Eight of the nine genes were induced under hypoxia, the remaining gene had a high number of 'absent calls' suggesting the microarray data were unreliable. Overall a statistically significant correlation was found between the microarray and qRT-PCR data ($r=0.46-0.97$) in seven of the genes (the two remaining had very small ranges of gene expression values). Only one gene was induced under hypoxia in the colorectal cells and, as no up-regulation was seen in the HIF-1 α knockouts, it appeared to be HIF-1 α dependent. Western blot analysis of the most upregulated and HIF-1 α dependent gene showed protein levels increased under hypoxia in all but the HIF-1 α knockout cells. By showing the hypoxic induction of genes identified using a knowledge-based microarray analysis, this work validates the novel approach used for deriving gene signatures that are radiobiologically relevant.

Why is HIF-1 α expression sometimes associated with a good prognosis?

Evidence for the presence and prognostic significance of hypoxia is lacking in gastro-oesophageal cancer. With the need to develop approaches for selecting patients likely to benefit from adjuvant radiotherapy, a retrospective study was proposed in patients with gastro-oesophageal adenocarcinoma who underwent surgery with curative intent. This work (Karim Sillah, Helen Valentine) involved collaboration with Mr Ian Welch and Dr Sue Pritchard (Wythenshawe Hospital). Hypoxia associated HIF-1 α is

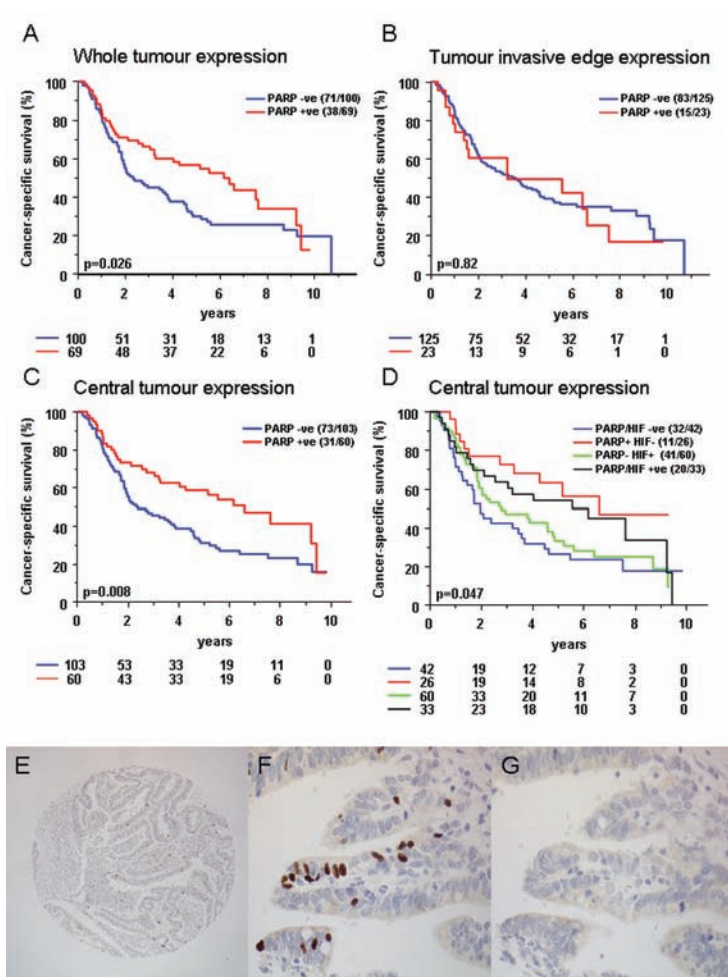
generally related to a poor prognosis, yet there are some reports of its association with good prognosis. In 177 patients, we found that, in comparison with tumours not expressing HIF-1 α , tumour invasive edge staining was associated with a hazard ratio of 1.6 (95% CI 1.0-2.5) and centrally positive staining with a hazard ratio of 0.7 (95% CI 0.5-1.2). It was hypothesised that the beneficial effect of central HIF-1 α expression might relate to its pro-apoptotic effects.

One approach for assessing apoptosis in tumours is to use cleaved poly (ADP-ribose) polymerase (PARP). This is an endo-nuclear enzyme that participates in DNA damage repair and also directs cells with defective DNA to undergo apoptosis. Currently no published studies have investigated apoptosis in gastro-oesophageal cancer using cleaved PARP. Our hypothesis was that high central tumour expression of cleaved PARP would be associated with a good prognosis. Using the same series of gastro-oesophageal adenocarcinoma previously studied for HIF-1 α , we identified cleaved PARP expression in 40%. Intestinal type, poorly differentiated tumours at the gastro-intestinal junction were more frequently positive for cleaved PARP. Expression of cleaved PARP was found to be associated with high TNM and overall stage, but also a good prognosis ($p=0.026$; see figure), which was more important when expressed centrally ($p=0.008$) rather than at the tumour invasive edge ($p=0.82$). Multivariate analysis revealed central PAR expression was an independent prognostic factor for cancer-specific survival ($p=0.02$). Concurrent expression of cleaved PARP and HIF-1 α in the centre of tumours was associated with a good prognosis ($p=0.047$). This suggests that when HIF-1 α expression in tumours is related to a good outcome it might be associated with non-hypoxic activation of HIF-1 α increasing apoptosis to prevent accumulation of mutated cells and promotion of an aggressive tumour phenotype.

RAPPER

Radiotherapy response is an inherited complex trait with patients varying in their intrinsic sensitivity to radiation. Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy (RAPPER) is a national study funded by CR-UK that aims to explore associations between single nucleotide polymorphisms (SNPs) in candidate genes and radiation toxicity. Planned recruitment is 2,200 patients with breast, prostate, gynaecological or rectal cancer. Collaborators include Drs Neil Burnet & Alison Dunning (Cambridge), Prof Søren Bentzen (Wisconsin) and numerous clinical oncologists locally and nationally. Day-to-day administration is carried out by Rebecca Elliott. Kathryn Fellow recruits patients at the Christie Hospital.

Recruitment is on target with blood samples collected from 1,757 patients since July 2005. High-quality DNA has been extracted from 382/384 blood samples (>99%), average yield 201 \pm 80 μ g, range 38-494 μ g. Current plans are to genotype 120 candidate genes using SNP-tags, focusing on genes in the cell-cycle checkpoint control, DNA damage response and cytokine pathways. The ultimate goal is to develop genetic profiles for greater individualisation of radiation dose to optimise tumour control while reducing toxicity.



Kaplan-Meier survival curves showing the cancer-specific survival of patients with gastro-oesophageal adenocarcinoma following surgery stratified according to tumour expression of: (A) PARP; (B) PARP at the invasive edge; (C) PARP centrally and (D) PARP and HIF-1 α centrally. Also shown are photomicrographs showing central tumour PARP expression (E, F) along with a negative control (G).

VORTEX-BIOBANK

One of our highlights in 2007 was collecting the first samples for VORTEX-BIOBANK. This is a CR-UK TRICC-funded translational study linked to the national VORTEX sarcoma trial. Based on the observation that hypoxia can predict for the likelihood of developing distant metastases in sarcoma patients, the aim is to validate and improve our hypoxia-associated tumour molecular profile derived in head and neck cancer. This could be used before treatment to identify patients with a poor prognosis who might benefit from adjuvant systemic therapy e.g. a hypoxia-modifying agent. A secondary aim is to investigate associations between SNPs and radiation toxicity.

Joely Irlam-Jones manages the prospective sample collection. Fresh tumour and normal tissue (for RNA microarrays), blood samples (for SNP analyses) and paraffin tissue blocks (for tissue microarrays) are being collected from each patient. Target accrual is 400 patients and after a lengthy set-up period we have collected 12 fresh tissue samples, 11 blood samples and 6 blocks – only 3 ½ years and another ~388 patients to go!

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Sai Daayana

(with HC Kitchener)

In the past year we have completed phase II trials of TroVax and Phase III trials have started. We are also expanding our interest in immune regulation and its effects on cancer immunotherapy. Likewise phase I trials of gene modified cell therapy are about to commence. The ongoing clinical programmes clearly require improved laboratory support and Eyad Elkord has been appointed to head the Medical Oncology programme in clinical immunological evaluation. This work will be facilitated by the completion (in the summer of 2008) of the laboratories designated for Good Clinical Laboratory Practice (GCLP) and which will house assessment of immune related clinical trials.

Immune responses in TroVax patients

In a trial of TroVax in patients with operable colorectal carcinoma (CRC) liver metastases, 20 received two vaccinations before surgery and 17 received at least two post-operative TroVax vaccinations. Seventeen of 19 colorectal carcinoma tumours expressed 5T4 in the tumour cells or surrounding stroma with two showing equivocal results. Eighteen of 19 CRC patients showed a 5T4-specific

immune response measured by lymphocyte proliferation (fresh), ELISPOT or ELISA. At ten weeks, eight patients showed *de novo* responses to 5T4 protein and 15 patients had produced an antibody response. Proliferative responses measured comparatively using cryopreserved peripheral blood mononuclear cell (PBMC) samples taken at baseline, 4, 10 and 14 weeks showed 12/20 patients with *de novo* responses to 5T4 protein and/or peptides; the peak of response was at 10 weeks in most patients. The tumour HLA class I loss phenotype, tumour infiltrating CD8/Treg ratios, systemic levels of Tregs and TGF beta were assessed for most patients and it is apparent that CD8 T cell effector mechanisms would be likely to be compromised in some cases. These observations reflect the complexity of interactions likely to influence the balance of immune factors contributing to clinical outcome.

T regulatory cells in cancer patients

T regulatory cells (Treg) are important regulators for the immune homeostasis but they also able to suppress anti-tumour immunity and consequently impair immunotherapy. Increased local and systemic frequencies of Treg have been reported in several types of cancers. We have shown that there is a significant increased frequency of Treg in renal cell carcinoma (Griffiths *et al.*, 2007) and colorectal cancer (unpublished data) patients, compared to normal donors. Higher Treg frequency is associated with adverse overall survival in Renal Cell Carcinoma (RCC) patients, compared to patients with normal Treg (high/normal $n = 22/27$; $P = 0.025$) (Griffiths *et al.*, 2007). These findings have led to the initiation of a phase I trial to assess the feasibility and safety of adoptive transfer of Treg-depleted autologous T cells following conditioning chemotherapy in six patients with advanced RCC. One patient showed an increased specific response to the tumour-associated antigen 5T4 coinciding with the nadir of Treg (Thistlethwaite *et al.*, 2007).

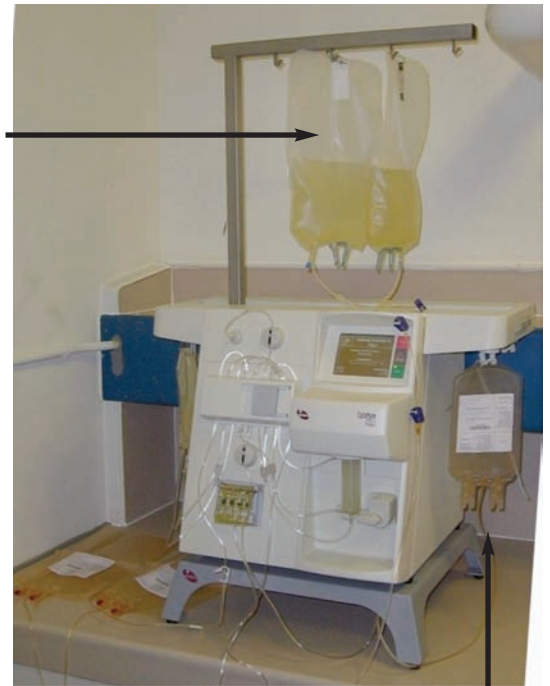
Immune regulation and cancer therapy

The use of antibodies to block negative regulators of the immune system is an attractive approach, and the most advanced molecular target in this aspect is the blocking of CTLA4-B7 interaction. CTLA4 is expressed by natural Treg and activated T cells. Anti-CTLA4 monoclonal antibody has shown promise in patients with melanoma and prostate cancer. We have performed a phase II clinical trial with a fully human anti-CTLA4 antibody (CP-675,206 or Tremelimumab, Pfizer) for treating patients with metastatic oesophageal or gastric adenocarcinomas. Eighteen patients were given 15mg/kg by infusion every 90 days; six patients received a second cycle of treatment, one a third and fourth. Four patients had stable disease on CT scan; in two their disease had been growing rapidly prior to entering the trial. Median time to progression was 87 days (95% CI 82-91 days). Median overall survival was 145 days (95%CI 122-167 days). One patient remains well on treatment after eleven months with reduction of disease burden. The phenotype type of peripheral blood lymphocytes and modulation of immune reactivity versus 5T4 oncofoetal antigen were assessed. In most patients, after 15 days increased proportions of T cells expressing CTLA4, FoxP3 (a Treg marker) and PD1 (a broadly expressed regulatory cell surface receptor) are detected. By day 60, in most patients these changes return to near baseline, but increased CTLA4 expression was found in a subset of CD4+CD25low lymphocytes. About half of the patients have been assessed for functional changes in lymphocyte proliferation responses to overlapping 5T4 peptides. Interesting these were maximal when the percentage of lymphocytes expressing FoxP3 expression was at its nadir and were seen in 3/9 patients, the most robust occurring in a patient with clear clinical benefit. This is consistent with the treatment releasing otherwise controlled anti-tumour associated antigen immunity. Larger trials are planned to further evaluate clinical benefits and potential immune predictors of both outcome and toxicity. The early results of this trial were presented at the NCI-EORTC TAT conference in Amsterdam in 2007.

Renal cancer trials

The group continues to refine the selection of patients for high-dose interleukin-2 therapy. Histological and clinical predictors identify a group who appear to have around a 50% response rate with around half of these achieving durable complete remissions. We are now investigating immune and genetic predictors of response to see if combining these can further refine the selection of patients. We are also evaluating combination of interleukin-2 with other therapies. The development of anti-angiogenic therapy for renal cancer also continues and we are a major centre for Phase II trials of a variety of such agents. We are also leading a Phase II/III trial of targeted superantigen therapy based on our previous clinical / translational studies (Shaw *et al.*, 2007).

Gene modified
and expanded
cells



Final washed and
concentrated product

The final steps in washing and concentrating engineered T cells for clinical trials. Trials are about to begin which target CEA (GI malignancies) and CD19 (B cell malignancies).

Planned immunotherapy trials

The trials of gene modified T cells to target both CEA and CD19 are about to commence after delays caused by regulatory authorities. A range of validated immune and molecular monitoring assays have been established to monitor these trials as well as the development of a GMP cell processing facility. Likewise we have established the ability to undertake innovative and complex clinical trials on Nathan House ward. This combination will facilitate the development of this type of work over the coming years. Trials targeting melanoma (in collaboration with Dr Paul Lorigan) using specific tumour infiltrating lymphocytes and engineered T cells targeting melanoma via specific T cell receptors are planned and the pre-clinical work is underway. In collaboration with the transplant group (Dr Effie Liakopoulou) we are also exploring the role of allogeneic transplantation in renal cell cancer and attempting to define additional specific targets for cellular therapy of renal cell cancer.

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Children's Cancer Group

Group Leader **Vaskar Saha**

Postdoctoral Fellows

Clare Dempsey
Mark Holland
Jizhong Liu

Scientific Officers

Seema Alexander
Naina Patel

Clinical Research Fellow

Shekhar Krishnan

Graduate Student

Shai Senderovich

Clinical Trials Manager

Carly Leighton

Administrator

Christine Connor

Our research focus is in improving the outcome of children with acute lymphoblastic leukaemia (ALL). On the clinical front, this involves designing and running novel international clinical trials for those with high risk disease. In the laboratory we are exploring biological explanations for the variations in response to therapy. Much of what we do is hypothesis-based. Our current research indicates that proteases produced by leukaemic blast cells are responsible for therapeutic failure in childhood ALL. Last year the laboratory relocated to the Paterson Institute for Cancer Research from Queen Mary University in London. After a period of reorganisation and upheaval, the group has almost completely been reorganised.

Clinical Trials

These are coordinated by Carly Leighton. A European phase II study of Clofarabine in relapsed and refractory childhood ALL has been completed. The data will be presented this year at the annual meeting of the American Society of Haematology. ALLR3 completed interim (mid-point) analyses and has received a clean bill of health from our statisticians and data monitoring committee. Both ALLR3 and EsPhALL underwent major amendments to allow translational studies to be incorporated. These studies have also been linked to

the frontline trial, ALL2003. Over the next 3 years, we aim to collect serial samples of marrow and blood from all children recruited to ALL trials in the UK, to evaluate prognostic and therapeutic biomarkers. Standards of procedures for collection and storage of samples have been developed by Shekhar Krishnan and Carly Leighton. Biomarker assay standardisation and validations have been carried out by Naina Patel and Jizhong Liu. A new clinical fellow will join in 2008 to help with this project. We are in the process of designing the next generation of clinical trials for childhood ALL. These trials will open in 2009-10. It is likely that a key focus will be on the optimisation of the drug Asparaginase and the assays being developed will become integral tools for the trials.

Studies in Asparaginase

With help from Dr Paul Bates and Marc Offman (Biomolecular Modelling Laboratory, London Research Institute), Naina Patel has examined the proteolytic degradation of the anti-leukaemic agent L-Asparaginase (L-Asp). She has discovered that principal enzymatic destruction is the result of the activity of cysteine proteases. The principal degradative enzyme in leukaemic blast cells, asparaginyl endopeptidase (AEP) cleaves L-Asp in at least three different sites. Using models predicted by Dr Bates's group, Naina has shown that the first cleavage site is important for tetramer formation, the structure required for the enzyme to be active. The second cleavage site is critical to enzymatic activity and tetramer formation. By a single amino acid replacement at the first cleavage site, she has been able to create a stable drug, which is active and not cleaved by AEP. This is subject of a patent with Cancer Research Technology and is being further characterised in collaboration with Medac GmbH. In collaboration with Cancer Research Technology, we are also investigating the synthesis and validation of AEP specific inhibitors.

The proposed biomarker study seeks to establish whether AEP expression relates to L-Asp inactivation in patients and affects survival. To do this, Jizhong and a new clinical fellow

will screen cells and sera from diagnostic samples for AEP expression using RT-RQPCR and ELISA. Jizhong and Shekhar have developed and validated assays for AEP including an AEP ELISA which is highly specific and has a sensitivity of 0.5ng/ml. We will correlate the expression of AEP with asparaginase activity (post drug administration) and antibody formation. We feel that if the study hypotheses are proven, then the results of the study will influence the design of the next generation of clinical trials.

Laboratory studies

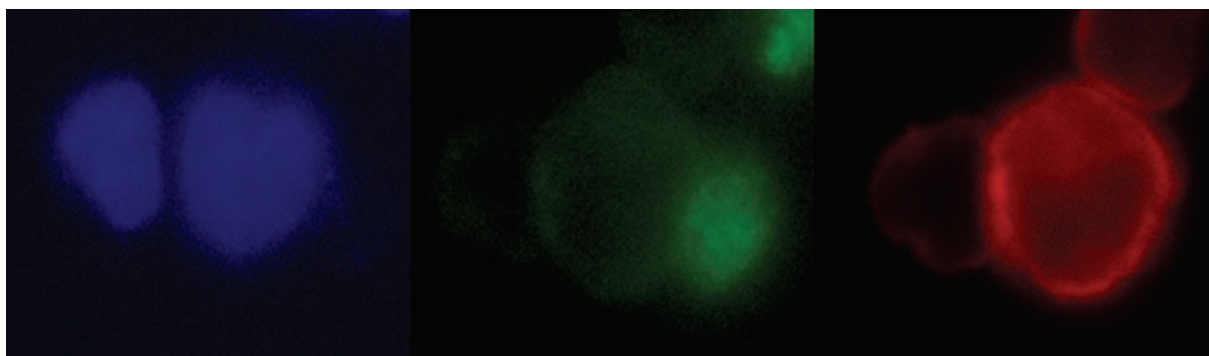
We have previously established that some drug-resistant leukaemic blast cells are able to migrate and invade across barriers. This phenomenon appears to be related to the high expression of AEP. As AEP is a lysosomal protease, a likely explanation is that AEP activates proteins on the plasma membrane which participate in the invasive process. Thus it is important to understand the changes that occur in the plasma membrane proteome of these cells. Mark Holland has been working on this in collaboration with Tony Whetton and Duncan Smith. He first developed a highly effective process of extracting the plasma membrane of cells growing in suspension. Next he showed using gel walking and mass spectrophotometry (MS) that the plasma membrane fractions of leukaemic cell lines were enriched for proteins associated with migration and invasion. Mark has now used a semi-quantitative proteomic approach using stable isotope labelling in culture (SILAC) to measure the differences in the plasma proteome between invasive and non-invasive cell lines. Early results suggest that the leukaemic cells invade by forming an actin cytoskeleton in a cdc42 dependent fashion.

To help Mark with his work, Seema Alexander is developing lentiviral transduced cell lines which aberrantly over express AEP. With Clare Dempsey, Seema will investigate the effect of AEP transduced cell lines *in vivo*, using a NOD-SCID mouse model. Shai Senderovich has shown that ALL cell lines will engraft in these mouse models, producing leukaemia. The question is will these mice develop extramedullary i.e invasive disease and what is the role of AEP in B cell differentiation

and proliferation? Clare will develop the lentiviral systems further to examine a highly resistant subtype of ALL. This is characterised by a fusion gene which is seen in all types of acute leukaemia. Shai has shown that the expression of this transgene in haematopoietic stem cell populations produces a myeloid arrest. Using cellular and murine models, Clare is seeking to investigate the effect of the fusion gene on early progenitor cells at different stages of development.

Shekhar has been involved in a number of the previously described studies and most importantly in standardisation of many of the assays. However, his primary focus is in examining the microscopic changes in structure of different leukaemic cells. In Mark's experiments with the invasive cells lines, using confocal microscopy, Shekhar has observed filamentous actin assembly in the form of sheets and villiform filamentous actin assembly (dot-like staining). This suggests that leukaemic blasts cells, like mesenchymal tumours, could invade extracellular matrix (ECM) through formation of actin-based structures. Shekhar will extend his observations to the cell lines created by Seema and Clare as well as using a mifepristone inducible cell line that he has created. He is testing the hypothesis that lymphoblasts are able to form invadopodia and that this phenomenon is related to AEP over-expression.

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SD1 cells were incubated for 1 hour with the AEP activity binding probe and then allowed to adhere to fibronectin coated 10mmx10mm circular glass coverslips. They were then formalin fixed and stained 3 hours later. DAPI stains the nucleus; F-actin is stained by phalloidin conjugated to far-red Alexa. The AEP activity binding probe is artificially coloured green - it is a Bodipy visible red spectrum stained probe (gift from Dr.Matthew Bogoy, Stanford University.). The probe is designed to fluoresce only when active AEP is detected. This figure shows active AEP expression by lymphoblasts. While AEP is a lysosomal protein, here it appears to be primarily lie within an exocytosed vesicle. Magnification 100X; Exposure 25msec for DAPI, 200 msec for AEP ABP and F-actin. Captured on a Zeiss 7D AxioVert 200M Time Lapse microscope.



Targeted Therapy Group

Group Leader **Tim Illidge**

Preclinical Group Postdoctoral Fellows

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Jamie Honeychurch
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Monique Melis

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Clinical Radioimmunotherapy Group Senior Clinical Scientist

Maureen Zivanovic

Clinical Scientist

Jill Tipping

Research Nurses

Tracy Gardener
Susan Neeson

The research goals of the Targeted Therapy Group are to increase our understanding of the mechanisms of clearance of tumour by radio-immunotherapy (RIT) and develop further applications to apply RIT to the treatment of cancer. We specifically investigate mechanisms involved in antibody and radiation induced ERK/MEK pro-death dependent pathways as well as how radiation-induced cell death interacts with host immune effector cells. We are developing strategies to enhance radiation-induced cell death with immunostimulatory antibodies and immunoregulatory ligands in the treatment of cancer and hope to translate our research findings into early phase clinical trials

The work performed over the last year has involved the following three research areas: i) mechanisms of action of radioimmunotherapy (RIT), ii) combining immunotherapy with radiotherapy (RT) and chemotherapy and iii) clinical translational early phase trial work

Mechanisms of action of radioimmunotherapy

Radioimmunotherapy is a highly effective treatment for some haematological malignancies. Over the last few years we have substantially increased our understanding of the relative contributions of antibody effector mechanisms and targeted

radiation to the eradication of tumour by using well defined syngeneic animal models of B-cell lymphoma. Using these well defined models we have demonstrated for the first time that successful RIT leading to long-term tumour protection consists of both targeted irradiation and monoclonal antibody (mAb) effector mechanisms (Du *et al.*, 2007). We have demonstrated that using one mAb to deliver larger doses of targeted radiation to tumour and a second mAb that induced cell signalling is an effective new strategy in RIT. Our work provides a scientific rationale for use of combinations of mAb in clinical RIT and for radiation dose escalation in combination with therapeutically active mAb.

Our more recent work has focused on the importance of the micro-distribution of radiolabelled mAb to targeting RT effectively to tumour. We have found important therapeutic differences between targeting different tumour antigens and their ability to deliver tumouricidal doses of RT at the microscopic level. In these complex studies we have specifically investigated how the microdistribution of the targeted RT component affects the long-term clearance of lymphoma. The profound differences in therapeutic outcome observed appeared independent of the levels of ¹³¹I -mAb tumour-binding or antibody-dependent cytotoxicity. Instead, the microscopic intratumoural dosimetry seemed to be critical with the more therapeutically effective ¹³¹I -anti-MHCII delivering more focused and thus substantially higher radiation dose to tumour cells (Du *et al.*, 2007). We believe that these new insights should influence the selection of new target antigens and the design of dosimetric methods in RIT of lymphoma.

A productive area of recent research has focused on investigating the cell death pathways that may underlie the impressive responses seen with radiolabelled anti-CD20 mAb in chemo-refractory patients in the clinic. We have investigated the downstream signalling events in a variety of human B-cell lymphomas treated with radiation treatment and anti-CD20 mAb. Increased cell death was observed with Tositumomab and RT and this was found to be MEK-

dependent and could be inhibited with pharmacological inhibitors (U0126, PD98059) as well as siRNA targeting MEK1 or MEK2. Furthermore the addition of U0126 reversed the loss of clonogenic survival triggered by combining Tositumomab with RT. Interestingly although Bcl₂ over-expression resulted in decreased apoptosis after RT alone, it had no impact on the additive cell death seen with Tositumomab plus RT. Our data indicate that activation and nuclear accumulation of ERK1/2 appear to be required to produce the synergistic effect produced by combining Tositumomab and RT. These findings suggest that the well documented survival pathway Ras–Raf–MEK–ERK1/2 can have a differential role and be stimulated by some anti-CD20 mAb to function as a pro-death pathway.

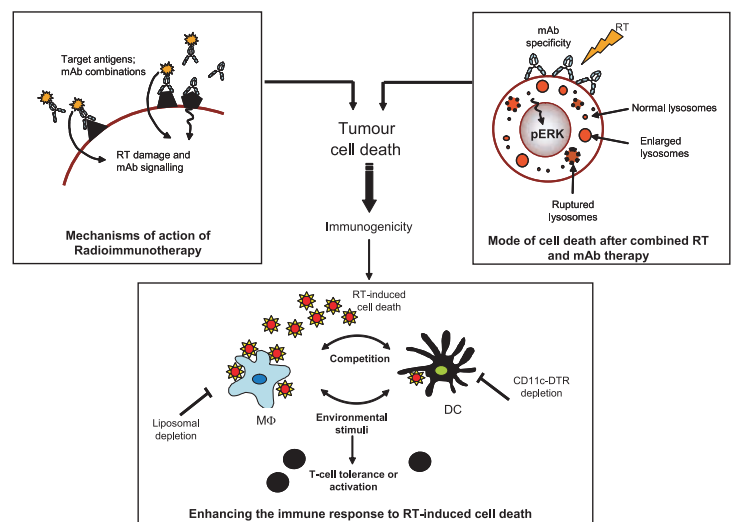
Radiotherapy and immunoregulation

In this work we aim to enhance the therapeutic potential of RT by combining it with immuno-modulatory mAb or immunoligands. Our initial novel “proof of principle” work focused on RT in combination with anti-CD40. We have been able to demonstrate that RT and anti-CD40 mAb can act in concert to eradicate lymphoma and induce long-term survival under conditions whereby either treatment alone is ineffective. Over the last year we have successfully further developed a number of powerful tools that will facilitate the future exploration of the roles of subsets of professional antigen presenting cells (APC) in generating immune responses to irradiated tumour cells. We have demonstrated that irradiated tumour cells are rapidly cleared by macrophages and that the degree of clearance correlates with the radiation dose. This approach allows the selective depletion of macrophages from various tissues and anatomical sites including secondary lymphoid organs such as the spleen. Depletion is transient, with all subpopulations of macrophages depleted but is of sufficient duration to enable us to investigate. We are also able to selectively deplete CD11c immune cells and thus determine the role of dendritic cells in antigen presentation.

A productive recent collaboration with Dr Richard Byers (senior lecturer in pathology, University of Manchester) has involved investigating the immune response in follicular lymphoma using real-time PCR to measure expression levels of candidate indicator genes, selected from microarray studies, to polyA cDNAs prepared from archived human frozen lymph nodes, in parallel with immunohistochemical analysis. Our results confirm the prognostic importance of the host immune response in outcome in Follicular lymphoma and have identified CCR1 as a potential new prognostic indicator and marker of immune switch between macrophage and a T-cell dominant response (Byers *et al.*, 2007).

Clinical Translational research

The chimeric anti-CD20 mAb, Rituximab, now plays a major role in the management of most B-cell non-Hodgkin



Abbreviations: mAb, monoclonal antibody; RT, radiation; DC, dendritic cell; Mφ, macrophage

lymphomas. One of the major successes from the laboratory programme has been the development of a high affinity mAb anti-idiotypic against Rituximab (MB2A4) that can be used to measure serum Rituximab levels in patients. We plan to apply this assay to measure serum Rituximab levels within an NCRI lymphoma study, in collaboration with the Clinical and Experimental Pharmacology Group led by Caroline Dive. This forms the basis of a current ongoing CR-UK full TRICC proposal.

The clinical RIT group has made considerable progress over the last year in leading early phase clinical trial design nationally and internationally. The largest Phase II study ever completed with ⁹⁰Y Ibritumomab in Diffuse Large B-cell lymphoma (DLBCL) demonstrating the high activity of this drug in DLBCL was recently published (Morschhauser *et al.*, 2007). The Phase I/II study incorporating ⁹⁰Y-Ibritumomab with R-CHOP in the treatment of DLBCL is recruiting well (n=22) and results will be presented at a major international meeting in 2008.

This year we opened this phase II study (FIZZ) in untreated follicular lymphoma and have developed key collaborations with Professor Morschhauser in Lille, Professor Bardies in Nantes and Professor Martinelli in Milan. This year we have also set up the SCHRIFT study (Short Chemotherapy Radioimmunotherapy In Follicular lymphoma Trial). This study will be the first UK national study using RIT, uses abbreviated chemotherapy followed by RIT in relapsed follicular lymphoma and has been adopted by the NCRI lymphoma group (awarded funding by the CR-UK Feasibility Study committee). There have been other notable successes in early clinical trial design especially in cutaneous T-cell lymphoma (CTCL) including the CR-UK CTAAC funded Gemcitabine and Bexarotene (GemBex) NCRI Phase II trial, which opened in late 2007.

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Medical Oncology: Cell and Gene Therapy Group

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Erik Alcantar Orozco
John Bridgeman
Grazyna Lipowska-Bhalla
Alex Smith

The research focus of the Cell and Gene Therapy group is upon the development of novel immunological therapies for cancer. Pre-clinical studies performed by our group have supported the initiation of phase I clinical trials testing engineered T cells to target cancer. Our recent work has centred upon understanding the factors which impact upon the power of these T cells after re-infusion into the patient with the aim of improving the overall potency of this approach to treating cancer. To this end, our research group has developed various model systems to explore the biology of engineered T cells.

Engineered T cells

T cells are powerful members of the immune system which play a pivotal role in the defence of the body against pathogens. T cells are also thought to play a role in protecting against the development of cancer. However, to counter this, tumours have evolved a number of mechanisms to avoid the activity of T cells. In order to re-programme T cells to target cancer, we have employed a gene therapy approach whereby T cells are collected from the peripheral blood of a patient and a gene encoding a tumour targeting receptor is introduced into the T cells. These engineered T cells are then re-infused into the patient in the hope that the T cells can home to tumour and then start to kill the tumour cells through the use of this new receptor. Our first trial sponsored by Cancer Research UK and targeting colorectal cancer has opened on the 28th November, 2007 with the

first patient due for treatment in December. Our second trial targeting B cell lymphoma is due to open early in 2008.

Whilst driving this technology into clinical trial represents a significant milestone it is also clear that, as with any new therapy, further development will be required to improve the overall potency of the approach. To this end, we are pursuing a number of specific areas relating to engineered T cell biology in order to generate information that will enhance our current trial protocols. In particular, we are investigating how the tumour targeting receptor interacts with other cell surface proteins present on the T cell. Our basic receptors consist of a tumour targeting domain (derived from an antibody) fused to the transmembrane and cytoplasmic domain of a key T cell receptor molecule (CD3 ζ). Interestingly, protein dimerisation appears to be a critical factor for the optimal function of the tumour targeting receptor. Further mutations in the CD3 ζ transmembrane domain of the receptor which destroy the ionic interactions thought to stabilise the immune synapse also significantly reduce the functional responses of the engineered T cell. Taken together, our current hypothesis is that the interaction of the tumour targeting receptor with the endogenous T cell receptor complex is critical to the optimal function of the engineered T cell. Based upon this hypothesis, we are engineering new receptor constructs which strengthen these protein-protein interactions in order to determine whether this improves the activity of the engineered T cell against tumour targets.

Aside from directly engineering the receptor, we are developing new culture protocols which can enhance the functionality of the T cell. The combination of interleukin (IL) 2 and 7 improves the survival of cultured T cells while the addition of IL-21 substantially increases the cytotoxic potential of mouse T cells. Overall, these protocol modifications are contributing to improved therapeutic read-outs *in vivo* which include the complete eradication of thirteen day established measurable B cell lymphoma tumours in immuno-compromised mice by anti-CD19 engineered T cells.

The development of realistic models to test engineered T cells is a prime target of our future studies. We are seeking to develop T cells which can target 'natural' antigens in mouse model systems (e.g. mouse 5T4 in collaboration with the Immunology group) in order to more fully explore the activity of these T cells in a background of normal tissue antigen expression. Furthermore, our collaboration with the Carcinogenesis group is formulating new ideas relating to combining selective chemotherapies with T cell immunotherapy for the more effective treatment of established tumours.

Trafficking of T cells

Ensuring that T cells reach the site of tumour will be critical to the likely effectiveness of engineered and non-engineered T cell therapy. We have reported that an unusual chemokine, Eotaxin-2, is present within tumours of colorectal origin (Cheadle *et al.*, 2007). Chemokines are small molecules which are thought to control the migration of cells around the body. The presence of high levels of Eotaxin-2 within colorectal tumours would suggest that cells which could respond to this chemokine would actively traffic into the tumour. However, this does not appear to be the case and this is potentially due to the balancing effects of other chemokines which counteract the effect of Eotaxin-2, such as IP-10 which is also found at high level in these tumours (figure 1). We are seeking to explore the reasons why colorectal tumours produce such high levels of these cytokines; however, in terms of our trial using engineered T cells to target colorectal cancer, exploiting the high levels of specific chemokines through the expression of the relevant chemokine receptor alongside the tumour targeting receptor is possible and we have shown in a T cell line that such a configuration of receptors can drive the movement of T cells towards Eotaxin-2 *in vitro*.

The ATTACK project

The ATTACK project is an EU-funded FP6 programme (co-ordinator Professor Hawkins) which brings together fourteen laboratories working upon the scientific and pre-clinical development of engineered T cells. A highly successful ATTACK / Cancer Research UK sponsored symposium was held in Manchester in December 2006 which brought together world leaders in the field of adoptive cellular therapy (Thistlethwaite *et al.*, 2007). The second annual meeting was held alongside the European Society of Gene and Cellular Therapy meeting in Rotterdam this year. Collaborations between the partner laboratories are important to the success of this project and this has been exemplified through a joint publication between our group and colleagues working at the University of Cologne (Hombach *et al.*, 2007).

TIL therapy for Melanoma

Cellular therapy of melanoma has been largely pioneered by the group of Dr Steven Rosenberg (Surgery Branch, NIH). Some spectacular clinical responses have been described using T cells isolated from tumour biopsies, so-called tumour

infiltrating lymphocytes (TIL). With the development of T cell handling facilities in collaboration with the National Blood Service (see BIGT report) and the development of key collaborations with our clinical colleagues in Medical Oncology (Dr Paul Lorigan) and with members of the Department of Surgery, CHT (Mr Gary Ross and colleagues), pre-clinical studies to examine the feasibility of generating TILs from melanoma biopsies have begun. Encouragingly, TILs have been successfully cultured from our first two patient donors (figure 2) and our aim is to further develop the technology in order to generate sufficient pre-clinical data to support regulatory application for phase I trial.

Summary

The Cell and Gene Therapy group is working with local, national and international collaborators to develop and deliver cellular therapies for cancer. The generation of relevant model systems is central to this aim in order to successfully provide a sufficient depth of scientific background support to clinical trial proposals. Many of these models are now developed and our hope is that these models will provide the springboard to provide clinical trial protocols for testing within the context of the BIGT group.

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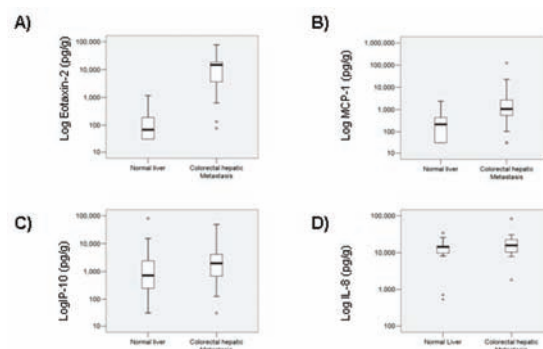


Figure 1. Analysis of chemokines produced from normal liver or patient matched biopsies of colorectal hepatic metastasis.

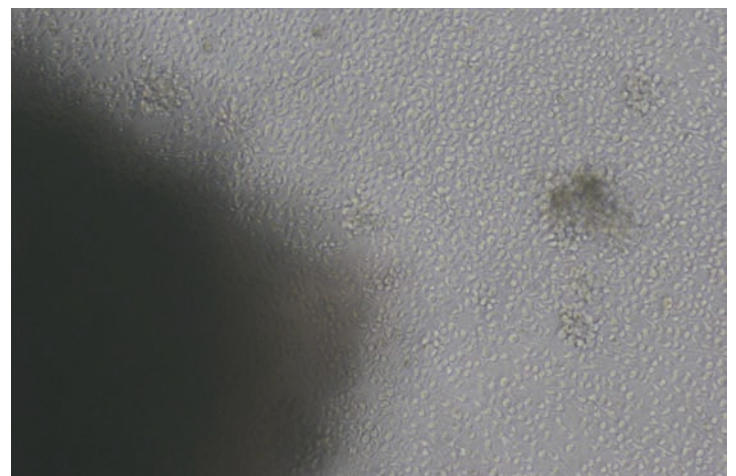


Figure 2. 'Walk-out' of tumour infiltrating lymphocytes from a tumour fragment (dark mass).

Medical Oncology: Glyco-Oncology Group

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Malcolm Lyon

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Scientific Officers

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Nijole Gasiunas

Graham Rushton

Placement Student

Nicole Yan

Loss of cell growth regulation is a major factor in the development of cancer. Heparan sulphate (HS) is a cell surface co-receptor for many of the growth factors and cytokines involved in tumour growth and tumour angiogenesis. Its mode of action is still unclear, although a considerable body of evidence indicates that patterns of sulphation along the HS chain act as binding sites for growth factors and enable their efficient engagement with tyrosine kinase receptors. We are investigating the molecular design and function of HS in different cell types, including embryonal stem (ES) cells, and examining how its unique domain structure and conformational flexibility drive the assembly of ligand-receptor signalling complexes on the plasma membrane.

Hepatocyte growth factor /scatter factor (HGF/SF): binding specificities and inhibition by sugar mimicry

The iduronate-containing glycosaminoglycans (GAGs) heparan sulphate (HS) and dermatan sulphate (DS) are co-receptors in the activation of the tyrosine kinase receptor, MET, by its ligand HGF/SF, an important growth factor in tumour progression and metastasis. We aim to elucidate the

specificity of GAG recognition by HGF/SF, as well as the potential for inhibiting its activity via disruption of these co-receptor interactions. Towards this aim, we have prepared all possible combinations of sulphation within HS tetrasaccharides, the minimal activating fragment size for HGF/SF. Comparisons of their HGF/SF-binding and activation profiles indicate that a single sulphate group per disaccharide, at any position (N-, 2-O or 6-O), appears to be the minimum for imparting weak HGF/SF interaction and activation, though affinity and efficacy increase with increasing sulphate density. This explains why DS, with its predominantly monosulphated disaccharide repeat, is also effective. NMR chemical shift mapping, in collaboration with Dr Dušan Uhrín (University of Edinburgh), confirms the proposition that HS and DS are alternative ligands for a single binding site within the N-terminal domain of HGF/SF. Recent development of a novel procedure for the paramagnetic tagging of GAG oligosaccharides (Gemma *et al.*, 2007) will now hopefully aid us in the future determination of the NMR solution structure of this growth factor–GAG co-receptor complex.

Additionally, in collaboration with Drs Jim Wilkinson & Sylvie Ducki (University of Salford), a series of sulphated non-sugar mimetics of a HS trisaccharide have been synthesised and screened for inhibition of MET activation by HGF/SF. Two compounds inhibited MET signalling and consequent cell migration, but had no observable effect on FGF-2 signalling, another major GAG-dependent growth factor (Raiber *et al.*, 2007). Simulated docking experiments suggest that one of these inhibitors binds to part of the GAG-interaction site in HGF/SF. These novel and promising results demonstrate the potential for selective inhibition of HGF/SF via the GAG co-receptor mechanism.

Heparin-mediated co-operative binding mechanism in the assembly of fibroblast growth factor (FGF) signalling complexes

HS is a mandatory co-receptor for the fibroblast growth factors (FGFs). We found that short heparin saccharides (chemical analogues of the sulphated regions of HS) of four-

to-six (dp4-dp6) sugars in length bind FGF2 monomerically and generate a weak mitogenic signal. In contrast, longer heparin saccharides (dp8+), at very low concentrations, stimulate a potent FGF2 mitogenic response which correlates with the ability of these saccharides to dimerise FGF2 in a co-operative manner. These results, together with parallel work on the interaction of FGFI/saccharide complexes with FGF signalling receptors (FGFRs), have revealed that co-operative ligand dimerisation by heparin is likely to be the main driving force in assembly of signalling complexes on the cell surface. This information is being used in the chemical design of saccharides that inhibit FGF activation (see Translational Angiogenesis Group).

To investigate the mechanism of FGF-dimerisation we have been using the multi-angle light scattering (MALLS) photometer at the Biomolecular Analysis Facility (University of Manchester) to investigate the binding of FGFs to various heparin saccharides. Preliminary data are promising and appear to support the stoichiometries we predict from size-exclusion chromatography. A particularly interesting observation was that saccharide concentration had no effect on the stoichiometry of FGFI-dp12 complexes. This is what we would expect from our published model of co-operative dimerisation (Robinson *et al.*, J Biol Chem 2005; 280: 42274). Further studies are underway to investigate the thermodynamic properties of monomeric and dimeric FGF-heparin complexes using both isothermal titration calorimetry (ITC) and surface-plasmon resonance (SPR).

We have continued to develop studies using FGFs to protect binding sequences in HS from enzymatic degradation. We hope to use this technique to purify HS oligosaccharides for crystallographic studies in collaboration with Tom Blundell's group at the University of Cambridge. Analyses of the composition of the FGFI-protected HS fragments show that these saccharides tend to be around dp10-14 in size and encompass both S-domain (dp6-8) and transition zone sequences. This is particularly interesting as these regions of HS chains tend to be the most variable in sulphation pattern.

Heparan sulphate in ES cell differentiation; dynamic regulation of structural motifs

ES cells provide a valuable model to study the role of HS during development. ES cell differentiation is accompanied by dramatic alterations in HS sulphation that may enable each cell/organ to respond characteristically to different growth factors and morphogenic proteins. Previously we have characterised HS from neural precursor cells compared to undifferentiated ES cells (Johnson *et al.*, 2007). We have also studied the changes in HS structure during mesodermal/haematopoietic differentiation. This work has led to the identification of a distinct HS epitope that is uniquely expressed during haemangioblast specification. In addition to the traditional haemangioblast marker Flk1, this HS epitope (designated HS4C3) can be used to fractionate a sub-population of Flk1+ cells which have significantly increased haemangioblast potential. This HS epitope is also expressed specifically in newly formed mesodermal layers in the mouse embryo during gastrulation, providing the first

example of a lineage- restricted HS structural motif.

To highlight the essential requirement for HS in differentiation we have characterised the differentiation potential of HS-deficient Ext1^{-/-} ES cells. These cells were found to be defective in both neural (Johnson *et al.*, 2007) and mesodermal/haematopoietic differentiation, mimicking the block in development seen in HS-deficient mouse embryos. However, remarkably, we have found that differentiation could be restored by the addition of soluble HS or heparin oligosaccharides to the growth media. These are very important findings as they open the way for investigating whether different HS species can dictate cell fate decisions in ES cells.

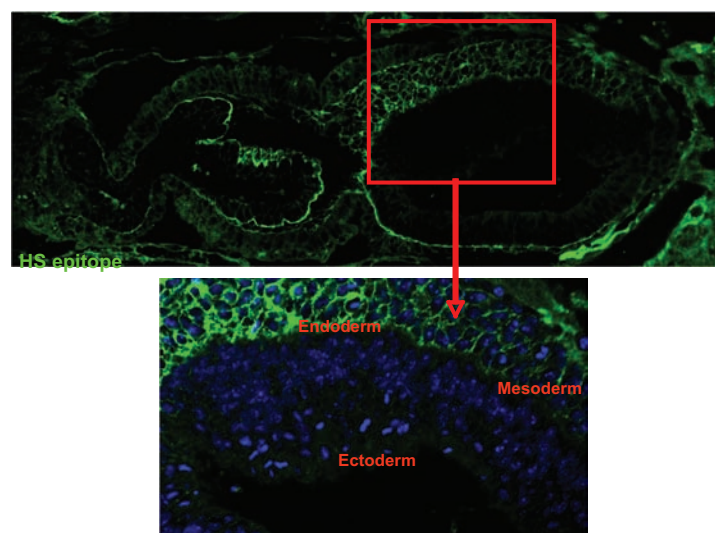
We are also examining the effect of addition of exogenous HS to wild-type ES cells. Addition of HS to HS-competent, Sox1-GFP cells (46C) affects differentiation to Sox1-positive neural progenitor cells, by either speeding up or restraining Sox1 expression. We are now investigating the effect of adding GAGs to the engineered 46C Ext1 RNAi line, which allows us to use the Sox1-GFP reporter in a HS-depleted background.

We have ongoing collaborations with Dr Catherine Merry (Materials Science Centre) directed towards immobilising GAGs to surfaces as a more optimal form of presentation to cells than as soluble factors.

Key collaborators

Valerie Kouskoff and Georges Lacaud (Stem Cell Biology and Haematopoiesis Groups), Gordon Jayson (Translational Angiogenesis Group), Catherine Merry and Brian Bigger (University of Manchester), Tom Blundell and Ermanno Gherardi (University of Cambridge), Dušan Uhrín (University of Edinburgh), Anne Dell and Berangere Tissot (Imperial College, London), Jim Wilkinson (University of Salford), Gerdy ten Dam (University of Nijmegen, Netherlands) and Marios Stavridis, (University of Dundee).

Publications listed on page 67



HS-specific antibody marks a specific stage in mesodermal differentiation. Staining of sectioned primitive streak stage mouse embryos with an antibody against a specific HS epitope (green). DAPI was used to stain nuclei blue.



Medical Oncology: Translational Angiogenesis Group

Group Leader **Gordon Jayson**

Postdoctoral Fellows

Alison Backen
Marek Barath
Claire Cole
Steen Hansen

Scientific Officer

Karen Brookes

Clinical Fellows

Gireesh Kumaran
Sin Lau
Claire Mitchell

We have established a Translational Angiogenesis Group in collaboration with Professor Caroline Dive with the aim of defining imaging, serological and tissue biomarkers for anti-angiogenic agents. We are running the translational research programme for two MRC/NCRN international randomised trials of anti-angiogenic agents in ovarian cancer. The laboratory programme has identified critical Fibroblast Growth factors (FGFs) in ovarian cancer and developed heparin oligosaccharide synthesis.

Ovarian cancer laboratory programme

The obligate dependency of the Fibroblast Growth Factors (FGF) on heparan sulphate (HS) for their biological activity led us to examine this axis in ovarian cancer. In previous work we had shown that stromal syndecan 1 was of prognostic significance in the disease and that syndecan 3, conventionally found on neuronal tissue, was aberrantly expressed in the tumour endothelium. Using a unique molecular probe we showed that the heparan sulphate on the tumour vascular endothelium has the capacity to activate FGF2. While the stroma bound this probe only moderately, the tumour cells were largely negative.

We investigated why the tumour cells do not express growth factor activating heparan sulphate and showed, using *in situ* hybridisation, that the tumour cells strongly express 6-O-

sulfotransferase, an enzyme implicated in the synthesis of biologically active heparan sulphate. In addition we showed that the core proteins for proteoglycans were present on the surface of ovarian cancer cells. These data implied that the tumour cells make heparan sulphate but that active heparan sulphate is not present on the cell surface. By adding exogenous FGF2 we were able to probe for non-activating sequences of HS but this was also reduced on the tumour cell surface suggesting that there is a global rather than sequence-specific reduction in cell surface HS. Both heparanase and H-sulf1 were expressed by the tumour cells but only heparanase could account for the findings. Thus these data suggest that heparanase is a key mediator of heparan sulphate depolymerisation at the tumour cell surface, augmenting its known role in invasion and angiogenesis.

In an extensive study of FGFs in ovarian cancer we have identified a switch in the receptor type upon transformation. This is associated with the response of the tumour cells to the appropriate ligand FGFs and enhanced chemosensitivity in inhibition studies. Critically we have validated this target in *in vivo* studies. Thus our data (manuscript in preparation) suggest that FGF3 and 7 play a role in transformation and the malignant phenotype, highlighting the potential of FGF inhibitors in the disease.

Therapeutic programme

We had previously shown that heparin octasaccharides inhibit angiogenesis *in vivo* and over the last year have developed the organic chemistry to make synthetic heparin octasaccharides. We have now completed the total organic synthesis of a range of heparan sulphate oligosaccharides and have demonstrated that the molecules have effects on specific HS-dependent growth factors *in vitro*. Over the next year we will optimise the structure-inhibition relationship and evaluate the molecules *in vivo*.

Clinical trial programme

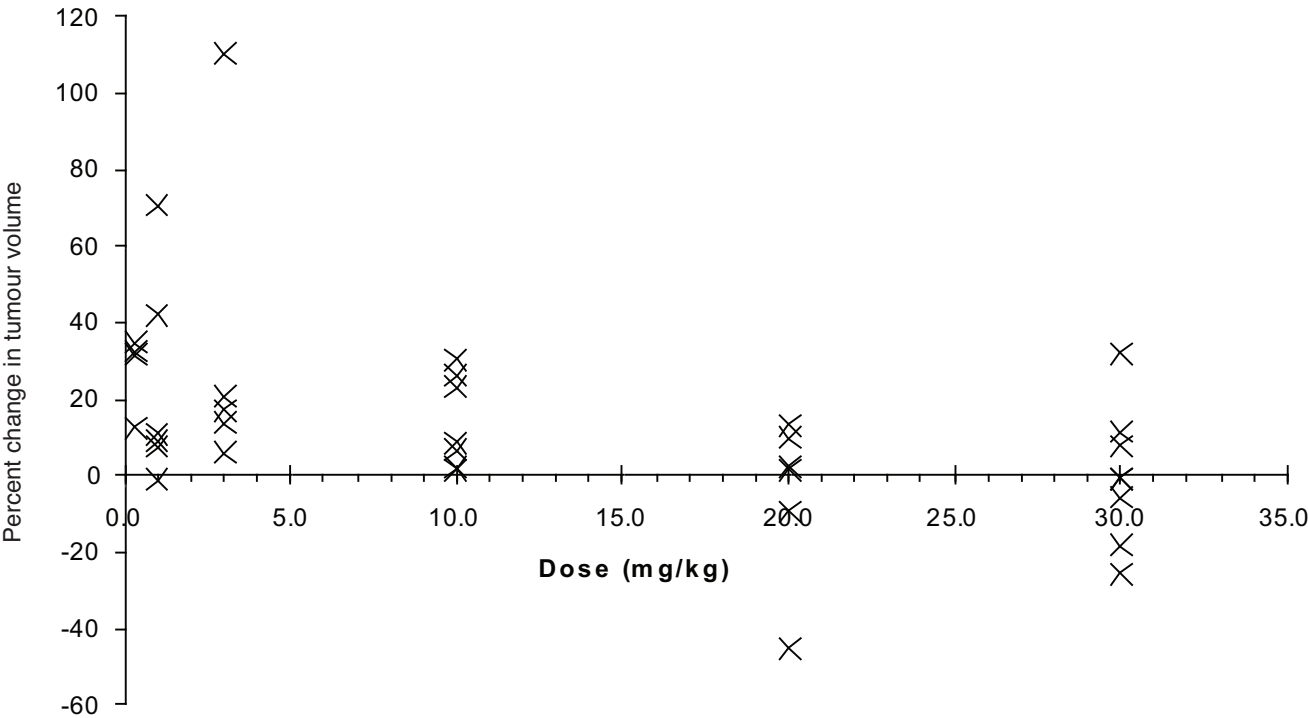
We have completed phase I trials of anti-angiogenic agents that include a pure anti- α_v integrin antibody and a pure anti-VEGFR2 antibody. These trials have yielded unique insights into the possible roles that the drugs might have. In the integrin trial we showed that the antibody was very well tolerated and that one patient with angiosarcoma underwent rapid improvement of her disease with single agent therapy. In the anti-VEGFR2 trial we demonstrated growth retardation using serial volumetric imaging strategies (collaborators: Jackson, Parker; University of Manchester) but did not see changes in vascular permeability, thereby challenging the dogma that all active VEGF inhibitors reduce vascular permeability as assessed by dynamic contrast enhanced Magnetic Resonance Imaging. On the other hand we have demonstrated the superiority of volumetrics over RECIST and 2-dimensional reporting with respect to response detection with biological therapeutics.

In collaboration with Prof. Sean Kehoe (Oxford) we have gained approval from CTAAC to expand the preliminary randomised study of neoadjuvant chemotherapy for the treatment of ovarian cancer. The CHORUS trial will now be completed over the next 2 years and will test the acceptability of deferring surgery in ovarian cancer through the use of pre-operative chemotherapy.

Translational angiogenesis research group

We have established a collaboration with Professor Caroline Dive (Clinical and Experimental Pharmacology) with the remit of identifying biomarkers for anti-angiogenic agents. A TRICC grant has been awarded (Jayson, Dive, Hall and Banks) to study samples from patients in ICON7, an NCRN/MRC randomised phase III clinical trial comparing conventional chemotherapy with the same regimen augmented with the anti-VEGF monoclonal antibody, bevacizumab. The trial is now open to accrual and samples will be collected and analysed over the next 3 years.

Publications listed on page 68



Volumetric Evaluation of Biological Agents

We completed a phase I evaluation of one of only a very few specific inhibitors of VEGFR2. Dynamic Contrast Enhanced Magnetic Resonance Imaging surprisingly did not demonstrate a change in the permeability surface area product (k^{trans}). However, the patients developed cutaneous hemangiomas that were biopsied. Immunohistochemical analysis demonstrated that the drug did achieve receptor de-phosphorylation. Although conventional 1- and 2-dimensional reporting did not detect a dose-level vs response relationship. However, volumetric assessment (above) demonstrated a statistically significant relationship between the dose level and change in whole tumour volume. Thus these data highlight the importance of volumetrics in the evaluation of biological agents.



Research Services

Head of Research Services **Jenny Varley**

The Paterson Institute Research Services continue to provide support for all core CR-UK funded groups as well as other research groups based within the Institute. The Transmission Electron Microscopy service is now up and running, aided by some investments in equipment as described below.

In 2007 a review of the Flow Cytometry Service was undertaken, and whilst commending the quality of the existing service, recommended an additional senior appointment to enable the unit to provide a facility better able to undertake developmental work. Associated with this was the purchase of a FACSArray system, and imminently of a third analyser. Central Services has also been reviewed recently; this previously encompassed the porters and media/sterile services. The two components are sufficiently distinct to warrant their separation particularly with ever-increasing requirements for the provision of sterile media, and a new head of Laboratory Services is currently being sought as a consequence.

We have purchased some large items of equipment for the research services during 2007, including a new mass spectrometer, a spinning disk microscope, a Zeiss Mirax Histology imaging system and a Xenogen IVIS Lumina *in vivo* optical imaging system. Each of these is discussed more fully in the appropriate sections below.

Last but not least, towards the end of 2007 we initiated some much needed refurbishments in some of the scientific service areas. The Molecular Biology Core Facility is being fitted with a new air conditioning system, the Flow Cytometry and Mass Spectrometry laboratories are being completely refurbished and the Histology unit will expand in early 2008. Fortunately we have been able to decant all the units for the duration of the works to ensure that there is minimal disruption to the users.

Advanced Imaging Facility

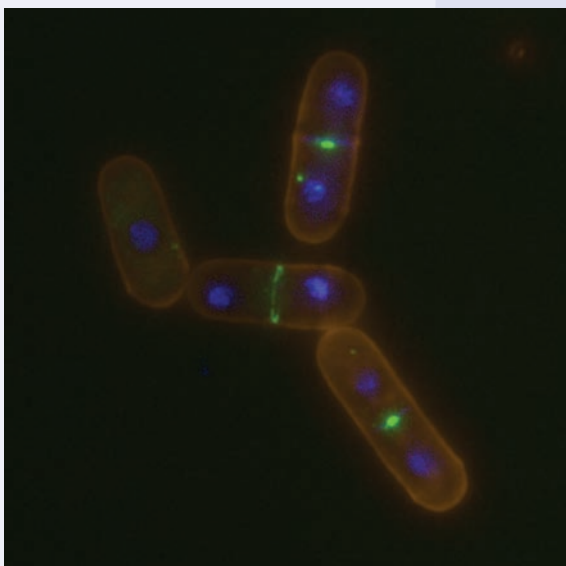
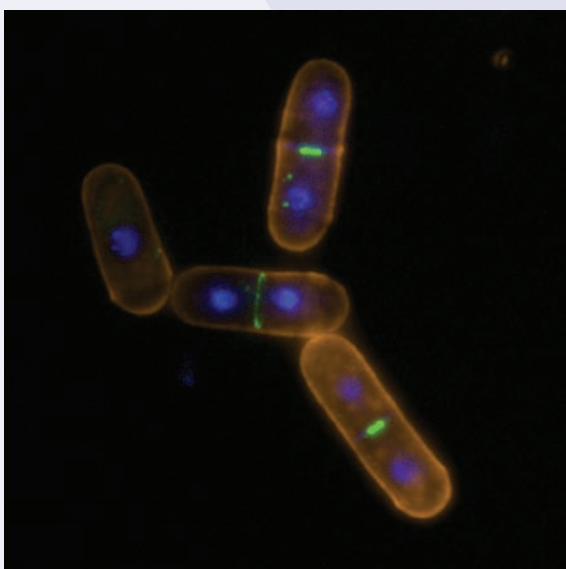
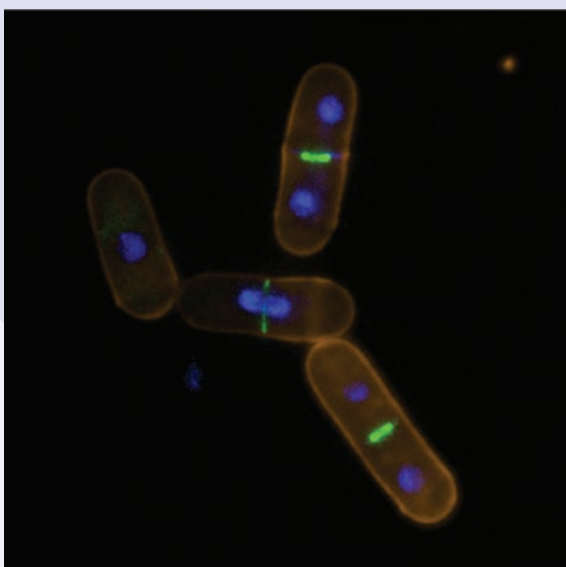
Head: **Steve Bagley**

The Advanced Imaging Facility's remit is to develop systems for the visualisation of biological processes, to facilitate good microscopy practice and to communicate new developments with regards to hardware, software and visualisation. The facility is utilised by nearly all of the groups within the Institute and offers consultation in microscopy systems to other research institutes.

Over the last year there has been ongoing development into the visualisation of live cells and examining cellular processes whilst reducing the affect of the visualisation process on the biology. As a result a microscope system has been developed which utilises EMCCD camera technology, light efficient optics and filters, and greater environmental control. The current systems have also been further enhanced to allow an image to be formed using minimal light, again lessening the environmental impact on cellular processes.

This year development of new techniques and imaging practices has been a priority and some of the projects being developed include:

- Development of spheroid imaging, refining the technique to allow the visualisation of hypoxic environmental studies
- Analysis of mitochondrial shape
- Photobleaching/FRAP investigations have been improved to allow numerical analysis of protein turnover and motility
- Improvements have been made in cellular array imaging so that multiple samples under different chemical environments can be imaged in the same visualisation environment
- Examining the effect and level of photo-induced stress when imaging under 'normal' conditions
- Investigation into the techniques required for correlative microscopy (photonic and electron microscopy).



Above: Cell division in fission yeast. Before these rod shaped cells (yellow) initiate genome separation they lay down a cytokinesis cleavage furrow (green) over the duplicated genome (blue). After the chromosomes have been pulled to either end of the cell, this ring contracts to separate the two daughter cells each of which now contains a single genome.

- Implementation of a laser autofocus method for utilisation with high numerical aperture lenses
- A new micro-injection system has been installed within the facility to allow the study of, amongst others, focal adhesion turnover in migrating cells along a wounded monolayer
- Assessing single molecule detection assays
- Refinement of GFP, mCherry and dTomato fluorescent protein imaging method

Over the next year, two major developments will be taking place in the facility. A new spinning disk imaging system will be installed and in conjunction with the histology service an automated whole section imaging system will be installed. After examining seven different real time confocal imaging systems which generate video rate images, a system and components have been ordered which will allow temporal 3D studies of proteins in conjunction with a real-time photobleaching system for protein generation/turnover studies.

The histology imaging system (to be installed in January 2008) will allow characterisation of tissue arrays and whole serial sections of tumours with a view to examination of histological staining in localised and tissue-wide areas. In previous investigations the investigator captured several fields of view that best described the data, whereas the new system will allow the whole slide to be imaged thus removing observer bias. It is hoped that this system will be further developed for three dimensional tissue imaging.

Biological Resources Unit

The Biological Resources unit at the Paterson is a modern transgenic facility that continues to support the scientific research programmes of the Institute. We provide the highest quality of welfare and the health status of the animals is paramount at all times. Continual refinements of techniques, reduction in numbers and replacement methods such as tissue culture are implemented. All animal work at the Paterson Institute that involves the use of rodents is covered by both Project and Personal licences that are issued by the Home Office and reviewed by a local ethical committee.

Transgenic Services

This area of the facility continues to increase with to date over 80 transgenic lines.

There are two dedicated transgenic technicians and an additional nine licensed technicians.

In order to create our novel transgenic mice we currently carry out two injection methods and there are several critical steps required for both processes.

Pronuclear Injection

- The design of an effective transgenic expression cassette
- Purification of high quality DNA



- Successful introduction of the DNA sequence by pronuclear injection into the mouse blastocyst

Embryonic Stem (ES) cell injection

- Engineering a gene targeting construct
- Transfection of ES cells
- Assessment of ES cells for homologous recombination
- Successful introduction of ES cells into the mouse blastocyst

For both methods blastocysts are then transferred into uteri of pseudo-pregnant fosters.

In addition to generating transgenic lines in house, we also bring in a number from collaborators and other sources. The rederivation of transgenic lines that are to be introduced into the facility is essential to ensure that the specific pathogen-free health status of the colonies is maintained. This year there have been a number of strains introduced such as the VE-Cadherin Cre mouse that is known to express the transgene in endothelial cells and so is a useful tool for studying lineage analysis and gene deletion, the OT-I Rag mouse that is T-cell deficient and thus useful for immunotherapy studies and the LCK Cre mouse for the studying the development of thymocytes. Because of the inherent value of the transgenic lines all strains are cryopreserved by isolating 2.5-day embryos into straws that are then stored in liquid nitrogen.

Experimental Services

This area of the facility is supported by two licenced technicians who carry out a range of surgical and non-surgical procedures. Some of the work is concerned with setting up timed matings, taking uteri and isolating embryos of a known gestation to support research into haematopoietic development and specifically the involvement of the *MOZ* gene in leukaemia. A large number of procedures involve xenograft models that require implantation of tumour cells, subsequent development of the tumour and then T-cell therapies. The biological resources staff dedicate a considerable amount of time to monitoring the experimental animals using established methods, for example measuring subcutaneous tumours and isolating *post mortem* tissues.

Equipment Purchase

To accommodate the increase in use within the facility additional IVC caging has been introduced and a laminar flow work station for the husbandry tasks and manipulation of the animals under procedure. We have also recently purchased an IVIS Lumina Imaging System which will allow us to perform *in vivo* optical imaging using fluorescent and bioluminescent reporters to track and monitor tumour growth. The system has integrated gas anaesthesia and a heated stage to maintain animal body temperature. The camera will be installed in early 2008 after the completion of minor building works.

Cancer Research UK GeneChip Microarray Service.

Head: Stuart Pepper

Over the last year we have seen significant developments in the area of microarray profiling with the release of new RNA extraction and labelling kits which are allowing researchers to work with archival samples in a way that has not previously been possible. This is an area that we have been working in for some time, and have previously completed two projects based on samples extracted from paraffin blocks. In both cases we were able to generate usable data, but the quality was not as good as we would normally achieve from fresh RNA extracts. During this year we have evaluated new reagents both for the extraction and labeling of RNA and have seen a major improvement in the quality of data that can be obtained. Essentially this leads to the possibility of conducting retrospective expression profiling studies on existing banks of archival material.

Last year we had introduced a new service run in conjunction with the CR-UK Genotyping facility headed by Mike Churchman. The service offers support for either linkage analysis or genomic copy number analysis using Affymetrix SNP arrays. This year we have conducted a pilot study using the new SNP6.0 array which has a stunning 1.8 million probe sets, making it the highest resolution platform for copy number analysis. Since presenting our pilot data at NCRI this year we have had significant interest in this service, with several projects already lined up.

The main workload for this service remains as expression profiling. Last year we introduced Exon arrays as an



alternative to standard 3' expression arrays. Exon arrays are able to detect alternative splicing as they have separate probe sets in each exon and intron of all known and predicted transcripts (1.4 million probe sets in total). The complexity of data that these arrays generate has made bioinformatics support more important for the service than ever. As well as our ongoing collaborations with the Paterson Bioinformatics Group (see page 10), over the last year we have had the benefit of having a bioinformatician working full time within the service team. As well as supporting users of the service this post has also allowed us to make sophisticated comparisons between array platforms and this year we were invited to present our work at the EMBL Data Analysis Workshop in Heidelberg.

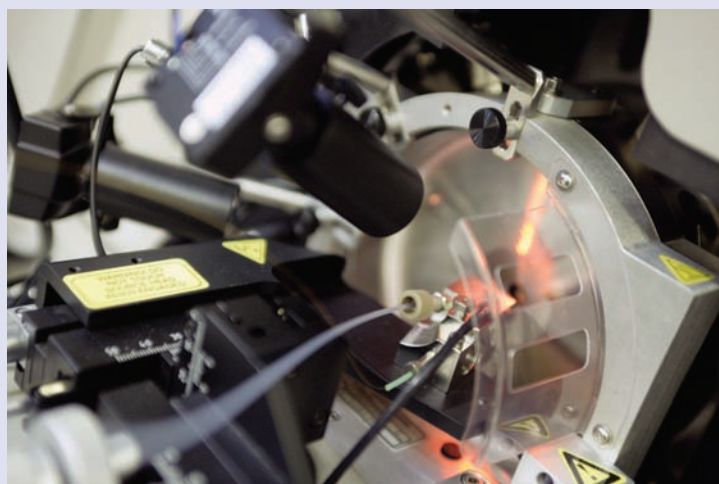
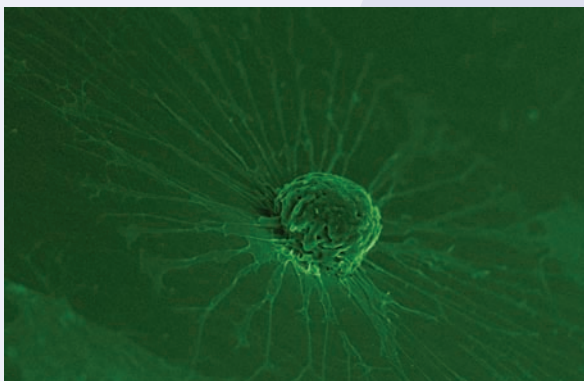
The latest generations of arrays have very large numbers of probes and take longer to scan than previous arrays. To allow us to process these new arrays we have added a second scanner to the lab. The array service now has a team of 4 people with a wealth of experience in microarray analysis of RNA and a good laboratory set up to meet the needs of cancer researchers for the year ahead.

Central Services

Head: Martin Chadwick (until September 2007),
Maurice Cowell (acting from September 2007)

Central Services comprises two distinct areas; porters and laboratory/sterile services. At the end of September Martin Chadwick left the Institute to embark on a career in recruitment, and at this time the nature of Central Services was reviewed. The outcome was the recommendation that the two components of the service were split.

The portering staff handle all deliveries to the Institute, distributing orders received and managing the Institute stores and the internal intranet ordering system. In addition they are involved in all aspects of waste management and recycling for both laboratories and the Institute as a whole. They also provide an invaluable service throughout the Institute in helping with moving equipment and furniture and ensuring that meeting rooms are set up as required.



Laboratory/sterile services are developing continually, particularly in the provision of sterile solutions and media. One main function is to provide assistance to each laboratory by a dedicated lab aide for a few hours a week to undertake basic technical duties and some laboratory management. This includes the daily (and sometimes twice daily) provision of sterile glassware and reagents and removal of used glassware for washing and autoclaving. Media supply is a rapidly growing area, and one in which we will be investing more in the future. In particular we have more and more critical requirements for specialist media for the growth of bacterial and yeast cells. We will shortly be appointing a new Laboratory Services Manager who will take over the running the current sterile/laboratory services, and develop the service, and in particular the media preparation, as required.

Flow Cytometry Unit

Head: Morgan Blaylock

The Flow Cytometry facility presently has two high speed sorters, the FACSVantage and the FACSria and two benchtop analysers, the FACSCalibur and the FACScan. The facility as a whole is going through a number of radical changes. Last February the facility underwent a review resulting in the recruitment of Dr. Morgan Blaylock as head of Flow Cytometry. He has recently moved to the Paterson from the Institute of Medical Sciences at the University of Aberdeen where he established a flow cytometry core facility more or less from scratch. At this time it was also decided to purchase a third benchtop analyser to assist with our increasing workload and allow us to move into the field of polychromatic flow, the new system should allow us to detect up to 18 colors from 4 different laser sources which a much higher cellular throughput. We have recently acquired a BD FACSArray which will allow us to move into high throughput multiplex bead based assays which will essentially allow us to multiplex ELISA based technology from very small samples. Furthermore, it supports cellular analysis; apoptosis, cytokine and chemokine profiling, and phosphorylation of key signal transduction proteins. We are finishing the year with a



complete refurbishment of the current lab space with removal of the current office and upgrade of the ventilation and water systems.

In June we had some problems with sterile sorts on the FACSaria due to bacterial contamination. We decided to remove the "Pall" filter from the sheath line and replace it with a sterile plastic Millipore filter with a 0.22 micron membrane that could be changed every day. Recently we have updated this system to incorporate more robust filter units which should mean an end to contamination worries.

We have developed a number of new projects this year involving the Genito-Urinary Cancer Research group; they have been looking into methods of enriching the side-population using Pyronin Y staining. This enables them to identify quiescent cells within the side-population to enhance the population of true stem cells. They have also used single cell sorting into 96 well plates to assess the colony forming efficiency of these cells and have found that there was a significantly greater colony forming efficiency in the G0 side-population cells compared to unselected side-population controls.

The Breast Biology group has used the FACSaria to sort MCF7 cells based upon the expression of various surface markers (eg. CD44, CD24, ESA). This has allowed them to assess the presence of stem cells within each cell sub-population using mammosphere culture. Initially there were a number of issues with high cell death however sorting at a reduced pressure (16PSI) and using HBSS as sheath fluid proved to be less damaging to their cells. They are now using this system to study the effect of different culture conditions e.g. inhibitors of signalling on the stem-like sub population.

The Stem Cell Research group has been using recently defined cell surface markers to isolate early haematopoietic precursors both from early mouse embryos and from *in vitro* differentiating embryonic stem cells. These first fully committed blood precursors can be identified and sorted according to their differential expression of alpha II integrin (CD41) and mucosialin (CD34). Nearly pure precursor subpopulations allow them to further understand and dissect

the molecular and cellular mechanisms that regulate self-renewal and differentiation at the onset of haematopoietic development.

Histology

Head: Garry Ashton

As in previous years the unit has prepared and analysed a wide range of samples including mouse tissues, whole mouse embryo preps, cell preps, zebrafish, human biopsies and tissue microarrays. All our key services have once again seen heavy demand, and as a result of this and the unit's development, we are recruiting another scientific officer:

The development of laser capture microdissection has continued. The extraction of high quality RNA from frozen sections is now a routine technique, demonstrated by the continued work with the Cell Signalling group, looking at the expression of Tiam1, STEF and pRex1 homologues in wild type, benign and malignant melanocytes in the zebrafish melanoma model.

In collaboration with the Cell Regulation group we have also been able to extract RNA from cells identified by their immuno phenotype. Using the islet-1 mAb, the hypoglossal nucleus within the brain was captured and high quality RNA extracted. Even more exiting and in collaboration with the Angiogenesis group, we captured both carcinoma and sarcoma cell populations from FFPE ovarian carcinosarcoma samples and using the Paradise reagent system, extracted RNA from these for analysis by real time PCR. This is a major development at it leads to possibility of extracting RNA from archival FFPE samples. In collaboration with the Molecular Biology Core Facility we are also evaluating alternative kits and the minimum number of cell required to extract RNA from LCM FFPE samples.

With the Clinical and Experimental Pharmacology group (CEP) immunohistochemistry (IHC) has been used as a pharmacodynamic method for both pre-clinical models and clinical trial use. Several staining protocols have been used,



some validated by CEP to GCLP. Staining of cleaved caspase 3, cleaved Parp and Hif 1 α has been undertaken on pre-clinical xenograft samples. Staining for one particular antigen the X-linked inhibitor of apoptosis (XIAP) needed extensive optimisation before clinical trial samples could be successfully stained and scored.

Also in collaboration with the Cell Regulation group, multiple IHC labelling was used to identify cells in the developing liver of ATF2/ATF7 mutant mouse embryos that display severe levels of apoptosis. Apoptotic markers were used with erythroid lineage (Ter119) and hepatoblast specific markers (cytokeratin 18). Furthermore, we demonstrated that elevated levels of stress-activated MAP kinase activities were present in erythroid and hepatocyte lineage cells of mutant embryonic livers, thus providing an underlying mechanism for the observed apoptosis in these cell types.

The unit has also recently purchased an automated IHC platform. Linked to this, two new epitope retrieval systems have also been introduced, allowing for higher throughput and improvements in standardisation. In collaboration with the Advanced Imaging Unit, a Zeiss Mirax slide scanning system has been purchased. This allows multiple fields to be imaged and stitched together, allowing for a final image that is not resized and characterises the section.

Finally from early 2008 the new Manchester Cancer Research Centre Biobank will be housed within the Histology unit. To accommodate this, together with the new equipment purchased and new staff, the unit will be expanded and reorganised over the coming months.

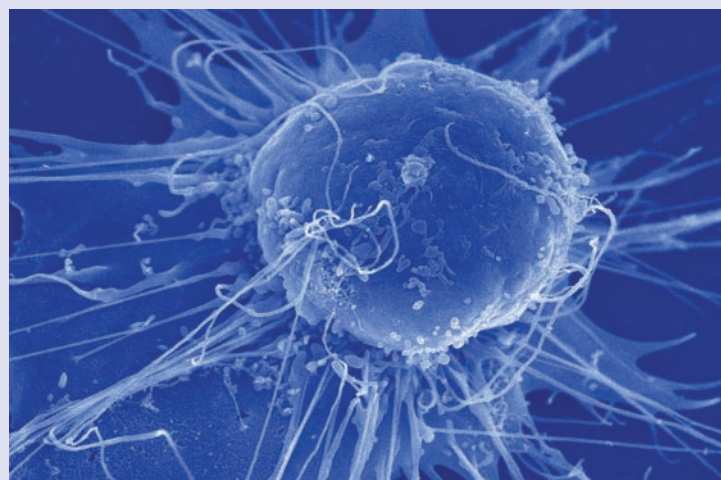
Kostoris Library

Head: Steve Glover

The Kostoris Library provides a service to staff and students at Christie Hospital Foundation NHS Trust and the Paterson Institute for Cancer Research. Sited near clinical oncology on the 2nd floor of the Nathan House courtyard, the library holds textbooks, PhD theses and journals.



Sven-Goran Eriksson hands out the runners-up certificate for the 2007 education team of the year award



Staff of the Paterson Institute can access the online journals of The University of Manchester from their desktop PCs within the Institute. Library staff work in close liaison with the health sciences and electronic resources teams at the university and provide an onsite point of contact for access to resources.

Library services are predominantly electronic and Institute staff access over 15,000 full text articles per annum. The library works with The University of Manchester to supply comprehensive access to the biomedical literature and provides access to over 10,000 journals.

Databases include PubMed, Medline, BIOSIS, ISI Web of Knowledge, EMBASE, and Scopus. The library also offers a personalised database surveillance service in which regular search queries are run on a monthly basis to keep staff up-to-date with the latest published research. The library also provides an electronic table of contents service with links to full text delivered to the desktop.

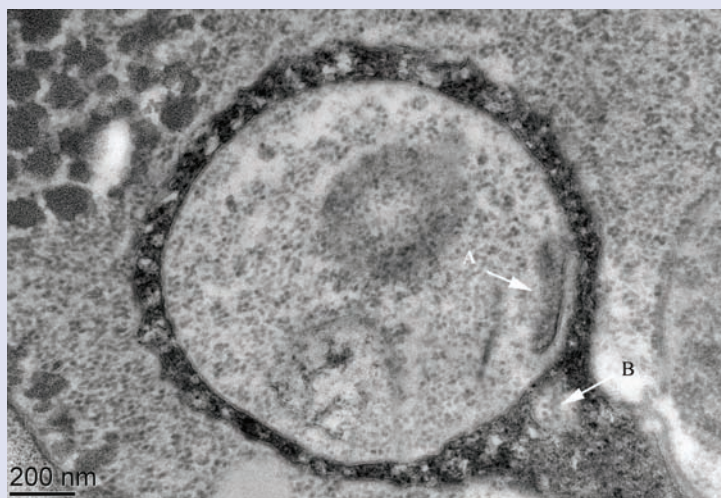
In 2008 the library will embark on creating a searchable database of all Christie Hospital and Paterson Institute publications as part of a web-based institutional repository. This will be accessible from the Christie Hospital website. 2008 may also see a library refurbishment with an updated facility offering a modern study environment.

Molecular Biology Core Facility

Head: Stuart Pepper

The Molecular Biology Core Facility is split into three service areas providing Mass Spectrometry based protein analysis, a variety of DNA and RNA services and a team running the CR-UK GeneChip Microarray service which is detailed separately in this section.

For the plasmid DNA extraction and sequencing services there have been few changes this year. Both services are



Electron micrograph of an autophagic body in *S. cerevisiae* showing partly lysed mitochondrion (A) and Lysosome (B)

supported by very robust equipment which provides reliable services. Users are able to have miniprep DNA isolated each morning in time for sequencing the same afternoon, and this 24- hour turnaround for bacterial culture to sequence information has remained a very popular combination. After 7 years of use our sequence machine has now run approximately 100,000 sequences, but is beginning to show signs of age. As the year ends we are upgrading the system to allow higher throughput as well as enhanced sequence analysis software.

For the last two years we have supported expression profiling on an ABI 7900 system, with a small robot to provide pipetting into a 384 well plate. This system has allowed us to offer very flexible support for quantitative PCR, however with the new Exon and tiling arrays the demand for qPCR is growing. To meet this growing demand we have been evaluating a novel system from Beckman which allows a high level of multiplexing of assays in each PCR. In a single day it is possible to analyse up to 25 genes in 96 samples, which is a significant increase in throughput compared to a 384-well plate. Initial trials have proved the value of this system in validating Exon microarray data (see GeneChip service section) and over the next year this platform will become an integral part of the facility.

During the last year we have continued to maintain our routine services for protein identification and phosphorylation site mapping. This year has also seen some major developments with new services being developed, particularly the introduction of quantitative protein expression profiling by SILAC (stable isotope labeling of cells) to complement our existing iTRAQ based profiling. The use of SILAC facilitates robust quantitation in the context of complex workflows such as immunoprecipitations or subcellular fractionations. This service was piloted on a project looking at the role of the plasma membrane

proteome in cellular invasion and is now offered as a standard service.

This year has also seen the development of quantitative phosphorylation mapping as a natural extension to our existing phosphorylation mapping service. We analysed the level of phosphorylation at 16 sites within Tiam1 and were able to identify which sites were cell cycle dependent. Towards the end of the year we have added a novel workflow to allow discovery of phosphorylation sites which have previously been impossible to map, this new method has already lead to the identification of novel phosphopeptides within a previously well characterized protein.

Looking ahead to next year events will be dominated by the installation of our newly delivered Orbitrap mass spectrometer and nanoUPLC system. This platform will initially be used to develop high throughput gel walking workflows that will allow sensitive characterisation of protein mixtures. Subsequently we will use the exceptional resolution, mass accuracy and sensitivity of this platform to develop other cutting edge services.

Transmission Electron Microscopy Service Facility

Head: Steve Murray

This facility provides a TEM service to the institute, complementing the well established Advanced Imaging Facility and providing users with the next step in imaging resolution of biological samples. With the recent advances in cryo technologies and in particular the development of commercially available equipment such as the high pressure freezer; the level of ultrastructural preservation attainable in electron microscopy has increased dramatically. The cell is a very dynamic entity with numerous process and interaction events occurring every second. To fully understand these dynamic events it is necessary to place them in their ultrastructural context. This requires a level of resolution which at the present time can only be achieved with electron microscopy.

Over the year the institute has upgraded the digital image acquisition system of the JEOL 1220 TEM with the addition of a 10 megapixel Gatan Orius CCD camera. As a result, there has been a ten-fold increase in the resolution of the captured images, which combined with the higher sensitivity and speed of the camera has enabled the imaging of specimens using higher accelerating and lower illumination levels without loss of image contrast. The new camera has been put to heavy use by the Cell Cycle group who are investigating the role of a novel cytokinesis protein and how the contraction of the actomyosin ring at the future cleavage site is coupled to ingression and abscission of the plasma membrane in *S. cerevisiae*. This study has required the extensive use of serial

section ultramicrotomy and subsequent TEM observation and tracking of individual cells through numerous sequential sections.

Other projects requiring electron microscopy have included the study of ultrastructural differences in the dorsal vagal nucleus and the hypoglossal nucleus of wild type, knock out and heterozygous mouse embryo brain stems.

The Stem Cell Biology group has used the service to gain further insight into the stages of early haematopoietic development and the presence of the haemangioblast using *en-face* and tangential sectioning techniques of chemically-fixed, resin-embedded cell cultures. The Immunology group has been studying the ultrastructural localisation of the 5T4 oncofoetal antigen in mouse ES cells during differentiation using TEM immuno-gold labelling methodologies.

The Targeted Therapy group has made use of the TEM service to further elucidate the ultrastructural changes which occur following combination monoclonal antibody treatment and irradiation. TEM was required to investigate the mode of cell death induced by the combined treatment with anti-CD20 antibodies and irradiation. This cell death is TUNEL-negative and does not respond to inhibition of caspases, therefore it is not apoptosis. TEM was needed to confirm that this is cytoplasmic cell death (accompanied by gross vacuolization of cytoplasm with relatively intact nuclei). Cell death is accompanied and functionally linked to antibody-induced homotypic adhesion of cells. For that reason, it was essential to investigate the ultrastructure of cell-cell contact areas and to see the "syncytium" (previously observed in phase contrast) at TEM level.

The recent purchase of an automatic freeze substitution unit with automatic solution changing facility has enabled the use of far more complex freeze substitution protocols leading to greater ultrastructural preservation and visualisation of cellular structures.

During the coming twelve months a project involving the use of 3D electron tomography will be instigated and in collaboration with the Institute's AIF the use of correlative light and electron microscopy will be further developed. The possible use of microwave processing for room temperature EM protocols will also be assessed.

Research Publications

Crispin Miller (page 10)

Applied Computational Biology and Bioinformatics Group

Refereed Research Papers

Meyer, S., Fergusson, W.D., Whetton, A.D., Moreira-Leite, F., Pepper, S.D., Miller, C., Saunders, E.K., White, D.J., Will, A.M., Eden, T., Ikeda, H., Ullmann, R., Tuerkmen, S., Gerlach, A., Klopocki, E. and Tonnies, H. (2007) Amplification and translocation of 3q26 with overexpression of EVI1 in Fanconi anemia-derived childhood acute myeloid leukemia with biallelic FANCD1/BRCA2 disruption. *Genes Chromosomes Cancer*, **46**, 359-372.

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Yates, T., Okoniewski, M.J. and Miller, C.J. (2007) X-Map: annotation and visualization of genome structure for Affymetrix exon array analysis. *Nucleic Acids Res*, epub Oct 11.

Geoff Margison (page 12)

Carcinogenesis Group

Refereed Research Papers

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Karim Labib (page 14)

Cell Cycle Group

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Iain Hagan (page 16)

Cell Division Group

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Nic Jones (page 18)

Cell Regulation Group

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Caroline Dive and Malcolm Ranson (page 22)

Clinical and Experimental Pharmacology Group

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Peter Stern (page 24)

Immunology Group

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Nullin Divecha (page 26)

Inostide Laboratory

Refereed Research Papers

Bins, A.D., van Rheenen, J., Jalink, K., Halstead, J.R., Divecha, N., Spencer, D.M., Haanen, J.B. and Schumacher, T.N. (2007) Intravital imaging of fluorescent markers and FRET probes by DNA tattooing. *BMC Biotechnol*, **7**, 2.

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Tim Somervaille (page 28)

Leukaemia Biology Group

Refereed Research Paper

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Jamal Zweit (page 30)

Radiochemical Targeting and Imaging Group

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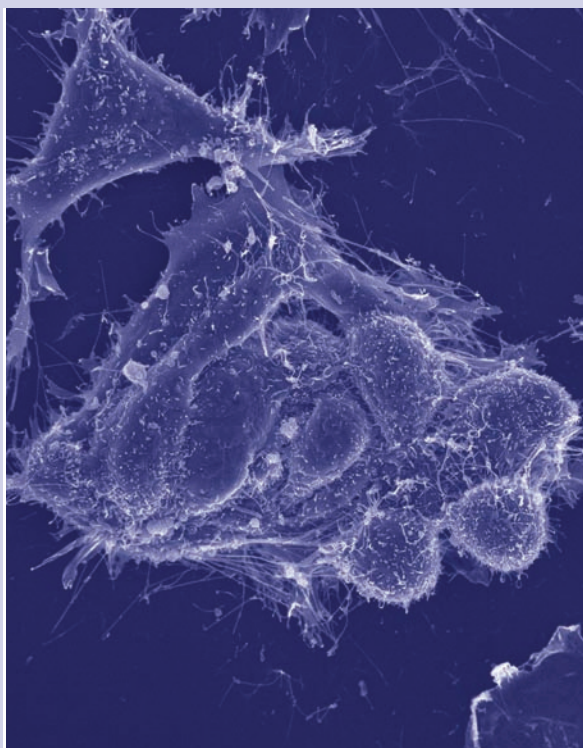
Georges Lacaud (page 32)

Stem Cell Biology Group

Refereed Research Papers

Breitwieser, W., Lyons, S., Flenniken, A.M., Ashton, G., Bruder, G., Willington, M., Lacaud, G., Kouskoff, V. and Jones, N. (2007) Feedback regulation of p38 activity via ATF2 is essential for survival of embryonic liver cells. *Genes Dev*, **21**, 2069-2082.

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Valerie Kouskoff (page 34)

Stem Cell and Haematopoiesis Group

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Akira Orimo (page 36)

Stromal-Tumour Interaction Group

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Catharine West (page 38)

Academic Radiation Oncology: Translational Radiobiology Group

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Robert Hawkins and Peter Stern (page 40)

Biological, Immune and Gene Therapy

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Vaskar Saha (page 42)

Children's Cancer Group

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Tim Illidge (page 44)

Targeted Therapy group

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Robert Hawkins (page 46)

Medical Oncology: Cell and Gene Therapy Group

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John Gallagher (page 48)

Medical Oncology: Glyco-Oncology Group

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Gordon Jayson (page 50)

Medical Oncology: Translational Angiogenesis Group

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Additional Publications

Allen, T.D., Rutherford, S.A., Murray, S., Gardiner, F., Kiseleva, E., Goldberg, M.W. and Drummond, S.P. (2007) Visualization of the nucleus and nuclear envelope in situ by SEM in tissue culture cells. *Nat Protoc*, **2**, 1180-1184.

Allen, T.D., Rutherford, S.A., Murray, S., Sanderson, H.S., Gardiner, F., Kiseleva, E., Goldberg, M.W. and Drummond, S.P. (2007) Generation of cell-free extracts of *Xenopus* eggs and demembranated sperm chromatin for the assembly and isolation of *in vitro*-formed nuclei for Western blotting and scanning electron microscopy (SEM). *Nat Protoc*, **2**, 1173-1179.

Allen, T.D., Rutherford, S.A., Murray, S., Sanderson, H.S., Gardiner, F., Kiseleva, E., Goldberg, M.W. and Drummond, S.P. (2007) A protocol for isolating *Xenopus* oocyte nuclear envelope for visualization and characterization by scanning electron microscopy (SEM) or transmission electron microscopy (TEM). *Nat Protoc*, **2**, 1166-1172.

Arhel, N.J., Souquere-Besse, S., Munier, S., Souque, P., Guadagnini, S., Rutherford, S., Prevost, M.C., Allen, T.D. and Charneau, P. (2007) HIV-1 DNA Flap formation promotes uncoating of the pre-integration complex at the nuclear pore. *Embo J*, **26**, 3025-3037.

Carrol, E.D., Salway, F., Pepper, S.D., Saunders, E., Mankhambo, L.A., Ollier, W.E., Hart, C.A. and Day, P. (2007) Successful downstream application of the Paxgene Blood RNA system from small blood samples in paediatric patients for quantitative PCR analysis. *BMC Immunol*, **8**, 20.

Cotter, L., Allen, T.D., Kiseleva, E. and Goldberg, M.W. (2007) Nuclear membrane disassembly and rupture. *J Mol Biol*, **369**, 683-695.

Kiseleva, E., Allen, T.D., Rutherford, S.A., Murray, S., Morozova, K., Gardiner, F., Goldberg, M.W. and Drummond, S.P. (2007) A protocol for isolation and visualization of yeast nuclei by scanning electron microscopy (SEM). *Nat Protoc*, **2**, 1943-1953.

Kiseleva, E., Morozova, K.N., Voeltz, G.K., Allen, T.D. and Goldberg, M.W. (2007) Reticulon 4a/NogoA localizes to regions of high membrane curvature and may have a role in nuclear envelope growth. *J Struct Biol*, **160**, 224-235.

Seminar Series 2007

We had another exceptionally good seminar series again in the Institute, with speakers from around the world. This series is complemented by seminars within the Christie Hospital, a Gene Therapy seminar series with a mixture of local, national and international speakers and many seminars at The University of Manchester. Finally the postdoctoral lunchtime seminar series was once again very popular.

Professor Yves Barral

Institut für Biochemie, Zurich, Switzerland.

Sir Tom Blundell FRS

Sir William Dunn Professor and Chair, School of Biological Sciences, University of Cambridge, Cambridge.

Professor Alan R Clarke

Cardiff School of Biosciences, Cardiff University, Cardiff, Wales.

Dr Vincenzo Costanzo

London Research Institute, Clare Hall Laboratories.

Dr Julian Downward

CR-UK London Research Institute, London.

Professor Yuri E Dubrova

University of Leicester, Leicester.

Dr Elaine Dzierzak

Erasmus Medical Center, Rotterdam, The Netherlands.

Dr Neil Gibson

OSI Pharmaceuticals, Melville, NY, USA.

Dr Bertie Gottgens

Cambridge Institute for Medical Research, Cambridge.

Professor Roger Griffin

Northern Institute for Cancer Research, Newcastle.

Professor Keith Gull

Sir William Dunn School of Pathology, Oxford.

Professor John Heath

School of Biosciences, University of Birmingham.

Dr Stefan Holdenrieder

University of Munich, Germany.

Dr Mike Hubank

Institute of Child Health, University College London, London.

Professor Steve Jackson

The Gurdon Institute, University of Cambridge, Cambridge.

Professor Yvonne Jones

The Division of Structural Biology, University of Oxford, Oxford.

Professor Bernd Kaina

University of Mainz, Germany.

Dr Robert Lucas

Faculty of Life Sciences, University of Manchester, Manchester.



Professor Olaf Nielsen

Institute of Molecular Biology and Physiology, University of Copenhagen, Denmark.

Dr Mark Petronczki

Institute of Molecular Pathology, Vienna, Austria.

Dr Aloys Schepers

Department of Gene Vectors, GSF, Munich, Germany.

Professor Gil Smith

National Cancer Institute, Bethesda, Maryland, USA.

Professor Hans Snoeck

Mount Sinai School of Medicine, New York, USA.

Dr Tomoyuki Tanaka

College of Life Sciences, University of Dundee, Scotland.

Professor Ian Tomlinson

Molecular & Population Genetics Laboratory, London Research Institute, London.

Professor Bryan Turner

Institute of Biomedical Research, University of Birmingham Medical School.

Dr Isabelle Wartelle

Project Manager – ATTACK, The University of Manchester, Manchester.



Postgraduate Education

Postgraduate Tutor **Graham Cowling**
Education Coordinator **Julie Edwards**

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

The first step in starting a successful career in cancer research is finding the right PhD project for you, an inspiring supervisor and a programme that will allow you to develop your skills as a junior researcher. At the Paterson, the year on year popularity of our projects would suggest that we offer such exciting research opportunities.

Our supervisors are all first class, many with years of experience of encouraging research students to realise their full potential. By registering our students through The University of Manchester Faculty of Medicine we can also offer robust training schemes allowing them to get the individual support they need in laboratory research, data handling, writing, presentation skills and careers advice. We strive to take carefully selected students who are research aware through stages of research competency and turn them into junior scientists capable of hard and productive postdoctoral research in any laboratory in the world.

The Paterson PhD Programme

In 2007 we welcomed seven new Paterson 4-year PhD students from the around the world, who were selected from over thirty interviewed candidates who were chosen from many hundreds of applications received by the Institute during the year. A further four new PhD clinical fellows also started work in the Institute. Two of these were CR-UK funded and two AstraZeneca Fellowships. The Faculty also awarded a strategic PhD studentship in cancer and we welcomed a CR-UK four year PhD student from the Netherlands and self-funding students from Portugal and Qatar. The contributions of all our growing number postgraduates to the scientific work of the Institute are described elsewhere in this report. The University of Manchester Faculty of Medical and Human Sciences underwent a major restructuring early in 2007. Our 80 PhD and MD students became part of a new School of Cancer and Imaging Sciences. While one of the smallest of the five new research Schools created from the old Medical School, we attempt to be one of the most dynamic with respect to

our postgraduate programmes and continue to maintain the highest possible standards in project quality, candidate selection and student progression and yet retain our policy of individual care for every student.

Why do a PhD here?

Postgraduate students entering the Institute to study for the degree of PhD are under the direct guidance of an appointed supervisor(s) but are also allocated an advisor, with whom they meet regularly to review and record progress, set new targets and identify any assistance required. In 2007, a Student Welfare Group was established to further support the supervisor and advisor system. This small subgroup links the Education Committee and Human Resources in the Institute to monitor the progress of all students and offers early help when things may not be progressing smoothly in their project or when there may be a communications problem with their supervisor or advisor.

All postgraduates are also required to participate in regular research meetings within their research group and to attend an organised series of seminars by national and international speakers, which runs throughout the year in the Paterson Institute, Christie Hospital and The University of Manchester. To further enhance our training programme, lead researchers in the Paterson and Christie Hospital explain the "what", the "how" and the "why" of their area of research at periodic and relaxed evening sessions. We also encourage students to attend postgraduate taught courses that are relevant to their project work. Students need to participate in a structured Faculty graduate training programme of short courses and workshops depending on their research needs. This includes

sessions on statistics and data handling, safety, innovation, written and oral presentations, careers, animal usage and ethics. The Faculty training sessions have continued to allow students to develop generic research and personal skills.

All new postgraduate research projects are assessed for quality. Based on Research Council principles, the Education Committee, with help from internal and external assessors, ensure that the work will form the basis of a stimulating and intellectually challenging postgraduate degree. The Education Committee, a body made up of senior scientists, postdoctoral fellows and student representatives, continue to assess

student progress throughout their 3-4 years of study and ensures, along with their supervisors and advisors, that the student achieves their degree goal. Assessment stages can be seen on our website. Students can ask advice of any member of the Education Committee or use their student representatives. There are always two student representatives within cancer and as well as representing the students' views on various committees they are usually found to be at the centre of many social events organised throughout the year.

Education Committee 2007

- | | |
|-----------------------|------------------------------|
| Iain Hagan – Chairman | Fiona Blackhall |
| Caroline Dive | Tim Illidge |
| Dave Gilham | Karim Labib |
| Valerie Kouskoff | Gordon Jayson |
| Crispin Miller | Noel Clarke (until June) |
| Jenny Varley | Vaskar Saha (from September) |
| | Graham Cowling |

Student Representative 2007

- Katalin Boros (until October)
Martin Brandenburg (until October)
Dorota Feret (from November)
Natalie Reeves (from November)





Operations Services

Director of Operations **Pippa McNichol**

Deputy **Margaret Lowe**

<http://www.paterson.man.ac.uk/operations/>

2007 has been a year of consolidation after the transfer of the Paterson Institute to The University of Manchester in 2006. It has also been a year of implementation as the new Cancer Research UK Contribution Review mechanism was put into practice for the first time. This has resulted in a fair and transparent system which rewards achievements and success. Staff were given the opportunity to elect their own representative onto the Contribution Review Panel and the union representative also became a member.

The acquirement of the new IT storage and archive system required us to use the European tendering process which provided a learning opportunity for everyone involved. Once the new system is fully installed, the Institute will have tremendous storage capacity and an incredibly robust archiving and Disaster Recovery system. The Procurement Manager has also been busy with the introduction of the Oracle on-line purchasing system which has meant that all staff who purchase items for their labs have had to be trained on the new University of Manchester system.

The Editorial team gave the Paterson's newsletter a facelift, which has resulted in a much better looking, more dynamic publication.

The Estates department has managed a large mixture of minor and major capital works programmes during the year, as well as having to deal with a very bad flood in our basement during the bad weather in July.

The Health & Safety Manager oversaw the introduction of a new freezer monitoring system for all the -80 freezers which has proved to be very successful and stops valuable research from being destroyed if a freezer breaks down.

We are proud of our continuing collaboration with 'Worklink', Manchester's Employment Service, which supports people with a disability or long term illness who are seeking employment. We have provided employment to five people and hope to increase this in the future.

The Postdoc Careers Club started in October with an exciting programme of external speakers providing information on a wide range of possible career choices for our postdocs and final year PhD students. It is planned to run an 'Introduction to Management course' in 2008, as part of the club.

All the Operations departments have been reviewed by Towers Perrin, external management consultants, and at the time of writing (early January 2008) their findings and recommendations are eagerly awaited. It is anticipated that these will be implemented during the first quarter of 2008.

Admin and Reception Services

Manager: **Julie Hallett**

Shirley Leonard, Carl Oluwole

During 2007 the team provided a considerable amount of behind the scenes organisation for the four site visits which took place, ranging from typing and collating papers to organising lunch and refreshments.

A new site manager at Mitie (the security company used on site) provided a good opportunity to review the procedures currently in place. New site instructions were drawn up and additional relief guards trained to ensure continuity of the Reception service. Internally, members of the Central Services Team were also trained to provide 'short period' cover such as lunch breaks.

2007 was the first year of the CR-UK Contribution Review Panel and the office provided valuable assistance to the Operations Manager with all the paperwork.

The Admin department helped the Assistant Director (Research) with the organisation of the annual Colloquium, which was a great success. The team also assisted Stuart Pepper with the organisation of the Student Day and Peter Stern with the Stem Cell Away Day.

Director's Office

Director's PA: Elaine Mercer

The Paterson Seminar Series continued to go from strength to strength throughout 2007 and provided a comprehensive and impressive list of guest speakers, both nationally and internationally. The forthcoming series for 2008 has now been planned and we can expect another exciting and enjoyable timetable of seminars. This series is very important to the Institute, particularly for the students and postdoctoral fellows since they become exposed to world-class research in the broad spectrum of cancer research.

Assistance was given to the MCRC Operations Manager for the planning and organisation of all the various MCRC Boards/Committees, of which there were many.

In 2007 the Paterson Newsletter took on a different more professional and aesthetically pleasing format, which has proved very popular with the readership. The circulation has grown considerably with a far greater distribution within the fundraising community.

Recruitment has been a major focus for the Director throughout the year, with potential Group Leader candidates being invited to give seminars and meet with existing Group Leaders and key members of staff within the Institute. Also recruitment associated with the MCRC began with particular priority on clinical research and molecular pathology.

Estates

Manager: Steve Alcock

Graham Hooley, Tony Woollam

2007 was a challenge for the Estate's team. There was a considerable number of major and minor capital works carried out in the Paterson. Funding was secured to allow the fitting out and refurbishment of new and existing laboratories on the second floor of the Paterson and in addition some essential refurbishment on the ground floor is ongoing. The minor schemes include the refurbishment of the IT server room area, a replacement chiller to the ventilation plant supplying the Kay Kendall laboratories, improved ventilation to the virus room, and many small schemes to improve facilities for the scientific groups.

In the middle of the capital works programme the team overcame the July floods which caused many thousands of pounds worth of damage. There have also been a number of mechanical and engineering issues associated with the Translational Research Facility (TRFI) which have been

addressed, including minor repairs and improvements to heating controls.

Estates have been pro-active throughout the year which has resulted in a positive response from the user group set up in 2006. The team members have attended relevant courses to improve skills and keep their knowledge up to date with current working practices and changing legislation.

Finance and Purchasing

Manager: Margaret Lowe

Liz Fletcher, David Jenkins, Denise Owen, Debbie Suthern

The provision of a comprehensive purchasing, travel and finance service on a daily basis to the Research Groups and Service Units within the Paterson Institute continued to keep the department working to full capacity. In addition to the Paterson core groups and service units, orders and invoices were also processed for the other University departments which are located within the Paterson Institute and the Kinnaird Road Laboratories.

Internet Procurement has been introduced throughout the Institute which initially required substantial input from the Procurement Section to ensure everybody was fully conversant with the system requirements, and they continue to provide support where required. This has now been in practice for several months and feedback has been mainly positive. Arrangements have been made for the new groups coming into the Institute to be trained on the system.

The Procurement Manager spent a large amount of time co-ordinating the tender process for the IT storage and archive system.

Relationships with the various University of Manchester departments have continued to develop. Since the beginning of the year all Paterson finances have fed through the Faculty of Medical and Human Sciences. This has resulted in a close relationship developing with the Faculty Accountant and the Faculty Research Team. We regularly attend Faculty meetings and are now included on all distribution lists so all relevant information is disseminated. As changes occur within the University more tasks are being devolved down from Faculty to School level. This will certainly involve the department and training courses are currently being arranged by the Faculty.

As the Institute continues to enhance its research activities with new group leaders and new initiatives, the department is looking forward to incorporating all the new developments into its structure.

Health & Safety

Manager: Colin Gleeson

A priority has been to improve the security and protection of much of the Institute's research and intellectual property housed in some thirty-five -80° freezers and within the information storage systems within the IT department. This was achieved by a successful roll-out of the -80° freezer monitoring and alarm system and an upgraded intruder alert system in the IT department. Building and personal security was also improved by an increase in the number of recorded surveillance cameras at key points around the building.

Safeguarding the health and safety of our staff is a continuing priority. Health and safety training has been provided in a number of areas including induction, risk assessment, work with biological agents and genetically modified (GM) organisms, COSHH, compressed and cryogenic gases, handling radioactivity, manual handling, fire and ladder safety. Informal and formal safety inspections have been carried out of a number of areas, and accidents and dangerous occurrences have been investigated. Performance testing of

the key laboratory engineering controls, fume cupboards and microbiological safety cabinets has been successfully completed. The annual Environment Agency inspection of our arrangements to handle and dispose of radioactivity was successful with no recommendations or remedial actions deemed necessary.

Our relationships with The University of Manchester and Christie Hospital NHS Foundation Trust are on a sound footing. This is demonstrated by reciprocated membership of various health and safety committees, attendance at regular meetings and informal contact. Liaison with the new Occupational Health provider in the Trust has enabled the continued health surveillance of our employees, especially of those carrying out GM work or using cytotoxic drugs.



The Operations team

HR

Manager: Anna Pearson

Laura Humes

Over the past year, the HR Department has provided both managers and staff with sound HR advice and offered guidance on all employment-related matters ensuring consistency and fairness across the Institute.

The team has undertaken a vast amount of recruitment this year covering a wide variety of areas. In September, the Paterson had an exhibition stand at a Science Career Fair in London to promote the Institute and in particular, attract postdoctoral candidates to apply for vacancies. This had a positive effect on both the quality and quantity of recruitment applications.

The recruitment and induction process from offer letter to the end of the probationary period has been improved and is currently undergoing a trial period to evaluate the effectiveness of the changes.

The HR Manager has successfully implemented several new and amended policies after ratification by the Joint Negotiation Committee including the Respect at Work Policy, Flexitime for Operations Staff Policy, No Smoking Policy, IT Policy, Associate Scientists Policy and a policy for monitoring Good Clinical Laboratory Practice. To coincide with the launch of these policies, training courses and seminars have taken place, for example, anti-bullying and harassment workshops were delivered by ACAS in conjunction with the implementation of the revised Respect at Work policy.

The HR Manager has developed the HR strategy with areas for priority and action points with timescales will enable the team to focus on proactive assignments in 2008 that will add value to the processes and practices of the Institute.

IT

Manager: Malik Pervez

Brain Poole, Steve Royle, Zhi Cheng Wang, Matt Young

For any 21st Century organisation, Information Technology and electronic communications have become crucial to the successful achievement of business objectives. In reality it is the actual data and information held within such system that is of greatest importance. Extensive amounts of data are now held electronically; therefore storage presents a complex challenge for organisations.

Research organisations such as the Paterson must seek solutions that are cost effective, scalable, secure, and robust and most importantly value for money. In 2007 one of the overriding priorities for the IT department has been to meet the current and future needs for the ever increasing storage requirements of the Institute. To meet this challenge the IT department has developed a strategy and implemented an infrastructure to meet the needs of the organisation for the next 5 years.

The solution includes an archive facility to meet future legal and regulatory compliance in this dynamic environment. Proven technologies and work processes have been adopted to ensure a robust solution and as a result of the radical changes this has led to in the IT infrastructure, a new disaster recovery strategy will be developed to ensure business continuity in the event of a catastrophe.

Cancer Research UK's Project Local

Cancer Research UK is the largest charity in the UK with one of the most recognizable and loved brands, and yet the general public is often unclear about the explicit role it plays in the prevention, diagnosis and treatment of cancer. One of the ways the Charity is seeking to address this is by bringing its vision - *Together we will beat cancer – to life locally*.

Generally, people rate local issues as more important to them than national or international ones. People like to support local causes because they can see the functional relevance of local charities to their daily lives. In an increasingly globalised world, people are valuing things closer to home, more than at any recent time.

Around 90% of the UK's population lives within 30 miles of a Cancer Research UK funded site. This presents the Charity with a huge opportunity for engaging supporters with its work at a local level, physically demonstrating how their money is used in the fight against cancer. Crucially, the Charity believes, this will help underpin and drive the huge fundraising income which is required year on year to meet the Charity's ambitious goals.

In the spring of 2007 the Paterson Institute was asked by Cancer Research UK to help it trial a new way of working, with the aim of raising awareness of the ground breaking research funded by the Charity within the Greater Manchester area. This trial was dubbed Project Local.

Cancer Research UK and the Paterson jointly appointed a manager to lead this local initiative. In the months since James Dunphy's arrival, the Institute has hosted many more laboratory tours and supporter receptions. Donors have had more opportunities to meet Cancer Research UK-funded scientists, doctors and nurses and have found the experience to be inspiring. For example the highest fundraiser of Manchester's Race for Life was invited as a guest of honour to the Institute. Sue Valentine had decided to enter the race because her three sisters had suffered from ovarian cancer. She raised over £1000 for the Charity and found her visit to be enlightening; she said "I found it particularly interesting and I think it is a very good idea to invite fund raisers along to the

Institute to see where the money they have helped raise is being spent. It makes you realise what an important job it is to keep the money rolling in! Thanks to everyone at the Institute for their time, and we will try and raise more money at our next Race for Life".

Paterson scientists have also undertaken more outreach activity since the presence of Project Local, attending and speaking at fundraising events, visibly linking the money that supporters donate with lifesaving research, exemplified by the work funded by Cancer Research UK at the Institute. This benefits the Charity, but, in a broader sense, it also takes science closer to the public, making it more accessible, so they can chart its significance in their daily lives.

Whilst the focus of Project Local has been to strengthen and deepen the relationship between Cancer Research UK and its supporters in the Manchester area, there have been benefits too for the relationship between the Charity and the Paterson. A review of Project Local after six months showed a marked improvement in communications between the Charity's national fundraising and communications departments and grantees. It seems the Charity no longer appears quite so remote to either its fundraising supporters or to the researchers it funds.

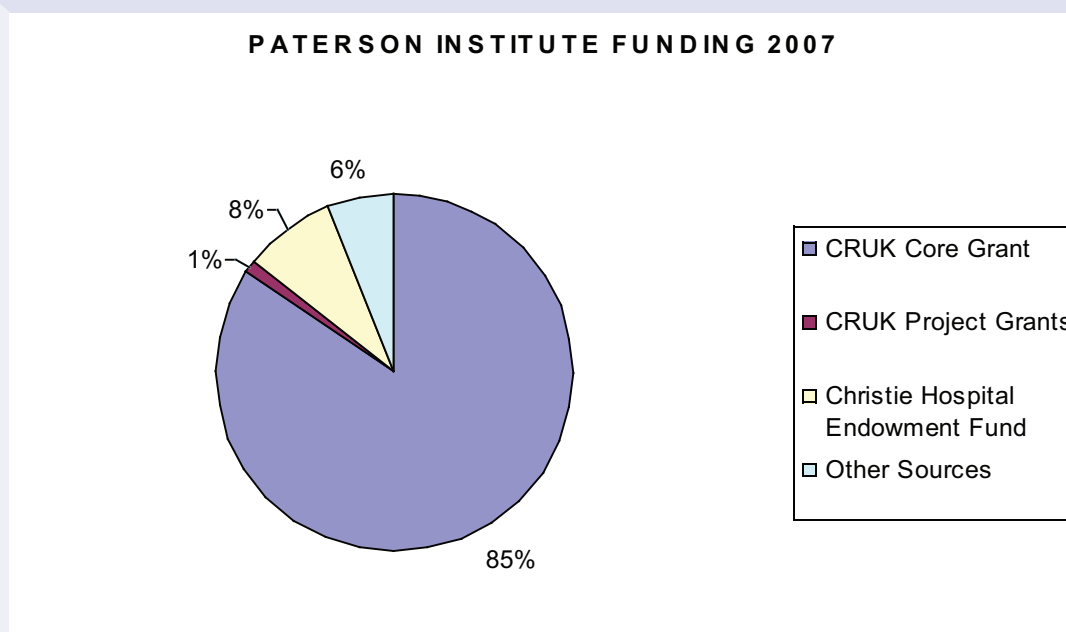
Due to the success of this pilot scheme in Manchester, James' position has been made permanent, and the Institute looks forward to being able to work with James to continue to support and develop this excellent initiative.



Nic Jones helping to give out medals at the end of Race for Life. It was here that he met the oldest participant at the race, the inspirational Doreen Rowlands who is 76 years old.

Acknowledgement for Funding of the Paterson Institute

The total funding of the Paterson Institute for 2007 was £14.4m. The major source of this funding (85%) is through a core grant from Cancer Research UK (CR-UK), the value of which was £12.2m. 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 a further 1% (£163k) of funding has been received from the CR-UK for Project Grant work.



The infrastructure of the Institute is funded by the Christie Hospital Endowment Fund and accounts for just over 8% (£1.2m) of the total income.

The final 6% 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
- European Commission
- Experimental Cancer Medicine Centre (ECMC)
- Wellcome Trust
- Trillium Therapeutics

We are immensely grateful to all our sponsors.

Donations to the Institute in 2007

- Mrs Susan Rowland
- Mrs M Emerson in memory of Mr Gordon Emerson
- Mrs D Geraghty in memory of Mrs Janet Brannick
- Mrs A C Archer in memory of her mother
- Mrs M Collier in memory of Mr Bill Collier
- Mellor March Charity
- Mrs P Johnson & Family
- Oldham SNU Church

We are once again tremendously appreciative of the generosity of the PACCAR foundation which has provided further funding via Cancer Research UK to support the Clinical and Experimental Pharmacology Group

Career Opportunities at the Paterson Institute

The Paterson Institute is located alongside the Christie Hospital, 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.

A Manchester Cancer Research Centre has been created with partners including the Paterson Institute, the Christie Hospital NHS Trust, The University of Manchester and Cancer Research UK.

This is an extremely exciting development which will enhance 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 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 (page 72). 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 always available for outstanding candidates. Interested applicants should contact

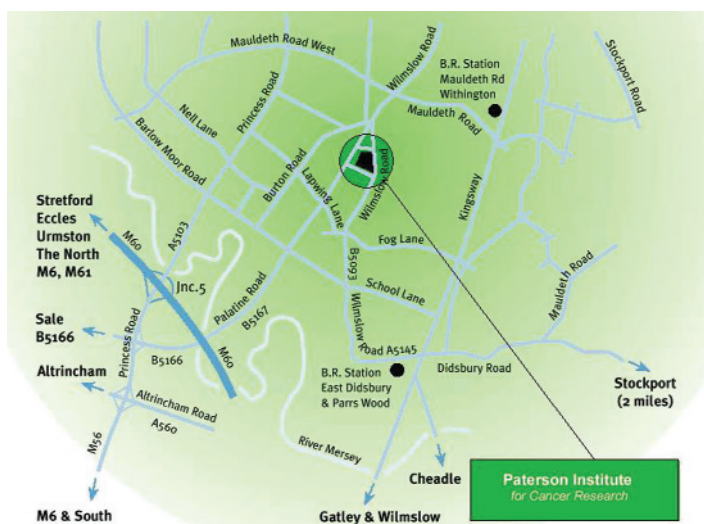
the Group Leaders directly, with details of their area of interest and recent experience.

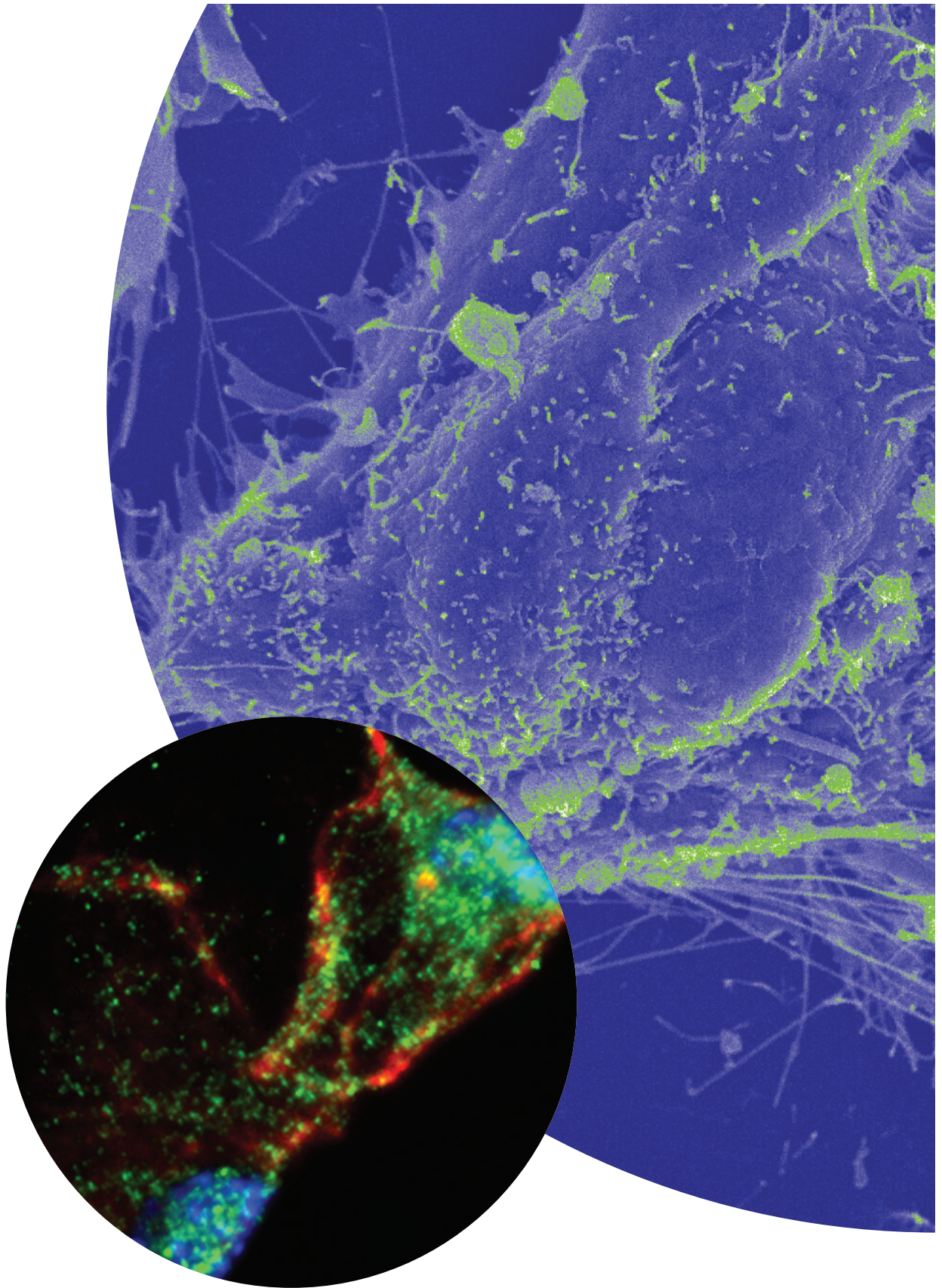
In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding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

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

Finding us

The Paterson is well placed for both national and international travel, with Manchester Airport only around a 30 minute drive away. The region is very well-served by the motorway network and the West Coast mainline rail service





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