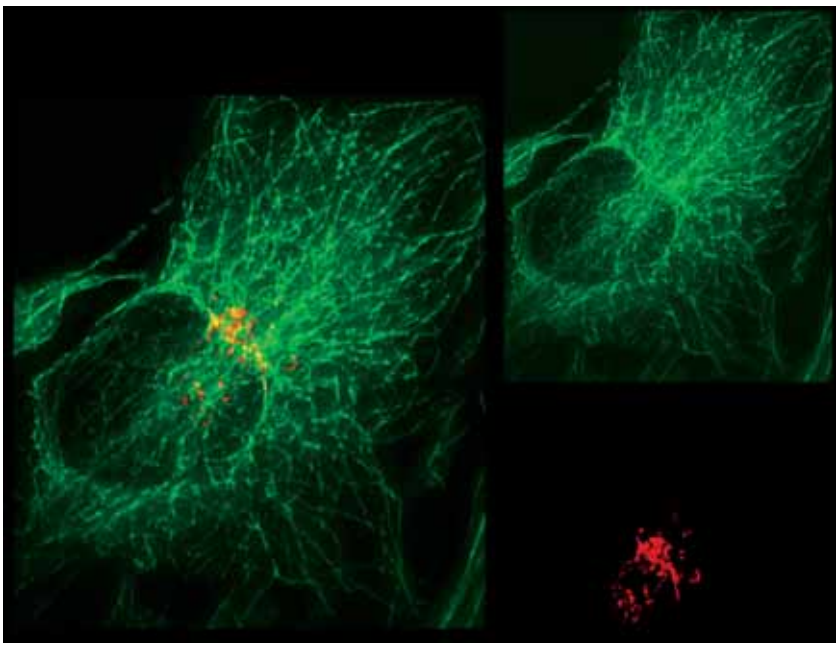
Paterson Institute for Cancer Research

SCIENTIFIC REPORT 2005

CANCER RESEARCH UK



Bovine pulmonary artery endothelial cells were labelled with quantum dots. Quantum dots are crystalline semiconductors composed of a few hundred or thousand atoms that emit different colours of light. The core of the particle is of a semi-conductor material (cadmium compounds) and are coated with a biological inert compound. The size of the quantum dot governs the wavelength of light that emits from them. 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

Illustration Credits

Many illustrations in this report were taken by staff of Medical Illustrations of the Christie Hospital, Jenny Varley and Steve Royle

Cancer Research UK

Paterson Institute
for Cancer Research

SCIENTIFIC REPORT 2005

Cancer Research UK Paterson Institute for Cancer Research Scientific Report 2005

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Director's Introduction

2005 has certainly been an eventful year in the Paterson Institute. Great progress has been made in strengthening interactions between the Institute and other research activities in Manchester which has led to the exciting development of the Manchester Cancer Research Centre.

Nic Jones, Director of the Paterson Institute

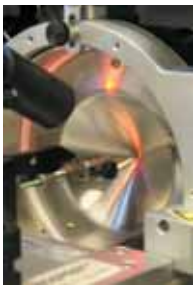
I reported last year the results of our quinquennial review in July 2004 which resoundingly endorsed our development and progress during the previous five years but also highlighted the enormous potential for the Institute and more generally for cancer research in Manchester by closely integrating our research efforts with those of the University of Manchester and the Christie NHS Trust. During 2005 very significant progress towards such integration has been made. Agreement has been reached for the transfer of the Institute from the Christie NHS Trust to the University although it will retain its current level of operational autonomy. Many practical issues needed to be resolved in order for the transfer to take place but through the concerted efforts of many, agreement was finalised in late December and the transfer will take effect on the 1st January, 2006. This transfer heralds the establishment of the Manchester Cancer Research Centre (MCRC) which will integrate the cancer research efforts of the University including the Paterson with those of the Christie NHS Trust and Cancer Research UK.

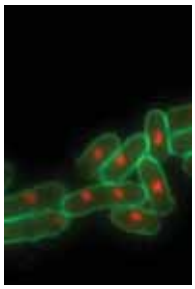
The timing of this development is most opportune. Over the last few years unprecedented advances have been made in understanding the molecular and cellular basis of many cancers and thereby providing real opportunities for the development of more effective and safer therapies and more meaningful and informative diagnostics. To realise these opportunities, an interdisciplinary approach where scientists and clinicians work closely together is vital and lies at the heart of the MCRC. The MCRC will increase its activities and strengths in basic, translational and clinical research and focus its efforts into a number of research themes/programmes the overlapping nature of which will facilitate integration of laboratory-based research into the clinically important areas of cancer therapeutics, diagnostics and individualisation of therapy. This will require significant new investment in cancer research and research facilities over the next five years centred at the Christie site and thereby closely integrated with the Paterson. These exciting plans and the opportunities they provide are a logical extension of the overall, major goals of the Institute: to be a world-class centre of cancer research with outstanding scientists and research infrastructure and to build on this platform together with our major research partners, a comprehensive approach to research that facilitates the development of extensive translational and clinical research programmes. The development of the MCRC and the strong partnerships it represents, will maximise our ability to fully achieve these goals.

Lez Fairbairn (centre) Pictured with Louise Earley (left) All Hallows RC High School, Macclesfield - and Becky Marchmont - Loreto Grammar School, Altrincham

During the year we lost two group leaders. Elmar Schiebel moved to the University of Heidelberg to take up a professorship at the Zentrum fur Moleculare Biologie. Elmar contributed greatly to the reputation and international recognition of the Institute and will be greatly lost. He is an outstanding scientist and has made a number of seminal findings related to the complex orchestration of events that regulate cell cycle progression in mitosis and ensures faithful chromatid segregration. We also lost Lez Fairbairn who died suddenly from a heart attack. It was a massive shock to all of us particularly given his relatively young age. Lez was a superb scientist and a fantastic colleague. He was an early pioneer of gene therapy approaches to cancer treatment at the Paterson and contributed much to this research area nationally and internationally which earned him considerable

respect with his peers. He enjoyed life to the full and is sorely missed by all of us. Lez was also passionate about communicating science to the outside world. An example of this was the open day he organised at the Institute for sixth form students from local schools. They spent the day conducting experiments and generally gaining an understanding of how research is conducted and the excitement it can provide. The feedback was highly positive and it would be a fitting tribute to Lez if one or more of these enthusiastic students developed a career in scientific research.





The major refurbishment of the north end of the Institute's laboratories continued through the year and is due to be completed by the middle of 2006. The work has caused disruption but is badly needed and will ensure that we maximise the space that we have for supporting our research efforts. The new facilities will provide excellent laboratory and office accommodation for some of our service activities, for modest expansion of the Paterson through the recruitment of new research groups and for the expansion of some current activities including the Clinical and Experimental Pharmacology Group headed by Caroline Dive and Malcolm Ranson. This group is crucial to our translational research efforts and demonstrates the synergy that exists from close interaction between the Paterson and the Department of Medical Oncology. The CEP group undertakes preclinical analysis of potential mechanism-based therapies and develops, validates and implements pharmacokinetic and pharmacodynamic analysis of Phase I trials. Its activities are closely integrated with those of the Derek Crowther Unit, a dedicated phase I/II clinical trial facility in the Christie Hospital which has become one of the major centres for such trials in the country. The development, testing and analysis of mechanism based therapies is a research area that is expanding rapidly and where we anticipate a significant increase in the number of such trials particularly focusing on areas and targets that match the biological strengths and expertise of the Institute and the MCRC. Specialised facilities are required to conform with ever-increasing regulations and the newly refurbished laboratories have been designed to meet the future needs of this group.

In terms of research, many significant advances were made in the last year. Below are just some examples both in basic and translational research. Iain Hagan and Janni Petersen identified a novel and important link between stress response pathways and cell cycle control. They showed that stress signalling could alter the activity of polo kinase which is associated with the spindle pole and regulates entry into mitosis (Petersen and Hagan, Nature 2005; 435: 507). Karim Labib's group characterised the composition and regulation of the replisome when DNA replication forks pause upon encountering proteins that are tightly bound to chromosomal DNA *in vivo*. Stability of the paused replisome does not require activation of a checkpoint response, and pausing is mediated by two proteins that are associated with the MCM helicase (Calzada et al., Genes Dev 2005; 19: 1905). Elmar Schiebel's group identified a kinase (Kin4) that plays a crucial role in ensuring that progression through mitosis is delayed until the mitotic spindle is properly orientated (Mol Cell 2005; 16: 209). Failure to do so could result in genome instability. Geoff Margison's group together with colleagues in Trinity college, Dublin, have continued their efforts in developing and testing potent inhibitors of clinically important DNA repair processes. In collaboration with clinical colleagues in the Christie Hospital, they have shown that one of these agents, PaTrin-2, can effectively reverse the resistance of a human breast tumour xenograft to the chemotherapy drug, Temozolomide (Br J Cancer 2005; 93: 1152). Studies are now ongoing in patients to establish the dose of PaTrin-2 required for Phase II trials of this combination. Peter Stern's group continue to work closely with the group of Robert Hawkins in the Medical Oncology Department to develop novel immunotherapies from initial concept through to clinical trials including target discovery, pre-clinical validation and immunological analysis in patients. These studies have established the 5T4 oncofoetal antigen as an immunological target and delivered preclinical studies leading to Christie-led phase I and II trials of antibody targeting and cancer vaccination. They are also developing the technology for using genetically modified T cells as effective cancer therapeutics (Journal of Immunotherapy 2005; 28: 203).

In summary it has been a big year for the Paterson culminating in its transfer to the University of Manchester and development of the MCRC. During the coming years the Institute will play a major role in the MCRC in what promises to be a very exciting and productive way ahead.

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http://www.paterson.man.ac.uk/groups/bioinf.jsp

Modern molecular biology generates enormous data sets that are too big to analyse by hand. Instead, computers must be used to sift through the information and find the relevant patterns and signals in the data. Bioinformatics is the study of how these programs must be written and applied to biological data. Our own research is focused on developing novel techniques and software tools for analysing microarray data and, increasingly, quantitative proteomics arising from iTRAQ based mass spectrometry.

Both microarrays and high throughput quantitative proteomics measure the expression of many thousands of genes in parallel, raising issues of data management, statistics and computing as well as biology and biochemistry. Successful analysis relies on understanding how each of these contributes to the data produced by an experiment. In addition, each sample can result in large amounts of data. Recent Affymetrix chips, for example, use ~500,000 features to probe for ~54,000 different transcripts; the simplest experiment comparing between two samples in triplicate generates data for about 3,000,000 features at once. A clinical study might involve hundreds of samples, and generate many millions of data points for further analysis.

Data management and analysis

Microarray analysis relies on the use of statistical tests to assess the significance of each change in gene expression; experiments are repeated a number of times to generate replicates, and the replicate data used to evaluate the consistency of the observed differences. These tests are often accompanied by calculations of fold-change, produced from the mean values for each set of samples. The majority of our data analysis work uses BioConductor (www.bioconductor.org), a collection of analysis tools built with the statistical programming language R. We contribute code to BioConductor and have also been continuing to develop our own package, 'simpleaffy', which implements a variety of analysis algorithms for Affymetrix data, including Quality Control, signal detection, expression level generation and a set of graph plotting and visualisation functions, and 'plier', which uses a wrapper around Affymetrix's SDK to provide access to their 'plier' algorithm.

Knowledge of the replicate structure of a microarray experiment is fundamental to its correct interpretation. We have developed a large MIAME compliant database that provides access to expression data via a Web interface. In order to allow the database to be searched for experiments in which specified genes are differentially expressed, the database must have access to information describing the replicate structure of each experiment, and use this to guide the statistical tests that underpin the search. We have developed an annotation system that uses a 'drag-and-drop' interface that allows users to build a pictorial representation of their experiment using a set of icons that represent the different stages of the experimental process. The system makes use of this apparently informal interaction to build a structured, and machine readable representation of experimental design. This is subsequently used by the database to group samples together to support a variety of tasks including data visualization and gene-centred searches. As datasets become larger and more complex to man-

age, MIAME VICE is becoming the main route of data exchange between the Molecular Biology Core Facility (page 52) and its users.

The complex relationship between genes, probes and transcripts

Affymetrix microarrays record the presence of a transcript in solution by measuring the level of hybridization between the transcript and a set of short (typically 25mer) oligonucleotide probes anchored to the array surface. Each 'probe-set' consists of a series of 'perfect match' (PM) probes, designed to match exactly to the transcript, and a series of 'mismatch probes' (MM), identical to the PM probes except that the middle residue has been changed. Hybridization conditions are controlled with the aim of maximizing the binding between a transcript and its PM probes, whilst minimizing the binding to its MM probes (see www.affymetrix.com for more details). The intention is that the PM probes record the presence of the transcript, whilst MM probes measure background and non-specific hybridization. One advantage of this approach is that the combination of short oligonucleotides and strict hybridization conditions makes it possible to use *in silico* searches to predict which probes are likely to bind to which transcripts; information that is important because many transcripts have similar sequences and certain probes are capable of binding to more than one mRNA molecule (for example because alternate splicing can lead to a set of transcripts being encoded by a single gene; due to homology; or due to repetitive or low complexity regions).

Not only do some probesets target multiple transcripts, the reverse is also true – there are multiple probesets that target a single transcript. This can occur, for example, with probe-sets designed to identify different splice-variants of the same gene, or where one probeset is designed to identify a gene family, whilst another targets a particular family member (see figure).

Identifying these situations is useful when considering experimental data in which evidence from a particular probeset is weak. If all the other probesets targeting the same transcript behave similarly, this can provide supporting evidence; if they behave differently it may be possible to discount the probeset from further analysis. We have developed an online database, ADAPT, that allows these complex relationships to be investigated.

Comparisons between protein and mRNA gene expression data

A significant opportunity exists to place proteomics and microarray data side by side, allowing comparison of changes in gene expression at the transcript and protein level. To do this requires complex mappings to be made between genomic, transcript and protein sequences; a difficult task because of the structure of existing databases and the complex many-many relationship that exists between genes and gene products. We have been developing software tools and analysis strategies to support these analyses in collaboration with Professor Tony Whetton at the University of Manchester and the Kouskoff and Lacaud groups at the Paterson Institute.

The complex many-many relationships that exist between Affymetrix probesets (blue balls) and transcripts (yellow balls) for the HGU133 array.

Publications listed on page 56

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The group's focus is on understanding the role of DNA damage and repair pathways in the biological effects of specific genotoxic agents and to exploit this in the treatment and prevention of cancer. Our main interest is in certain types of alkylating agents one example of which is the CR-UK drug, Temozolomide. Our clinical strategy is to inactivate the DNA repair protein, O6-alkylguanine-DNA alkyltransferase (MGMT, also known as ATase), using our pseudosubstrate, PaTrin-2 (now officially named Lomeguatrib) in order to sensitise tumours to the killing effect of this agent. We are also involved in a number of preclinical studies investigating ways in which bone marrow toxicity, can be reduced using gene therapy; the relationship between MGMT polymorphisms and cancer risk, and a novel DNA alkylation damage repair pathway.

Background

The treatment of several types of cancers involves alkylating agents of various types. Their effectiveness against the cancers is dogged by inherent or treatment-induced tumour resistance along with the life-threatening toxic side effects of the treatment, which limit the maximum doses that the patients receive. Understanding the mechanisms of tumour resistance and normal tissue sensitivity should allow the development of strategies that will improve the effectiveness of these agents. Studies of their DNA damaging, carcinogenic and toxic properties and of the DNA repair processes that can attenuate these biological effects, are now enabling this.

Chemotherapeutic alkylating agents generate varying amounts of a dozen different types of lesions in DNA and there is increasing understanding of the mechanism by which some of these lesions result in cell killing. Thus, methylating agents such as DTIC (Dacarbazine) and Temozolomide produce O^6-methylguanine in DNA and this kills cells via the action of the post replication mismatch repair (MMR) system. They also generate 3-methyladenine, which kills cells by blocking DNA replication.

Repair pathways that probably evolved to deal with low levels of endogenously produced damage reduce both the therapeutic efficacy and also the collateral toxicity of alkylating agents. Thus there is increasing interest in attenuating the expression of such pathways in tumours, and to enhancing them in normal tissues in order to improve clinical outcome. These processes, especially that repairing damage at the O^6-position of guanine, i.e. MGMT, and more recently the base excision repair system that processes 3-methyladenine, have therefore become targets for modulation. ATase 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. Alkylpurine-DNA-N-glycosylase (APNG, also known as AAG: alkyladenine glycosylase) removes 3- and 7-alkylpurines generating apurinic sites in DNA that activate and are processed by poly-ADP-ribose polymerase.

One approach to increasing the efficacy of such agents is the use of non-toxic pseudosubstrates that inactivate ATase and transiently, i.e. until an additional supply of active MGMT is synthesised, sensitise cells to killing. In collaboration with Prof Brian McMurry and the late* Dr Stanley McElhinney (and their group at the Chemistry Department, Trinity College, Dublin), we have developed Lomeguatrib. This highly potent inactivator of MGMT is licensed by Cancer Research Technology to KuDOS Pharmaceuticals.

[*footnote: sadly, Stanley was diagnosed with lung cancer which progressed rapidly and he died in October: he is greatly missed].

Clinical trials involving Lomeguatrib

Phase I clinical trials of Lomeguatrib were carried out here at Christie Hospital and at University College, London and established a combination dose of Lomeguatrib and Temozolomide for Phase II trials. These are mainly in melanoma patients recruited from the UK and several Australian centres, and are under the auspices of KuDOS Pharmaceuticals. The dose and schedule of the two drugs is currently being optimised. In addition, another trial is addressing the effectiveness of PaTrin-2 in inactivating ATase in a number of tumour types in order to establish the doses required for complete inactivation of ATase. These trials have required us to develop and validate to Good Clinical Laboratory Practice standards, a quantitative assay for total ATase protein to run alongside the previously validated functional assay. Brain, prostate, breast and colorectal tumours are being examined in dose escalation studies. Once the effective dose is established, Phase II trials will be undertaken in such patients.

MGMT polymorphism studies

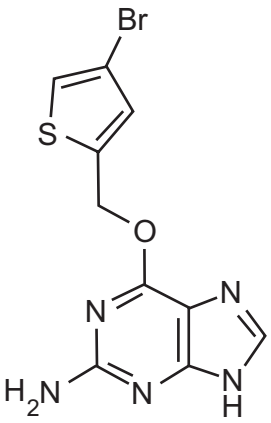
MGMT expression levels vary widely between individuals but the underlying causes of this variability are not known. We are therefore investigating whether or not there are single nucleotide polymorphisms (SNP) in the human gene that might be used to predict the basal levels of expression of ATase. We have used quantitative trait locus analysis using intragenic SNPs, and found that there are at least two sites influencing inter-individual variation in human peripheral blood mononuclear cell MGMT activity. One of them, $I_{143}V$, affects an amino acid close to the Cys^{145} residue at the active site of MGMT. We further showed using *in vitro* assays that while the Val^{143} variant did not affect the activity of the protein on methylated DNA substrate, it was more resistant to inactivation by Lomeguatrib. These findings warrant further investigations of the potential epidemiological and clinical significance of inherited differences in MGMT expression and activity.

Preclinical studies

We have continued to contribute our MGMT expertise in collaborations with the groups of the late Lez Fairbairn (here at PICR) and Prof Chris Baum (Hamburg and Cincinnatti) who have been working towards clinical trials of chemoprotective MGMT gene therapy. We have thus shown that the $P_{140}K$ mutant of MGMT is highly resistant to inactivation by Lomeguatrib and that a human haemopoietic cell line (K562) transduced with a retroviral vector encoding MGMT($P_{140}K$) is highly resistant to the cytotoxic effects of Lomeguatrib in combination with temozolomide. These and other data show that MGMT($P_{140}K$) is a suitable candidate for chemoprotective gene therapy where Lomeguatrib is being used in conjunction with temozolomide. We have also shown that self-inactivating gammaretroviral and lentiviral vectors with comparable internal expression cassettes had similar MGMT expression properties. These vectors are of interest because of the safety concerns associated with the use of the standard retroviral vectors.

Alkyltransferase-*like* (ATL) proteins: a novel DNA repair pathway

DNA sequence comparisons have revealed a new class of putative DNA repair proteins, the alkyltransferase-like (ATL) proteins. These contain primary sequence motifs resembling those found in DNA repair O^6-alkylguanine-DNA alkyltransferase proteins. However, in the putative active site of ATL proteins a tryptophan (W^{83}) residue replaces the cysteine at the known active site of alkyltransferases. The *E. coli atl* gene has been expressed as a fusion protein and purified. Gel shift assays show that ATL specifically binds to short single- or double-stranded oligonucleotides containing O^6-meG, but there was no evidence of demethylation of O^6-meG or of glycosylase or endonuclease activity. This binding strongly inhibits the action of MGMT on O^6-meG in DNA. These and other results suggest that ATL may act as a damage sensor that flags O^6-meG and possibly other O^6-alkylation lesions for processing by other repair pathways. The further characterization and possible exploitation of this finding in various aspects of cancer chemotherapy is being actively pursued.



Lomeguatrib (aka, PaTrin-2, Patrin™ and O^6-(4-bromothenyl)guanine

Publications listed on page 56

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Our group studies chromosome replication and cytokinesis in eukaryotic cells. During 2005 we identified the Replisome Progression Complex (or RPC) that controls the advance of eukaryotic DNA replication forks. The RPC is built around the MCM helicase at origins during the initiation of chromosome replication. The four-protein GINS complex is a key component of the RPC as it allows other important factors to associate with MCM; these comprise Cdc45 that is thought to activate the MCM helicase, Tof1 and Csm3 that cause the replisome to pause upon encountering tight protein-DNA complexes, Mrc1 that allows checkpoint activation in response to problems in DNA synthesis, and Ctf4 that promotes the establishment of cohesion between the newly formed sister chromatids. The RPC also contains a histone chaperone that may facilitate the passage of the replisome through chromatin. In parallel with this work, we are also investigating how Cyclin-Dependent Kinase (CDK) represses cytokinesis until the end of mitosis.

The role of GINS during the initiation of chromosome replication

We carried out a systematic analysis of all the essential budding yeast proteins of previously unknown function in order to identify novel cell cycle factors. We generated yeast strains in which we modified each of the corresponding genes so that the encoded protein carried the "heat-inducible degron" cassette at the amino terminus. This allowed us to induce rapid degradation of the target protein and then examine the immediate consequences. In this way we identified four proteins required for chromosome replication; remarkably these four proteins interact with each other to form a complex called GINS. During 2005, we have shown that GINS is essential for the activation of "pre-Replication Complexes" (or pre-RCs) that are built at every origin of DNA replication during the G1-phase of the cell cycle. The pre-RC contains an inactive form of the MCM helicase; activation occurs subsequently during S-phase when two kinases, Cdc7 and CDK, promote the recruitment of GINS to origins. GINS allows MCM to associate stably with another protein, Cdc45, which is thought to be an essential component of the active helicase. Loading of Cdc45 and GINS is a complicated process that also requires another factor Sld3. We showed that Sld3 is displaced from the origin during the loading reaction, indicating that it only acts during initiation, whereas GINS and Cdc45 then travel with MCM as part of the nascent replisome.

Building the Replisome Progression Complex

We purified GINS from yeast extracts and used mass spectrometry to show that it interacts with MCM and a group of other factors that mediate or control the progression of DNA replication forks. We found that all these proteins assemble around MCM during the establishment of DNA replication forks, to form what we call the "Replisome Progression Complex". GINS is essential for assembly of the RPC, as it allows a specific set of proteins to associate stably with MCM. These include Cdc45, and GINS is thus essential for unwinding of the origin and for activation of the pre-RC. In addition, GINS is needed for Mrc1, Tof1, Csm3 and Ctf4 to be incorporated into the RPC. The role of Mrc1, Tof1 and Csm3 is discussed below. Ctf4 is required for establishment of cohesion between the newly formed sister-chromatids, though the mechanism remains to be established.

Unwinding of the parental DNA duplex at forks causes the accumulation of positive supercoils that block further progression and must therefore be removed. We have shown that GINS allows the RPC to recruit Topoisomerase I to forks; it thus appears that Topoisomerase I normally carries out this important role ahead of the replisome. Progression of the fork also requires "unpacking" of chromatin so that the replisome can gain access to the chromosomal DNA. We have shown that

the RPC contains a two-subunit histone chaperone called "FACT" (Facilitates Chromatin Transcription) that is likely to be essential for progression of the replisome past nucleosomes, though this remains to be demonstrated directly.

The regulation of paused eukaryotic replisomes

DNA replication forks pause upon encountering tight non-nucleosomal protein-DNA complexes throughout the genome. We have generated a yeast strain in which efficient pausing can be induced at specific chromosomal loci, allowing us to study the nature and regulation of the paused replisome. We found that the RPC components Tof1 and Csm3 are essential for pausing of DNA replication forks, which is therefore a property of the paused replisome itself. In contrast, Mrc1 is not required for pausing, and is instead required for the activation of a checkpoint response when DNA synthesis is inhibited or defective. We have shown that a stable replisome remains associated with the paused fork, comprising the RPC together with DNA polymerases alpha and epsilon.

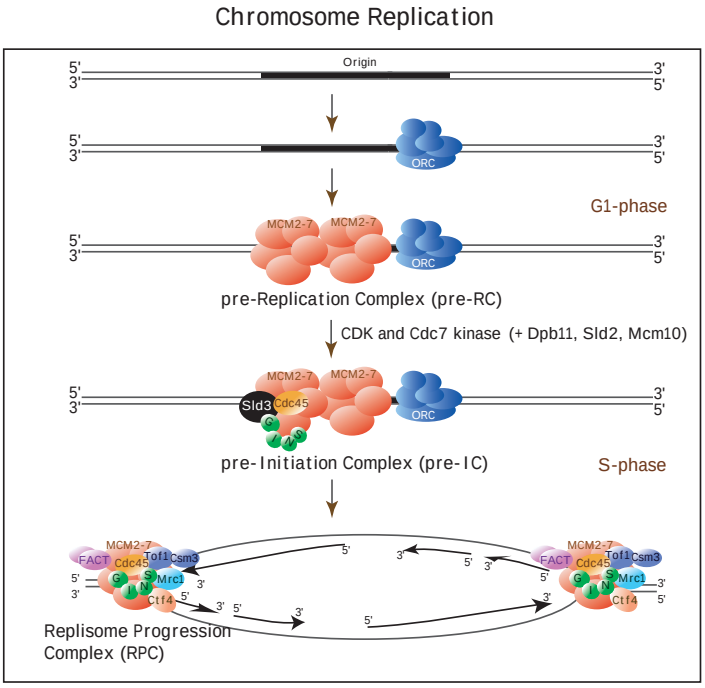

Figure 1:
The Replisome Progression Complex (RPC) controls the advance of eukaryotic DNA replication forks. The RPC is built around the MCM helicase during the initiation of chromosome replication, by recruitment of other proteins to pre-Replication Complexes. See text for details.

Cdc104 is essential for cytokinesis and is negatively regulated by CDK

We identified Cdc104 in our degron screen as a novel factor that is essential for cytokinesis. We are now studying the function and regulation of Cdc104 and have shown that it forms part of the actomyosin ring that assembles at the future site of cell division; contraction of the ring at the end of mitosis is an essential requirement for cytokinesis. Cdc104 is required for a very late step in cytokinesis, and only associates with the ring when CDK is inactivated at the end of anaphase.

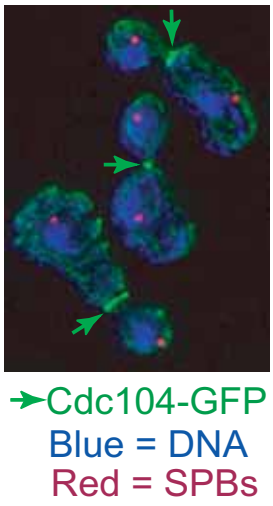

Figure 2:
Cdc104-GFP localises to the contractile actomyosin ring at the end of mitosis and is essential for a late stage of cytokinesis. The picture shows cells that also express a fusion of Red Fluorescent Protein (RFP) to a Spindle Pole Body component; cells were fixed and stained with a DNA binding dye.

Publications listed on page 57

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Errors in chromosome transmission alter the balance of tumour suppressor and tumour promoter genes. This imbalance favours changes in genome composition in the ensuing cell divisions that can lead to cancer. Chromosome segregation during mitosis is initiated by the attachment of the microtubules of the mitotic spindle to the chromosomes. Once all chromosomes have become attached to both spindle poles the chromosomes split into two identical chromatids that then move to the poles. Because the regulatory networks that regulate mitotic progression are highly conserved, studying the complexities of cell division in the relatively simple unicellular yeasts greatly accelerates the analysis of the more complex issue of the control of cell division in man.

We study cell division in the fission yeast *Schizosaccharomyces pombe* because it is a simple, unicellular organism with excellent genetics that is cheap to grow and divides rapidly. Commitment to mitosis in *S. pombe* is regulated by the activity of a protein kinase called MPF. MPF is composed of a catalytic sub-unit encoded by the *cdc2*$^{+}$ gene and a regulatory sub-unit called cyclin B. Prior to mitosis MPF is inhibited via phosphorylation by the protein kinase Wee1 on a residue (tyrosine 15) that lies in the ATP binding pocket of p34$^{cdc2.}$ This phosphate can be removed by a protein phosphatase encoded by the *cdc25*$^{+}$ gene. The balance of activity between Cdc25 and Wee1 is the critical factor in determining when MPF will be activated to drive mitotic commitment. Once a critical threshold level of MPF is activated a positive feedback loop is promoted to boost Cdc25 activity and suppress Wee1 activity, thereby driving full-scale commitment to mitosis. Fully activated MPF then activates a number of highly conserved kinases that are named after the founder members of each group Polo, aurora and NIMA.

Events on the spindle pole regulate MPF activation during commitment to mitosis.

Previous work in our lab has identified a critical role for events on the spindle pole in mitotic control as they suggest that the MPF positive feedback loop is primed from the spindle pole body (SPB). Mutations in the SPB component Cut12 allow cells to divide without Cdc25 protein. They appear to do this by promoting polo kinase activity in interphase so triggering the feedback loop independently of the normal requirement for a priming impetus from a small amount of active MPF. The observations in human cells by Jon Pines group (Gurdon Institute Cambridge) that active MPF first appears on the centrosomes strongly suggest that the networks we are studying in yeast occur in human cells. In other words key decisions about whether to divide or not do not arise from the gradual accumulation of a "pro mitosis" state. Rather, they are taken at a discrete location, the spindle pole.

Phosphorylation of polo kinase by the stress response kinase Spc1/Sty1 coordinates cell division with the environment

We recently extended our understanding of how Plo1 promotes mitosis from events on the SPB by studying the phosphorylation of the serine that lies at position 402 of Plo1. Like human cells, fission yeast activate a MAP kinase cascade in response to a variety of different stresses. In addition to responding to stresses, the fission yeast pathway has a level of constitutive signalling that is required to control the timing of commitment to mitosis. Importantly the amount of signalling through the pathway changes in response to the nutritional context of the cell. Signalling is strong in minimal medium and significantly reduced in highly nutritious environments. Abolishing this SRP signalling delays entrance into mitosis in any nutritional context. While this connection between SRP signalling and cell cycle control has been appreciated for over a decade the molecular basis of how SRP signalling modulated mitotic control had remained obscure. We found that the phosphorylation of serine 402 of

Plo1 regulated its affinity for the spindle pole and so directly influenced the entrance of mitosis. For example the ability of the *cut12.s11* mutation to suppress *cdc25* defects was dependent upon the phosphorylation status of serine 402. Importantly serine 402 phosphorylation was entirely dependent upon SRP signalling and the delay in commitment to mitosis that occurs when SRP signalling is abolished was considerably reduced by substitution of glutamic acid for serine at position 402. Thus it appears that a major part of the way by which SRP signalling ensures that division occurs at the right rate for the nutritional context is via phosphorylating Plo1 on serine 402 to regulate its affinity for the SPB.

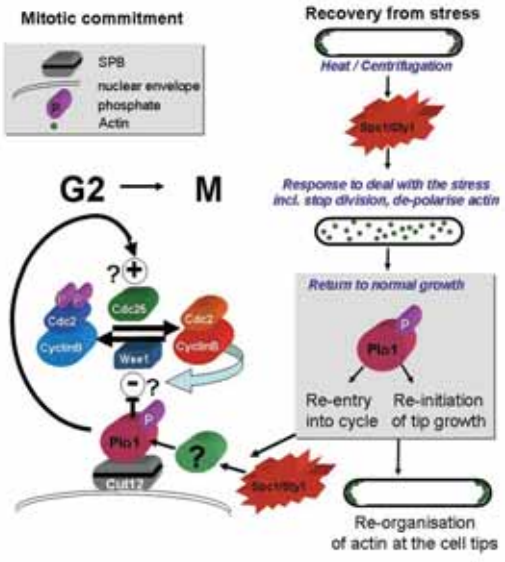
Plo1 regulates the cytoskeleton and cell cycle to promote recovery from stress

In addition to being phosphorylated to modulate the timing of commitment to mitosis, serine 402 is phosphorylated in response to the stresses of heat and centrifugation. In fact far more of the Plo1 molecules in the cell are phosphorylated on serine 402 following stress than during a normal cell cycle. The large peak in SRP signalling that is triggered by exposure to these stresses has largely subsided before serine 402 phosphorylation accumulates to significant levels suggesting that serine 402 phosphorylation is not an immediate stress response, rather, it coincides with recovery from the stress. This recovery function revealed a previously unappreciated response to stress and function for polo kinase as both heat and centrifugation depolarised the actin cytoekeleton and so completely halted cell growth. The phosphorylation of Plo1 on serine 402 was then required to promote the re-polarisation of the actin cytoskeleton and entrance into mitosis that accompanies the resumption of growth. Thus, in addition to its more widely appreciated role in controlling cell cycle progression, polo kinase controls the cytoskeleton.

Lessons from yeast

The ability to manipulate genes at will in a simple organism whose primary purpose is to divide is enabling us to explore the finer points of the pathways that co-ordinate a successful cell division. 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.

A number of polo kinase inhibitors will enter clinical trial next year and the realisation that polo is part of stress response pathways and controls cell migration and morphogenesis via modulation of the actin cytoskeleton identifies novel opportunities in the design of these trials. As most tumour cells are growing in sub-optimal environments that impose a range of stresses, perturbation of the stress response maybe a highly effective therapy if combined with other treatments that target the vasculature such as Taxol.



Polo phosphorylation controls division and actin

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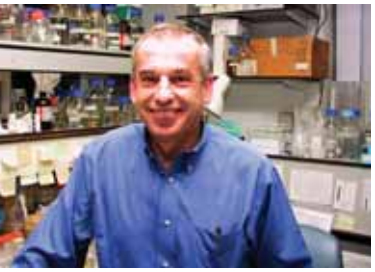

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Cells commonly respond to extracellular signals by modulating the activity of specific transcription factors and subsequently the expression of many target genes. We are particularly interested in the response to cytotoxic and genotoxic stress which 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 as well as apoptosis. On the organismal level AP-1 plays important roles in tissue stress responses such as inflammation and ischemia and is implicated in the onset and progression of tumours. The factor and its regulation is complex since it is not a single entity but rather a mixture of dimeric complexes composed of members of the Jun, Fos, ATF and MAF protein families. Different dimeric combinations can recognise slightly different sequence elements and be regulated by distinct signalling pathways. A well characterised signalling cascade involves the activation of the mitogen-activated protein (MAP) kinases ERK and stress-induced MAP kinases JNK and p38 which directly phosphorylate and modulate the activity of various members of the AP-1 complex. Over the last few years considerable progress has been made in elucidating the function of individual AP-1 proteins through the characterisation of genetically modified mice and cells that derive from them.

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 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 ATF-2

ATF-2 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. ATF-2 is activated by the p38 or JNK kinases through phosphorylation of two N-terminal threonine residues T69 and T71. Many reports, mostly using *in vitro* systems, have implicated ATF-2 in numerous growth and developmental programs and in response pathways after stimulation with geno- and cytotoxic stresses. Depending upon cellular context, ATF-2 has been implicated in the regulation of cell proliferation and apoptosis. Other biological functions for ATF-2 include responses to neuronal stresses, as well as a role in inflammatory responses, such as the activation of T-cell specific genes after immunological stimulation. In most of these cases however, the potential role of ATF-2 has not been verified in whole animal models.

To address the biological importance of ATF-2, its interaction with other AP-1 family members and its regulation by stress-activated MAP kinases we have generated a number of genetically modified mice where ATF-2 activity is compromised. Germline

inactivation of ATF-2 results in death shortly after birth due to severe respiratory distress. A role for ATF-2 in earlier development was revealed by the genetic inactivation of ATF-2 together with its closest homologue, ATFa. At least during embryonic development ATF-2 and ATFa appear to have partly redundant functions since deletion of both genes (ATF-2-/-ATFa-/-) results in lethality during mid-gestation with significant morphological defects in embryonic liver and heart. In the case of the liver, massive apoptosis is observed. We also generated mice containing an allele of ATF-2 where the phospho-acceptor threonine residues (T69 and T71) are altered to alanines (ATF-2 AA) and thus rendering ATF-2 inactive for response to upstream MAP kinases. On an ATFa-/- background, these mice demonstrate the same embryonic phenotypes. The results show that phosphorylation of ATF-2 is crucial and suggests that ATF-2 may mediate important functions of JNK and p38 signalling in development. In support of this, mice with defective JNK signalling also demonstrate massive liver apoptosis at mid-gestation. Studies are ongoing to identify the crucial ATF-2 target genes involved in survival signalling in the liver and in heart development. In addition, cells derived from the mutant mice are being characterised with respect to growth regulation, sensitivity to apoptosis and response to various stress-related signals.

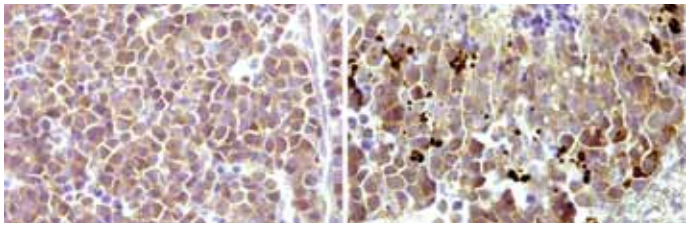
To circumvent the early lethality that occurs in the ATF-2 knockouts we have also generated mice containing a floxed ATF-2 allele to allow tissue-specific Cre/loxP mediated inactivation of the gene. ATF-2-floxed mice have been crossed to a number of lines expressing Cre recombinase in a tissue-specific manner. We are using these models to better understand the role of ATF-2 in stress-induced responses in various tissues and to address the question of whether ATF-2 can modulate tumourigenesis or the response of tumour cells to therapeutic approaches.

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. All cells sense and react to changes in their environment. Single-celled organisms, in particular, must contend with fluctuations in nutrients, pH, temperature and external osmolarity, as well as exposure to UV irradiation and a range of potentially toxic environmental compounds. Appropriate responses to these environmental stresses must be induced for cell survival and proliferation. A comprehensive characterisation of these responses - the mechanisms involved in sensing stress, the signalling pathways transmitting this information within the cell, and the resulting compensatory changes in physiology and gene expression - is essential to understand how cells adapt and survive under non-ideal conditions.

In collaboration with Jurg Bahler at the Sanger Centre in Cambridge, we carried out a comprehensive global microarray analysis of the transcriptional response to oxidative, heat, heavy metal, osmotic and DNA damage stress. This analysis provided a comprehensive overview of cellular responses to environmental stress and insights into how the cell integrates information concerning the state of the environment and orchestrates the expression of the appropriate set of genes. It is clear from these studies that Atf1 and Pap1 are major factors that mediate many of the stress responses. More detailed analysis of the transcriptional changes following oxidative stress has illustrated the need to respond appropriately to the level as well as the nature of the stress. Using mass-spectrometry based approaches we have identified a number of proteins that interact specifically with Atf1 and Pap1. Characterisation of these interactions will lead to a better understanding of how these factors are regulated, how they mediate transcriptional activation and how they intersect with other pathways resulting in signal integration.

The stress activated pathway has also been implicated in cell cycle control and functional interactions between Sty1, Atf1 and the cell cycle/checkpoint machinery have been identified and are being characterised.



Lack of ATF-2 causes severe apoptosis in embryonic liver as shown by staining for activated Caspase 3.

Publications listed on page 57

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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 actin cytoskeleton organisation and gene transcription. In this way, Rho proteins influence cell morphology, adhesion, motility, as well as cell cycle progression and cell survival. Rho proteins are essential for Ras-mediated *in vitro* transformation. Recently, data has emerged to directly implicate Rho proteins in tumour initiation and progression *in vivo*. Our group's focus is on identifying signalling events downstream of Rho proteins that modulate tumour susceptibility and disease progression.

Similarly to Ras, 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 this way, Rho proteins govern cell morphology, adhesion, motility, as well as cell cycle progression and cell survival. Of relevance to cancer, Rho proteins are transforming in *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. Activation of Rho GTPases results in binding to various effector molecules that elicit downstream responses.

Tiam1/Rac signalling and Ras-induced skin tumorigenesis

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. Interestingly, Tiam1 preferentially associates with activated GTP-bound Ras through a Ras-binding domain (RBD). Activated Ras and Tiam1 synergize to induce formation of Rac-GTP (Lambert *et al.*, Nature Cell Biol 2002; 4: 621). Further, Tiam1-deficient cells are resistant to Ras-induced cellular transformation (Malliri *et al.*, Nature 2002; 417: 867). Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by application of a two-stage chemical carcinogenesis protocol (Malliri *et al.*, Nature 2002; 417: 867). This protocol entails tumour initiation in epidermal keratinocytes by treatment with the carcinogen 7,12-dimethyl-benzanthracene, which induces oncogenic activation of the c-*Ha-Ras* gene. Subsequent repeated treatments with the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) result in the outgrowth and progression of initiated cells. 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/Rac signalling and malignant progression

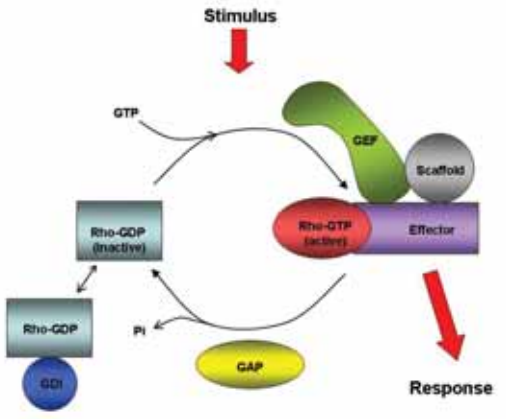
The skin carcinogenesis model revealed an additional role for Tiam1 in tumourigenesis. The few skin tumours arising in Tiam1-deficient mice progressed more frequently to malignancy than those in wild-type mice, suggesting that Tiam1 deficiency promotes malignant conversion (Malliri *et al.*, Nature 2002; 417: 867). Analysis of Tiam1 expression in skin tumours of wild-type mice revealed that benign papillomas maintained high levels of Tiam1 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 Tiam1 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 Tiam1/Rac co-operate with Ras in establishing tumours, they antagonize Ras during tumour invasion.

One probable mechanism by which Tiam1/Rac antagonizes malignant progression is through their positive effect on cell-cell adhesion. *In vitro* studies have shown that over-expression of activated Rac or Tiam1 can promote the formation of adherens junctions and the accompanying induction of an epithelioid phenotype in a number of cell lines (Malliri & Collard, Curr Opin Cell Biol 2003; 15: 583). Moreover, using both RNA interference and cells derived from Tiam1-deficient mice, it was recently shown that endogenous Tiam1 is required for both the formation as well as the maintenance of cadherin-based adhesions (Malliri *et al.*, J Biol Chem 2004; 279: 30092). Intriguingly, Asef, another Rac specific exchange factor, promotes intestinal tumour cell migration and invasiveness in *in vitro* models through down-regulating cadherin-mediated adhesion (Kawasaki *et al.*, Nature Cell Biol 2003; 5: 211).

Tiam1/Rac signalling and intestinal tumourigenesis

Recently we have shown that Tiam1 is a potent modifier of intestinal tumourigenesis. The majority of intestinal tumours are caused by mutations in the canonical Wnt signaling pathway, leading to its activation. However, few genes targeted by this pathway have been demonstrated to affect tumour development *in vivo*. We determined that *Tiam1* is a Wnt-responsive gene. It 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 gene. Further, by comparing tumour development in Min mice expressing or lacking Tiam1, we found that Tiam1 deficiency significantly reduces the formation as well as growth of polyps *in vivo*. However, invasion of malignant intestinal tumours is enhanced by a lack of Tiam1. In line with this, knock-down of Tiam1 reduced the growth potential of human colorectal cancer (CRC) cells and their ability to form E-cadherin-based adhesions, a prerequisite for local invasion of tumour cells (Malliri *et al.*, J Biol Chem epub Oct 05). The two studies on tumourigenesis *in vivo* demonstrate that two independent oncogenic signalling pathways of major clinical significance (Ras and Wnt) recruit the Tiam1-Rac signalling pathway by specific, albeit distinct mechanisms. In the context of oncogenesis, activation of this signalling module promotes tumour initiation and growth.

Thus, Tiam1 appears to make a unique contribution to the development of the tumour types studied so far, since its loss cannot be compensated for by other Rho GEFs. Moreover, the diverse roles of Rho GEFs in certain processes clearly indicate that Rho GEFs do more than simply activate Rho molecules, and 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. Tiam1 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 (Buchsbaum *et al.*, Mol Cell Biol 2002; 22: 4073). Tiam1 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 supresses the ability of Tiam1 to activate Pak1, a different Rac effector (Buchsbaum *et al.*, J Biol Chem 2003; 278: 18833). In our lab we are taking a biochemical approach to identify Rac effectors involved in different aspects of transformation including malignant progression. We have also started investigating the potential role of the Tiam1 homologue, Stef, in tumourigenesis and the impact of down-regulating more than one GEF simultaneously in different aspects of the transformed phenotype.



The Rho GTPase cycle. Rho-like GTPases cycle between an active GTP-bound and an inactive GDP-bound form. This is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Guanine nucleotide dissociation inhibitors (GDIs) inhibit nucleotide dissociation and control cycling of Rho GTPases between membrane and cytosol. Active GTPases interact with effector molecules to elicit various cellular responses including cytoskeletal reorganisation, transcription, cell-cycle progression, apoptosis, membrane trafficking. Additionally GEFs could work as scaffold proteins by either binding directly to Rac effectors or other scaffold proteins that bind to effectors.

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Our research focus remained on the roles of c-Src and hypoxia in tumours and their response to therapy. We discovered that in contrast to established literature on pro-proliferative effects of v-Src in fibroblasts, induction of c-Src in human colorectal cancer cells has no effect of cell growth *in vitro* or *in vivo*. Induction to high level of a constitutively active mutant of c-Src, however delays G_2/M progression and promotes a non-apoptotic cell death. In ductal carcinoma *in situ* in the breast however, activated c-Src was seen in HER2 positive, high grade lesions with a high proliferation index. We have also demonstrated that hypoxia drives drug resistance in colorectal cancer cells and in neuroblastomas.

Colon cancer and c-Src

The ubiquitously expressed, non-receptor tyrosine kinase c-Src is thought to participate in multiple signalling pathways that regulate cell proliferation, survival, differentiation, adhesion and motility. Multiple studies suggest roles for c-Src early in tumour development and later during tumour metastases in several cancers including colorectal (CRC) and breast cancers. Increases in the level and/or activity of c-Src are observed in >70% of human CRC. High c-Src activity in CRC is associated with poor clinical prognosis but activating mutations in c-Src are rare. Due to this association with human malignancy and c-Src's tractability as a drug target, many companies have developed small molecule Src inhibitors that are approaching or have already entered Phase I trials. Until recently surprisingly little had been published regarding the impact of c-Src in CRC cells making it difficult to predict with confidence the efficacy of Src inhibitors in the clinic. In order to study the consequences of c-Src up-regulation in later stages of CRC, we developed a single step, high throughput system to generate CRC cell lines in which wild-type (wt), and low or high levels of constitutively activated c-Src (Y527F) could be inducibly and tightly regulated and the impact on CRC cell growth and drug responsiveness could be compared. Neither induction of wt c-Src nor low levels of constitutively active c-Src had any effect on cell proliferation. In contrast, high levels of c-SrcY527F resulted in "Src crisis" characterized by changes in cell morphology; delay in G_2 phase of the cell cycle accompanied by adhesion-dependent inhibitory phosphorylation of the G_2/M control protein Cdk1 on Tyr15 and increased non-apoptotic, autophagic-like cell death. Induction of c-Src Y527F in a CRC xenograft model did not result in an obvious increase in tumour growth. Together these data argue against a growth promoting role of elevated c-Src in later stages of CRC and might provide explanation for low frequency of activating c-Src mutations in colon cancers.

c-Src in Breast Cancer

c-Src functions downstream of HER2 and activation of c-Src influences response to Herceptin, tumour progression and metastasis. In order to determine whether expression of activated c-Src in pure ductal carcinoma *in situ* (DCIS) correlates with HER2 expression and clinicopathological parameters in DCIS, immunohistochemical expression of activated c-Src was evaluated in 110 patients with 'pure' DCIS and a median follow-up of 60 months. Levels of HER2, HER4, ER and Ki67 levels were also evaluated. Activated c-Src staining was evaluated as 1 (weak), 2 (intermediate) and 3 (strong). Strong expression of activated c-Src was associated with high tumour grade ($p<0.0005$) and epithelial

proliferation (measured by Ki67, p=0.015) but not tumour size, ER status and HER4 expression. HER2 positive DCIS had a higher recurrence rate at 5 years (p= 0.0076). Activation of c-Src is seen in HER2 positive, high grade DCIS lesions with a higher proliferation index. Thus, interruption of c-Src signalling with small molecule inhibitors may be therapeutically useful.
In collaboration with Mr Nigel Bundred, University of Manchester, Dept of Surgery.

PI-3K Mutations in colorectal Cancer

The lipid kinase phosphatidylinositol 3-kinase (PI3-K), is activated downstream of c-Src in CRC. PI-3K regulates pathways involved in cell death, growth and proliferation. Several studies identified a series of highly conserved mis-sense mutations in the gene encoding the catalytic PI3-K subunit p110α in a variety of human cancers including colorectal cancer (CRC), although their ramifications are not clear. A panel of CRC cell lines was screened for these p110α mutations and three lines (HCT116, LS147-T, DLD-1) were identified. Constitutive and IGF-1-stimulated activity of PI-3K, together with kinetics of PI-3K activation was assessed. No differences that distinguished cells carrying p110α mutations from those with wild-type p110α were detected.

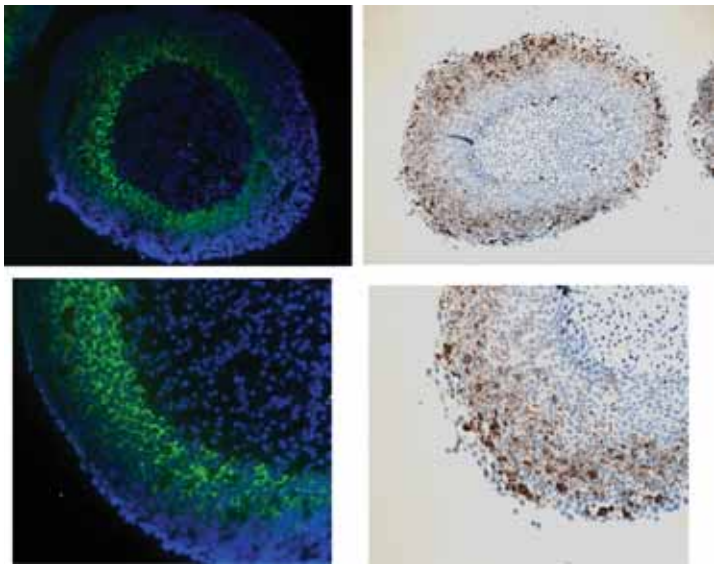
Hypoxia and colorectal cancer

Solid tumours contain sub-populations of hypoxic cells that are refractory to radiotherapy and some forms of chemotherapy. A key question for us is how does hypoxia affect drug-induced apoptosis in tumours? Hypoxia down regulates several pro-apoptotic members of the Bcl-2 family (Bax, Bad, and Bid) in a panel of CRC cell lines grown as monolayers *in vitro* or as human tumour xenografts. The hypoxia mediated down regulation of Bid occurs via a Hypoxia Inducible Factor-1 (HIF-1) dependent process in these cell lines and contributes to drug resistance under hypoxic conditions. In addition, in a subset of clinical CRC, nuclear HIF-1 expression was inversely correlated with Bid expression suggesting that this pathway, predicted to lower the threshold for apoptosis, is likely to be operational in patients' tumours. Further studies of the role HIF-1 plays in modulating drug responses is now under investigation including the use of a colorectal cancer spheroid model (see Figure). By utilising a reporter system based on hypoxic response elements (HREs) and Hoechst 33342 as a perfusion marker, oxygenated and hypoxic cell subpopulations could be isolated from disaggregated spheroids and subjected to clonogenic assay after treatment with oxaliplatin. Hypoxic cells were clonogenically resistant to oxaliplatin we are now determining the relative contribution to this resistance of drug penetrance and hypoxia. Subsequent studies in tumour xenografts are planned using these methodologies.
Collaborators: Professor Ian J Stratford, Dr Kaye Williams (School of Pharmacy, University of Manchester), Miss Sarah T O'Dwyer, Mr Andrew Renehan and Dr Mark Saunders (Depts Surgery and Medical Oncology, Christie Hospital NHS Trust).

Hypoxia and neuroblastoma

Neuroblastoma is the commonest extracranial solid tumour of childhood. These tumours often display adverse biological features and are highly resistant to current chemotherapy. Little is known about the importance of hypoxia in paediatric tumours. We evaluated the effects of hypoxia on the response of the SH-SY5Y neuroblastoma cells to the clinically relevant drugs, vincristine and etoposide. Short periods of hypoxia (1% 0_2) of up to 16 hours had no effect upon drug-induced apoptosis or clonogenic survival. Prolonged hypoxia of 1 to 7 days lead to reduction in vincristine and etoposide-induced apoptosis which was reflected in increased clonogenic survival. Transcriptionally active HIF1α was less detectable beyond 48 hrs of hypoxia, except when treating cells with vincristine or etoposide. Down regulation of HIF1α by shRNAi reduced hypoxia-induced drug resistance, and increased apoptosis, as did prolonged inhibition of its transcriptional activity by YC-1. These results suggest that prolonged hypoxia leads to resistance to clinically relevant drugs in neuroblastomas.

Collaborator. Dr Eddy Estlin, Royal Manchester Childrens' Hospital. Funding from BBSRC and The Friends of Rosie



Fixed, sectioned colon carcinoma cell spheroids (~500μm diameter) labelled with anti-pimonidazole antibody to mark low oxygen tension (green) with blue DAPI-stained nuclei (left images), or treated with Oxaliplatin and stained for active caspase-3 (brown) to detect apoptosis.

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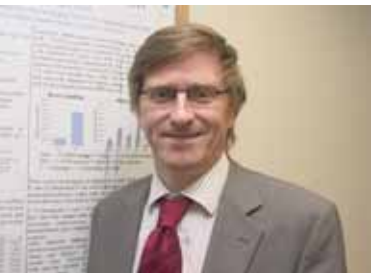

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CEP develops, validates and implements pharmacokinetic (PK) and pharmacodynamic (PD) assays for Phase I trial at the Christie Hospital's Derek Crowther Unit (DCU). Our research focuses on novel agents targeted at apoptosis pathways and at angiogenesis. PK and PD analyses continued on the CR-UK sponsored trial of AEG 35156 (antisense oligonucleotide to the inhibitor of apoptosis protein X-IAP, Aegera Therapeutics). Preclinical studies on a small molecule inhibitor of XIAP, Xiapuradimib commenced (with John Reed, Burnham Institute) and on inhibitors of anti-apoptotic members of the Bcl-2 family. PD assay validation nears completion for the upcoming CR-UK sponsored trial of an anti-angiogenic drug GSAO. A strategy to hasten trials on novel agents in childhood malignancies was implemented whereby preclinical studies are initiated in the paediatric setting as adult Phase I trials begin. We also established a Biomarker Discovery team and a CR-UK/AstraZeneca funded fellowship scheme for clinical pharmacologists.

Clinical trial facilities at the Christie Hospital's Derek Crowther Unit (DCU)

The translational research of CEP is directly associated with the DCU that provides world class facilities for Phase I/II trials. During 2004/2005 the DCU had 106 trials on its database involving 725 patients and almost 5,500 patient visits. The Christie Hospital NHS trust is a CR-UK Key Centre for Phase I trials and also a Key Centre for the National Translational Cancer Research network (NTRAC).

Development of a good clinical laboratory practice (GCLP) quality system in CEP

In order to comply with the new European Directive (2001/20/EC) on Clinical Trials which entered the UK statute book on the 1st of May 2004 (Statutory Instrument, 1031, HMSO) and facilitate greater collaboration with the Cancer Research UK Drug Development Office and the Pharmaceutical Industry, CEP further developed its quality assurance (QA) system based on the principles of good laboratory practise (GLP) and good clinical laboratory practise (GCLP). The CEP QA web pages plot the progress of the groups QA system(http://www.paterson.man.ac.uk/groups/CEP/glp_home.jsp). We move to the purpose built PK/PD PACCAR laboratories designed for GCLP studies in 2006.

Pharmacodynamic assays for novel agents that induce tumour cell apoptosis

We have validated or are in mid-validation of a series of PD assays for tumour apoptosis, including IHC for active caspase 3, cleaved PARP and cleaved cytokeratin 18 (CK18). In addition have validated or are validating surrogate markers of tumour apoptosis e.g. the M30 ELISA that detects cleaved CK18 shed into peripheral blood, the M65 ELISA that detects blood borne full length CK18, and an assay for serum nucleosomal DNA. The clinical utility of these ELISAs is being evaluated in several clinical trials of novel and conventional agents in the Christie hospital.

Pharmacodynamic evaluation of an antisense therapeutic AEG 35156 targeting the inhibitor of apoptosis protein (XIAP) during a Phase I clinical trial.

XIAP is a potent anti-apoptotic protein whose over expression is associated with resistance to chemotherapy and radiotherapy. AEG35156 is a synthetic 19-mer, 2nd generation, mixed backbone antisense oligonucleotide to human XIAP. Inhibition of XIAP expression by AEG35156 enhances cancer cell apoptosis *in vitro* and *in vivo* as a single agent or in combination with chemotherapeutics. Study objectives were to establish the MTD of AEG35156 as a 7-day continuous infusion every 3 weeks, determine its PK profile, evaluate XIAP inhibition in PBLs and tumour cells and document anti-tumour activity. 15 evaluable patients have been treated with 36 cycles administered. The most common toxicities observed were thrombocytopenia and elevated transaminases. AEG35156 steady-state plasma levels ranged from 0.5 to 3.0μg/mL. A ~ 30% decrease in XIAP mRNA was seen in PBLs 3 days after infusion at 160mg/m2. A patient with non-Hodgkin's lymphoma had marked transient decreases in peripheral lymphoblasts during AEG35156 administration associated with ~ 80% XIAP mRNA knockdown. A patient with breast cancer had an unconfirmed partial response. In conclusion, AEG35156 can be safely delivered as a protracted infusion with preliminary evidence of XIAP mRNA knockdown and antitumour activity.

Collaborators John Durkin & Eric LaCasse at Aegera Therapeutics

Anti-angiogenic Drug Development.

We are establishing a range of preclinical models to confirm anti-angiogenic drug action and to facilitate PK and PD assay development. The anti-angiogenic trivalent arsenical, GSAO will enter clinical trial at the Christie in 2006 with Dr Jayson as PI. In addition to PK evaluation, a comprehensive package of pharmacodynamic endpoint assays will be performed. Biopsies will by examined for the expression of endothelial markers (CD31, CD34 and CD105, see Figure 1) that allow discrimination between the effects of the drugs on established and new vessels. Tumour apoptosis will be detected by expression of activated caspase 3 and cleaved cytokeratin 18 and the surrogate apoptosis endpoint assay M30 will also be used. Imaging approaches (DCE-MRI and/or PET) will also be conducted in these trials.

AZMU Biomarkers Club was established as a forum to discuss biomarkers of efficacy and toxicity with colleagues at AstraZeneca. There were 50 participants at the inaugural meeting, considered a great success, on biomarkers of angiogenesis.

Co-founders of AZMU Biomarkers Club, Andrew Hughes and Ruth Roberts, AstraZeneca.

Pre-clinical evaluation of novel agents in paediatric tumours

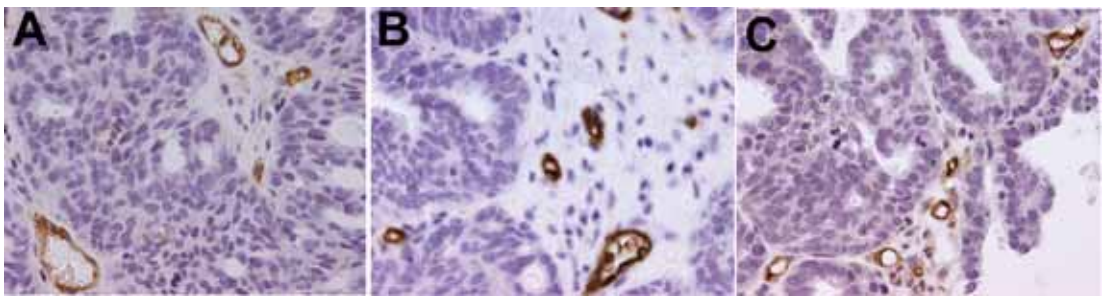
To address a relative lack of new agents for childhood cancer we established a system for evaluating novel agents against paediatric tumour cell lines at the same time these agents enter adult phase 1 study, a strategy strongly supported by the United Kingdom Childhood Cancer Study Group and CR-UK Development Committee. We are currently investigating the Aegera XIAP antisense, a XIAP small molecule inhibitor and the bioreductive agent, RH1.

Collaborators; Eddie Estlin (Royal Manchester Children's Hospital), Sue Burchall (CRUK Clinical Centre, Leeds), Debbie Tweddle (Northern Institute for Cancer Research, Newcastle).

Funding; CR-UK Development Committee, Christie Hospital Endowment Fund

The Clinical Pharmacology Fellowship Training Scheme was established in 2005 with equal funding from Cancer Research UK and AstraZeneca. Two fellows will enter the scheme in early 2006 to study biomarker discovery and implementation in clinical trials at the Christie Hospital from the academic and industrial perspectives.

Collaborators Andrew Hughes and Donna Johnstone, Discovery Medicine, AstraZeneca.



Immunohistochemical staining of endothelial cells in human ovarian tumours Panel A. CD31. Panel B CD34. Panel C CD105.

Publications listed on page 58

GROUP LEADER
Lez Fairbairn

POSTDOCTORAL FELLOWS
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Tom Southgate
Barbara Verbeek

SCIENTIFIC OFFICERS
Dorothy Gagen (50%)
Lorna Woolford

GRADUATE STUDENTS
Laura Edwards
(née Hollins, with Crispin Miller)
Caroline Truman

UNDERGRADUATE STUDENTS
Rebecca Schofield

Gene Therapy Group

http://www.paterson.man.ac.uk/groups/gt.jsp

The work of the Cancer Research UK Gene Therapy Group has continued to focus on two key areas in the treatment of cancer, i) the genetic chemo/radioprotection of haematopoietic stem and progenitor cells, and within this area, the selection of these cells and potential clinical consequences of selection, and ii) the targeting of tumours utilising macrophages. Within the group we have expanded our usage of viral vectors for gene transfer to these cells types by developing lentiviral and adenoviral vectors to complement the retroviral vectors we have previously used extensively within the haematopoietic system.

In July this year the Gene Therapy Unit group leader Lez Fairbairn unexpectedly and tragically died. The group continues to draw its enthusiasm and inspirations from the work programmes that Lez had instigated but this has obviously had an effect on the direction of the work that the group has been undertaking. With a strong emphasis on the efficacy of retroviral gene transfer and the subsequent consequences of this genetic manipulation we have continued to study gene therapy strategies for chemo and radioprotection, their potential to aid selection, and their ability to induce a repopulation advantage after transplantation to the haematopoietic compartment. We have focussed our studies on a series of candidate genes that allow us to examine each of these areas individually, and utilised both *in vitro* and *in vivo* models to do so.

HOXB4 Friend or Foe?

Our investigations into the potential of the homeobox transcription factor, HOXB4, for usage in gene therapy has taken us down two avenues; one utilising the gene for it's potential to aid repopulation of the haematopoietic compartment following transplant, and the second to determine the molecular events underlying these effects. Whilst the biological effects of HOXB4-overexpression on *in vitro* expansion and *in vivo* engraftment of haemopoietic stem and progenitor cells is well documented, the molecular events that underlie these effects remain unknown. To ascertain and understand these events, Dorothy Gagen has introduced a tamoxifen-inducible HOXB4 in FDCP-mix haemopoietic progenitor cells. Laura Edwards, a PhD student, has been phenotypically characterising these cells and investigating the transcriptional consequences of HOXB4 activation. She collected mRNA from cells cultured in the absence of tamoxifen and at various times following tamoxifen addition, and has used this to probe Affymetrix GeneChips. Data analysis is still underway with putative targets being validated using quantitative real-time PCR. An alternative to the *in vivo* selection strategy is to impart a proliferative advantage upon transduced haematopoietic stem cells (HSC). Over-expression of HOXB4 in bone marrow cells has been shown to expand the number of long-term repopulating cells from bone marrow *in vitro* and *in vivo*. Apart from a role in the self-renewal of HSC, ectopic expression of HOXB4 inhibits the differentiation of primary murine bone marrow and purified cord blood $CD34^+$ cells. This disruption seems to be not a result of a complete block of differentiation, but from a reduction in the rate of commitment. Barbara Verbeek has been examining this effect highlighting the need to carefully determine the therapeutic window of HOXB4 expression. As part of this work we also undertook a study investigating whether HOXB4 could mediate an increased risk of leukaemogenesis within an *in vivo* model. The results of this study are still under examination and analysis of the viral integration sites will be key to any conclusions that can be drawn. It however, appears to demonstrate that there is no increased risk of leukaemogenesis as a direct consequence of overexpression of HOXB4.

Chemo- and Radioprotection Studies

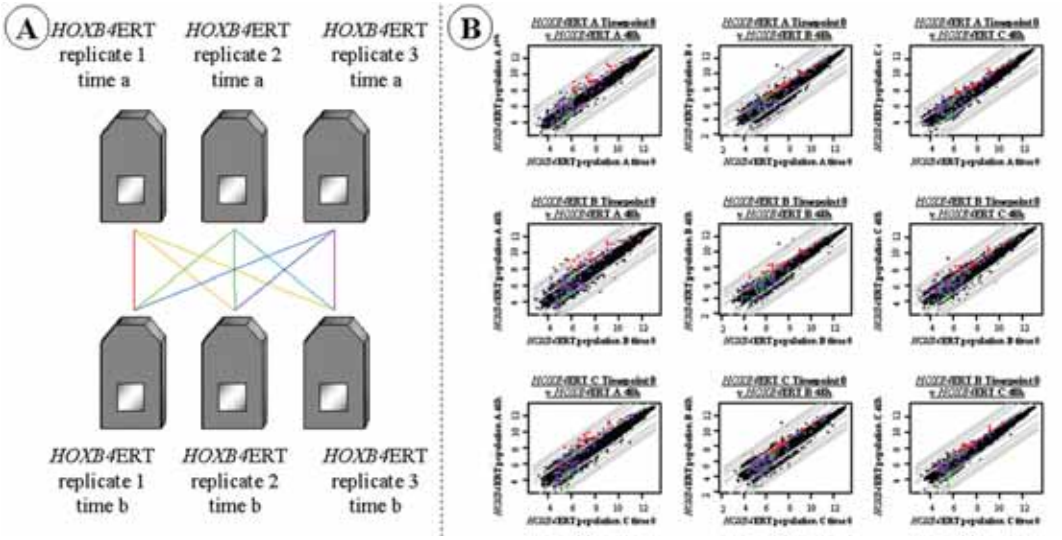
Effective chemo- or radiotherapy of malignant disease is achieved through balancing efficient tumour kill with toxicity in normal proliferating tissues, primarily the haematopoietic system. We have contin-

ued our studies utililising the drug-resistance genes encoding either the inactivator-insensitive $P_{140}K$ mutant version of methylguanine-DNA-methlytransferase (MGMT), or multi-factoral chemoprotection by the co-expression of MGMT with either MDR1 or MRP1, both *in vitro* and *in vivo*. Lorna Woolford has been investigating the potential of MGMT $P_{140}K$ resistance to inactivation by PaTrin2. In this study we have tested the potential of MGMT $P_{140}K$ to confer inactivation-insensitive protection to HSCs. She has shown *in vitro* that mutant MGMT confers significant resistance to the combination of PaTrin2 and temozolamide when compared with controls and subsequently *in vivo* in cells transduced with MGMT $P_{140}K$-2a-eGFP used to reconstitute haematopoiesis in an *in vivo* model. In this model she has shown protection of the gene–modified cells following cytotoxic challenge and an *in vivo* selective advantage. Secondary transplantation experiments indicated that this selection occurred at the level of the stem cell. To further these studies Barbara Verbeek has developed inducible version of MGMT that will add increased safety and efficacy to the gene therapy selection strategies that we have been developing.

We have also initiated a study into the potential of enhancing DNA repair as a way of protecting the HSCs against other types of chemotherapeutics such as topoisomerase I inhibitors. Tom Southgate and Dorothy Gagen have over-expressed XRCC1 within HSCs and demonstrated protection against a class of chemotherapeutic agents which induce high levels of collateral toxicity. This is now being tested *in vivo* in order to assess whether XRCC1 may also be used to aid selection. Along with Rebecca Schofield, Tom has also developed gene therapy strategies that confer radioprotection. Previously Tom had demonstrated that SOD2 conferred significant protection of the HSCs against ionising radiation. They are now working on enhancing this protection though the co-expression of Catalase which will aid in the removal of reactive oxygen species and their downstream consequences within the cell. Furthermore, Tom has examined the ability of XRCC1 to confer enhanced DNA repair after ionising radiation damage. He has shown that overexpression of XRCC1 mediates the greatest level of radiation protection yet conferred to HSCs and that the mechanism of action is likely to be through a non-classical action of the XCRR1 protein. This investigation is still ongoing but suggests that XRCC1 may be a useful tool in both chemo - and radioprotection of tissues.

Modifying Macrophages to Infiltrate and Attack Tumours

Previous work by our group has demonstrated that chimeric immune receptors targeted against tumour antigens can specifically direct the Antibody-Dependent Cellular Cytotoxicity (ADCC) pathway of macrophages against tumour cells expressing these antigens. Michael Lie-A-Ling has continued this work by investigating the use of lentiviral vectors in delivering chimeric immune receptors to bone marrow cells. The goal of this is not only to incorporate the chimeric receptors in a wider range of effector cells but also to incorporate these receptors in stem and progenitor cells ensuring long term production of gene modified effector cells *in vivo*. We are aiming to use the lentiviral technology to express chimeric immune receptors in the myeloid compartment of the immune system.



(A) Illustrates comparisons made between *HOXB4*ERT arrays at two timepoints. Equivalent comparisons were also made for ERT-control arrays and fold changes calculated for each probeset. (B) Illustrates scatterplots for each of these comparisons (*HOXB4*ERT time 0 v 48h time point shown). Colours identify probesets with fold changes of ≥ |1.5|; red in all 9 comparisons; blue in 8; green in 7; and purple in 6.

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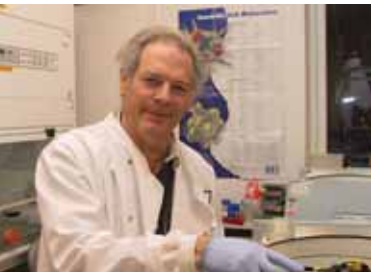

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Lucy Hopcraft
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Helen Spencer

Immunology Group

http://www.paterson.man.ac.uk/groups/immuno.jsp

Ongoing and future work is focused on the evaluation of responses against tumour associated antigen (TAA) locally and systemically in immunotherapy clinical trial and other cancer patients. We are testing and further developing (pre-clinical studies, early phase clinical trials): 1) 5T4 antibody directed superantigen therapy; 2) Cancer vaccination versus HPV, 5T4 and other TAA; 3) Engineered T cells versus 5T4 antigen; 4) Combination therapies; 5) Enhancement of tumour immunity by prevention of T regulatory activity. We work closely with Medical Oncology to form the Manchester Immunotherapy Group and key translational research goals include the development of GLP assays of tumour immunity.

HPV associated neoplasia

We have initiated clinical trials to further investigate therapeutic immunization of HPV associated neoplasia (with Professor Henry Kitchener, St Mary's Hospital Manchester). Thus 20 patients with high grade vulval intraepithelial neoplasia (VIN) have received treatment with imiquimod followed by photodynamic therapy (PDT); another group will receive imiquimod plus HPV oncogene vaccination. The imiquimod acts partly through inducing a local inflammatory response, and the PDT or vaccination have the potential to stimulate T cell mediated immunity effecting resolution of persistent VIN. The early clinical results are encouraging. Having completed the active treatment protocol with 26 week follow up, eight of nine women show partial and one a complete response after the imiquimod phase; following the PDT there is evidence of additional clinical responses. Preliminary immunohistochemistry analysis of tumour infiltrating lymphocytes in biopsies from 5 imiquimod responders and 5 non-responders has shown higher lesion-associated CD4, CD8 and CD1a immune cells pre-treatment in the responders. This supports data from our previous study of therapeutic vaccination with TA-HPV (Davidson *et al.*, Cancer Res 2003; 63: 6032). Ten available specimens from this study were examined for the presence of T regulatory cells by staining for the transcription factor FOXP3. Interestingly, in women who made a favourable response the numbers of T regs within the lesions fell in response to vaccination, but rose in those women who did not respond favourably.

5T4 oncofetal antigen and development

We have utilized the associated loss of pluripotency of murine ES cells with the early upregulation of 5T4 expression to search for other changes in gene expression using an Affymetrix GeneChip approach. The ES cells were grown with or without leukemia inhibitory factor (LIF) for 3 days and the disaggregated cells sorted for expression or not of cell surface 5T4 expression. The preliminary analysis of data generated from one ES cell line has identified a set of candidate genes for further validation as relevant to processes common to embryonic morphogenesis (eg 5T4: Barrow *et al.*, Dev Dynamics 2005; 233: 1535) and tumour cell behaviour.

Preclinical studies of cancer vaccines

We have investigated homologous and heterologous vaccine combinations using human 5T4 expressing replication defective adenoviral vectors and retrovirally transduced dendritic cell (DC) lines for induction of tumour protection and therapy of human 5T4 expressing B16 melanoma cells. Importantly, the best treatment regimen for *in vivo* tumour protection does not give similar beneficial effects in active therapy. In the active therapy model, vaccination with Adh5T4 followed by DCh5T4 is the best treatment regimen, whereas vaccinations with DCh5T4+Adh5T4 consistently failed to improve survival. The tumour challenge *per se* induces a Th2 immune bias to 5T4 and the differences in treatment efficacy correlate with the first immunization of either Adh5T4 or DCh5T4, respectively driving a Th1 or Th2 response. This in turn differentially influences CD25+ regulatory cell activity. Importantly, depletion of CD25 T regula-

tory cells following tumour challenge, but prior to immunization, improves the vaccine efficacy offering useful strategies for optimizing 5T4 vaccine immunotherapies (Ali *et al.*, submitted).

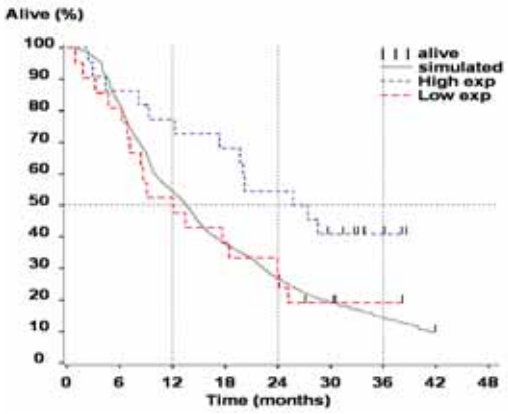
We have generated murine T cells expressing chimeric receptors (CR) against human 5T4 and evaluated their tumour therapeutic efficacy alone and in combination with immunization using Adh5T4 and bone marrow derived DC (BMDC). In the B16h5T4 melanoma model, early local subcutaneous, but not systemic administration of syngeneic 5T4 specific CR T cells resulted in significantly improved survival. This anti-tumour activity was enhanced when combined with a post tumour challenge immunization with Adh5T4 followed by post CR T cell treatment with BMDC. This synergistic effect was lost without delivery of the BMDCs. Our findings suggest that combining engineered T cells with specific vaccination strategies can improve the active tumour therapy (Jiang *et al.*, submitted).

Clinical trials of immunotherapy

The pivotal role of regulatory T cells in immune homeostasis, preventing autoimmunity as well as potentiating cancer is emerging. In support of this we have shown that in renal cell carcinoma (RCC) patients there are significantly increased numbers of CD4+CD25 high regulatory cells which express the FOXP3 marker (Griffiths *et al.*, submitted). These results have prompted a clinical trial aimed at evaluating the safety, immune and clinical consequences of a T regulatory cell depletion protocol in patients with RCC.

The RCCs strongly express 5T4 oncofoetal antigen (Griffiths *et al.*, Br J Cancer 2005; 93: 670). RCC patients have been treated with individualized doses of ABR-214936, a Fab anti-5T4 fused to a modified bacterial superantigen. Median survival for all 43 patients with a minimum follow up of 26 months was 19.7 months with 13 patients still alive to date. When stratified by the Motzer prognostic criteria, there is a prolonged survival compared to published expectation. As can be seen in the figure, the group of patients receiving the highest drug exposure lived almost twice as long as expected, while the low dose group lived as expected from their Motzer scores. The increase in circulating IL-2 levels after treatment provides a useful biomarker for clinical effect since patients with the highest increase in IL-2 at the second treatment day lived significantly longer. The high degree of disease control in this study and the encouraging survival times suggest that this treatment can be effective in a significant number of RCC patients (Shaw *et al.*, submitted). The effective dose correlated with better outcome and thus the next generation of drug, with reduced antigenicity and toxicity, should offer increased efficacy. This drug has been successfully labelled with the positron-emitting nuclide ^{124}I and has been used in two RCC patients to demonstrate a selective accumulation in tumour.

A CR-UK sponsored trial in collaboration with Oxford BioMedica evaluating 5T4-MVA vaccine in patients undergoing surgical resection of colorectal liver metastases has finished recruitment (20 patients). The first 18 patients have had their primary immunological assays completed. From investigations performed at the Paterson Institute all demonstrated T-cell responses to MVA and MVA-5T4 in proliferation assays, 12 showed baseline responses to 5T4 protein or subunit peptides, 8 showed boosting of some responses following vaccination, and 11 demonstrated new vaccine induced responses.



Patient survival following treatment with ABR-214936 stratified by drug exposure
Patients receiving the highest drug exposure survived significantly longer than predicted and longer than the low exposure group, who survived as long as predicted by their Motzer prognostic scores

Publications listed on page 60

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Mitotic Spindle Function and Cell Cycle Control Group

The overall aim of our research is to understand the molecular mechanisms that achieve sister chromatid segregation and regulate cell cycle progression in mitosis. This is a very active and rapidly evolving research area that is driven by the parallel analysis of mitosis in organisms such as yeast, *Drosophila, C. elegans* and human cells, advances in cell imaging and proteomics, and genome wide screens for genes that function in mitosis. Our initial studies on the spindle checkpoint and the function of kinetochore, spindle and centrosome components were performed in budding yeast as a model system. Now, we have reached the point that our knowledge and expertise allows us to switch to higher eukaryotes. For the past year we have been studying the function of the phosphatase Cdc14 in chicken and mammalian cells. Recently, we have started to analyse the human family of Mob proteins.

Kin4 and the spindle position checkpoint

Yeast Cdc14 is a conserved phosphatase, which is entrapped in the nucleolus during most of the cell cycle through complex formation with the nucleolar protein Net1. This entrapment effectively inactivates the phosphatase. At the beginning of anaphase, Cdc14 becomes activated through release from the nucleolus in a stepwise manner. First, in early anaphase, the FEAR network (cdc Fourteen Early Anaphase Release), which includes separase Esp1, promotes a partial release of Cdc14 from the nucleolus. In a second phase the mitotic exit network (MEN), a GTPase driven signalling cascade, triggers the release of the remaining Cdc14. The Ras-like GTPase Tem1 is one of the most upstream MEN components and is regulated by the putative GEF Lte1 (an activator of the MEN) and the GAP complex Bfa1-Bub2 (MEN inhibitor). The full activation of Cdc14 by the MEN is essential to reduce Cdk1 activity, which is a prerequisite for cells to exit mitosis, not only in yeast but also in mammalian cells. How the MEN controls Cdc14 is an important and outstanding question. It is only known that the Dbf2-Mob1 kinase complex is the most downstream component in the MEN signalling cascade and as such should regulate Cdc14.

The association of the MEN activator Lte1 with the bud cortex led to the proposal that the SPB-associated Bfa1-Bub2 GAP inactivates Tem1 until the SPB enters the bud in anaphase where the MEN becomes activated through Lte1. This coupling of mitotic exit with nuclear migration prevents premature mitotic exit in mutants with defects in spindle orientation and was termed "the spindle position checkpoint" (SPOC). The distinct polar cellular distribution of MEN activators and inhibitors is only one element of the SPOC. An additional component is the inhibition of the phosphorylation of Bfa1 by Cdc5 in cells with a misaligned anaphase spindle. This keeps the MEN inactive and prevents mitotic exit.

Recently, we have shown that the Kin4 kinase is an essential component of the SPOC. Interestingly, Kin4 only becomes essential when spindles are misaligned but not when microtubules are depolymerised for example by the drug nocodazole. A number of data support the notion that Kin4 inhibits the MEN upstream of the Bub2-Bfa1 complex in response to spindle misalignment. For example overexpression of *KIN4* is lethal and this lethality is suppressed by the deletion of either *BFA1* or *BUB2*. Kin4 may inhibit the capability of Cdc5 to phosphorylate and thereby inactivate Bfa1 with anaphase onset when the anaphase spindle is misaligned.

An indication of how Kin4 could regulate Bub2-Bfa1 and Cdc5 comes from localisation studies. In interphase and early mitosis Kin4 is associated with the cell cortex of the mother cell and binds in mid-anaphase predominantly to the SPB in the mother cell body. In contrast, Bub2-Bfa1 are associated with the SPB in the daughter cell while Cdc5 is at

both SPBs. Due to this non-overlapping localisation Kin4 cannot regulate Bfa1-Bub2 at SPBs. However, in response to a misaligned anaphase spindle Kin4 localises to both SPBs. This enables Kin4 to regulate Bub2-Bfa1 for example by inhibiting Cdc5.

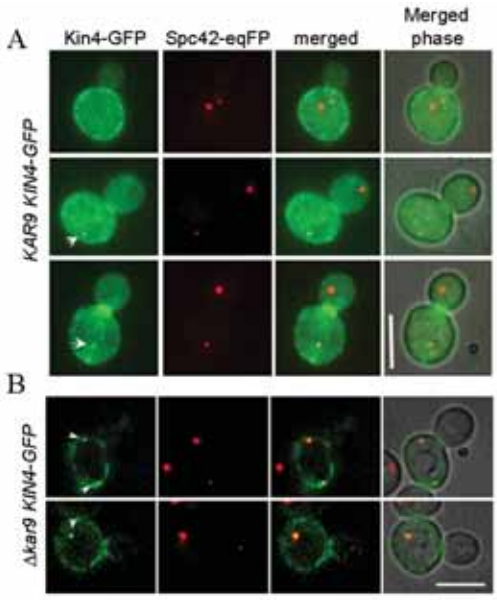
SPB duplication

The SPB in *Saccharomyces cerevisiae* functions to nucleate and organize spindle microtubules. The SPB is duplicated once per cell cycle and it is embedded in the nuclear envelope throughout the yeast life cycle. However, the mechanism of membrane insertion of the SPB has not been elucidated. Ndc1 is an integral membrane protein that localizes to SPBs, and it is required for insertion of the SPB into the nuclear envelope during SPB duplication. We identified the essential SPB component Nbp1 as an interactor of Ndc1. *NBP1* shows genetic interactions with several SPB genes in addition to *NDC1*, and two-hybrid analysis revealed that Nbp1 binds to Ndc1. Furthermore, Nbp1 is in the Mps2-Bbp1 complex in the SPB. Immunoelectron microscopy confirmed that Nbp1 localizes to the SPB, suggesting a function at this location. Consistent with this hypothesis, *nbp1-td* (a degron allele) cells fail in SPB duplication upon depletion of Nbp1. Importantly, these cells exhibit a "dead" SPB phenotype, similar to cells mutant in *MPS2, NDC1* or *BBP1*. These results demonstrate that Nbp1 is a SPB component that acts in SPB duplication at the point of SPB insertion into the nuclear envelope.

Human

In human cells two ORFs named hCdc14A and hCdc14B that have high homology to *CDC14* have been identified. Notably, the cell cycle arrest of conditional lethal *cdc14-1* cells was complemented by hCdc14A and hCdc14B indicating that the hCdc14 proteins fulfil at least some functions of Cdc14. Despite this complementation, the molecular roles of hCdc14A and hCdc14B in human cells are poorly understood. Cdc14A seems to have a role in centrosome duplication and cytokinesis. hCdc14B is a nucleolar protein of unknown function. Whether Cdc14A/B are essential in metazoae as it is in budding yeast is a matter of debate due to conflicting reports from *C. elegans*.

Whether a MEN-like pathway regulates Cdc14A and Cdc14B in mammalian cells is unclear. However, at least some components of the MEN are conserved in mammalian cells. The MEN proteins Mob1 and Dbf2 have homologues in mammalian cells. Dbf2 belongs to a family of conserved kinases, including the mammalian tumour suppressor gene LATS, that are important regulators of cell morphogenesis, cell proliferation, and cell cycle regulation. Mob4A and Mob4B with high homology to yeast Mob1 are associated with centrosomes and the midbody and have an essential role in cytokinesis.



Kin4 becomes targeted to both SPBs in response to spindle misalignment.
(A) Kin4 (green) is associated with the cortex of the mother cell and binds in early anaphase to the SPB (Spc42-eqFP, red) in the mother cell body (marked by arrows) but not the SPB in the bud. Kin4 is also associated with the site of cytokinesis. (B) When the anaphase spindle becomes misaligned in the mother cell body (*Δkar9* cells) Kin4 associated in 95% of the cells with both SPBs (top panel, arrow). However, when the spindle of *Δkar9* cells was correctly aligned along the mother-bud axis, Kin4 bound in a polar manner with only the SPB in the mother cell (bottom panel, arrow).

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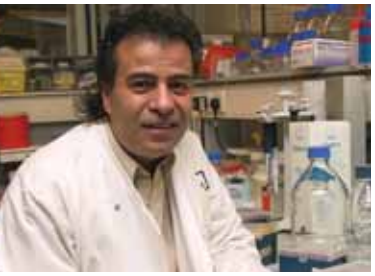

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Radiochemical Targeting and Imaging Group

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Our research programme over the last year continued to focus on developing paradigms for molecular targeting and imaging assays. The overall aim is to develop means by which various tumour activities can be related using specifically targeted probes combined with molecular imaging. We have shown that detection of radiolabelled Z-vad-fmk, a peptide inhibitor of apoptosis, is more sensitive than radiolabelled annexin V *in vitro*, and *in vivo*.

We have compared the kinetics of F-18-fluorothymidine in tumour cell lines and observed variation in the extent of uptake depending on the tumour cell type. In a NSCL cancer model, we have demonstrated changes in cell proliferation in response to cell death induction. Our translational research activity over the last year focussed on the development of a radiolabelled tumour targeted superantigen antibody which is currently being tested in phase I clinical studies. As part of this development we have designed, optimised and validated a clinical labelling strategy to label the drug with the positron emitting isotope ^{124}I. In the renal cancer patients treated so far, we have shown antigen-specific accumulation and retention of the labelled drug in their tumours.

Targeting and Imaging Cell Death Pathways

A prominent feature of programmed cell death is the activation of caspases - cysteine proteases that cleave their substrates after aspartate residues. Our results so far show that BOC-D-fmk inhibits DEVD-AMC cleavage *in vitro*. We have subsequently found that it inhibits apoptosis in camptothecin treated Jurkat cells and hepatic apoptosis in mice treated with anti-Fas antibody. We have made a radiolabelled derivative of this molecule, [^{125}I]4IB-D-fmk. Our preliminary data show that this is a more sensitive probe than the annexin-PS binding and experiments to see whether this molecule could be used to detect small changes in programmed cell death *in vivo* are in progress.

Cell proliferation PET probes

We have been investigating the merit of various radiolabelled thymidine analogues including [^{124}I]-IUdR, [^{124}I]-FIAU and [^{18}F]-FLT. In terms of thymidine phosphorylase activity, [^{124}I]-IUdR was found to have the highest number of iudouracil cleaved molecules with [^{124}I]-FIAU the least. In both normal and tumour bearing animals, thymidine kinase activity was reflected in proliferative tissues such as spleen, bone marrow and tumours significantly more than in other tissues. A pilot clinical imaging and metabolite analysis study using [^{124}I]-IUdR in NSCLC patients, revealed kinetic information which identified the contribution of the various metabolized species to the PET signal. This limited data however, has not identified the contribution to the PET signal from [^{124}I]-IUdR-DNA as the circulating ^{124}I-metabolites were eliminated more slowly than anticipated, resulting in a biodistribution which predominantly reflected ^{124}I-iodide accumulation.

Imaging drug targeted therapy

In collaboration with Immunology and Medical Oncology, we are using molecular PET imaging to study tumour targeted superantigen (TTS) therapy directly in cancer patients. Bacterial superantigens are secreted proteins produced by some bacteria as an immune-evasion mechanism. They bind to antigen-presenting cells (APC) and to T cells, causing their activation, and thus the death of the APCs. This is a very potent killing mechanism which we can harness for tumour therapy by fusing the superantigen to an antibody that recognises an antigen expressed by tumour cells. Clinical trials of TTS therapy directed against the tumour associated antigen 5T4 have shown clinical efficacy in lung cancer (unpublished) and in renal cancer (Shaw *et al.*, submitted). In the latter study, a sub-set of patients were assessed for clinical effect using FDG-PET, which gave a reliable, early indication of disease control which correlated with long term survival.

A new generation of TTS drug, ANYARA™, with

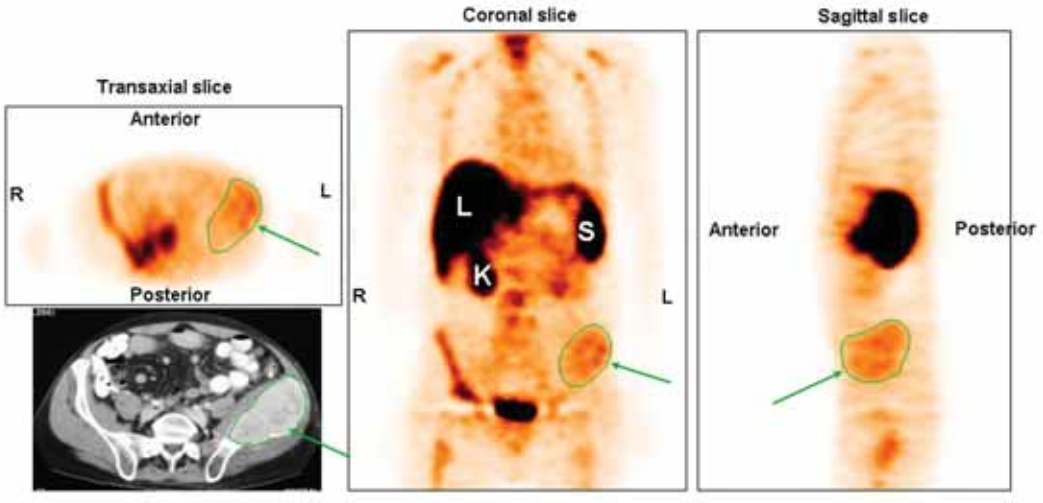
an improved toxicity profile, is currently being tested in phase I clinical studies. As part of this development we have designed, optimised and validated a clinical labelling strategy to label the drug with the positron emitting isotope ^{124}I. In the renal cancer patients treated so far, we have shown antigen-specific accumulation and retention of the labelled drug in their tumours see figure.

Pharmacokinetics and pharmacodynamics of anti-angiogenic agents

The aim of this project is to develop an ^{18}F-labelled anti-angiogenic agent with validated pharmacodynamic endpoints by comparing a biological response, related to the expression of a protein biomarker, with PET-derived intratumoral concentrations of the anti-angiogenic agent. As a biomarker we have synthesised an anti-integrin RGD-peptide which labelled with fluorine-18 using a novel fluorinating agent, [^{18}F]-Fluoroacetaldehyde. Unlike previously developed nucleophilic fluorinating agents, the advantage of this new compound in reductive N-alkylation reactions is that the conditions are mild, water and low temperature compatible, and can be used in protein radiolabelling. [^{18}F]Fluoroethyltosylate is produced, with a radioactive yield ranging from 75 to 90%, from ethylene di-*p*-toluenesulfonate by fluorination with [^{18}F]FK. It is then oxidized to [^{18}F]Fluoroacetaldehyde which is distilled, radioactive yield 60-70%. [^{18}F]Fluoroacetaldehyde is thus produced in 30 minutes from [^{18}F]FH coming from the cyclotron target with an overall radioactive yield ranging from 45 to 63%. This synthesis is easy to automate. Following the synthesis of the stable N-Fluoroethylbenzylamine derivative, the new compound has been characterised by mass spectroscopy and NMR and serves as a reference compound. The N-[^{18}F]Fluoroethylbenzylamine is analysed by HPLC under reverse phase conditions found to match the reference compound

Radiotracer quantum dots

We have initiated the development of quantum dot probes to target and image cellular pathways within tumours and their environment. We have achieved the synthesis of a new class of quantum dots, which we term "radiotracer quantum dots". These are nanoparticles synthesized from tracer quantities of imaging and therapeutic radionuclides. We have demonstrated greater than 90% incorporation of radioactivity into dots, as well as retention of fluorescence, stability and other properties of stable dots. We have demonstrated no toxicity of CdSe dots at this tracer (nM) level. These multi-functional particles were localized to various tumour cell lines where 50-60% uptake was observed within 30 minutes. The radiotracer dots were successfully injected into animals and the pattern of their localisation was studied. The non-conjugated dots were taken up by various tissues predominantly in accordance with blood flow. We are currently studying the pattern of accumulation of these nanoparticles in individual cells and organelles. Our analyses includes radioimaging, microautoradiography, optical detection and electron microscopy enabling therefore multi-modality detection and imaging.



Sagittal, coronal and transaxial PET images captured at 5-6 hours show accumulation of $_{124}$I-labelled ANYARA™ in a destructive metastatic lesion of the left iliac blade of a renal cell carcinoma patient (arrow). The comparable CT scan is shown for reference. Non-specific accumulation is also seen in the liver (L), spleen (S) and right kidney (K).

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The *AML1/Runx1* transcription factor is a frequent target of gene rearrangements and mutations in human acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). Consistent with its initial implication in leukemias, *Runx1* has been shown to be critical for normal haematopoietic development. The *MOZ* gene is involved in three independent myeloid chromosomal translocations fusing MOZ to the partner genes CBP, P300 or TIF2. MOZ, CBP and P300 are histone acetyltransferase (HAT) that are required as cofactors by many DNA-binding transcription factors. Using the *in vitro* differentiation system based on mouse embryonic stem (ES) cells, our goals are to further define the role of *Runx1* and *Moz* in early haematopoietic development and how alterations of their function leads to leukemogenesis.

Early haematopoietic development

The earliest site of haematopoiesis in the mouse embryo is the yolk sac where blood islands, derived from mesodermal cells, develop at approximately day 7.5 of gestation. The yolk sac blood islands consist of two lineages, a population of primitive erythroid cells surrounded by a layer of angioblasts that eventually form the developing vasculature. 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. Definitive haematopoiesis is defined by the generation of all lineages other than primitive erythroid cells and includes the production of definitive erythroid, myeloid and lymphoid cells.

Attempts to identify, isolate and characterize the precursors representing these initial stages of lineage development, including the elusive haemangioblast, have been largely hampered by the inaccessibility of the early mammalian embryo. The differentiation of embryonic stem (ES) cells in culture offers a powerful alternative approach to study the development of such lineages that are established very early in embryonic life. When removed from conditions that maintain them *in vitro* in an undifferentiated state, ES cells will spontaneously differentiate and form colonies or embryoid bodies (EBs) that contain precursors for multiple lineages including those of the haematopoietic and endothelial systems. Several studies have established that the early events of haematopoietic and endothelial commitment in the ES/EB model are comparable, if not identical, to that of the early embryo. Using this model system, a precursor was identified that generates blast colonies containing precursors of endothelial and haematopoietic lineages. The blast colony-forming cells (BL-CFC) that generate these colonies represent a transient population that appears in the 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. More recently the existence of the haemangioblast has been demonstrated *in vivo* in early embryos.

A critical function of *Runx1* in haemangioblast development.

To investigate the role of *Runx1* at the earliest stage of haematopoietic commitment, we have analyzed its expression pattern and function during ES/EB differentiation and in early yolk sac development. Expression analyses indicated that *Runx1* is expressed in yolk sac mesodermal cells prior to the establishment of the blood islands and within the BL-CFC in EBs. Embryoid bodies generated from the *Runx1*$^{-/-}$ ES cells did not contain definitive haematopoietic precursors, reflecting the defect observed in the mutant mice. Analysis of early EBs revealed a profound defect in the potential of the *Runx1*$^{-/-}$ ES cells to generate blast colonies. Fewer colonies were generated by the mutant ES cells and

their potential was restricted to endothelial and primitive haematopoietic development. In contrast, *Runx1*$^{+/+}$ blast colonies displayed endothelial as well as both primitive and definitive developmental potential. Altogether these results provide evidence that *Runx1* does function at the haemangioblast stage of development and suggest that its functional requirement may define subpopulations of BL-CFC precursors.

Downstream transcriptional targets of Runx1

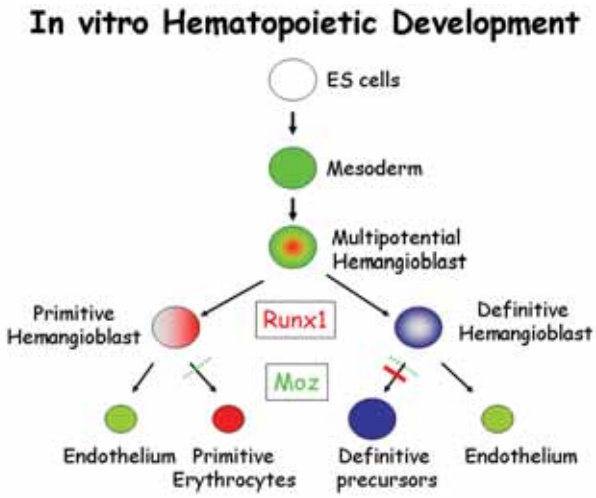
Runx1 has been shown to participate in the regulation of expression of a number of different genes including IL-3, myeloperoxidase, neutrophil elastase, M-CSF, GM-CSF and T cell receptors that are involved in relatively late stages of haematopoietic development. Our results have indicated that *Runx1* is required for definitive haematopoietic development at the level of the BL-CFC and is therefore likely to regulate a different set of genes at this time of development. To identify these genes, we have 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. The possibility to isolate large number of cells at the precise stage of the haematopoietic defect represents a unique advantage of the ES/EB system. We have validated the differential expression of several candidates on samples generated from the ES/EB system. We are now establishing the direct regulation of the transcription of some of these genes by *Runx1* by promoter assays or chromatin immunoprecipitation and are further determining the function of these genes during early haematopoietic development.

Runx1 isoforms

Previous studies have shown that *Runx1* is expressed as multiple naturally occurring spliced isoforms that generate proteins with distinct activities on target promoters. Recent experiments have indicated that expressions of some of these isoforms are differentially regulated during development. 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. We are testing this hypothesis by further defining the pattern of expression of the different *Runx1* isoforms and evaluating their respective functions.

Moz and haematopoietic development.

MOZ was found translocated in AML with CREB binding protein (CBP), the nuclear receptor TIF2 or the P300 transcriptional co-activator. 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 have created ES cells in which one or both allele of *MOZ* are mutated for the HAT activity. We have evaluated the consequences of such alteration upon *in vitro* ES cell differentiation. Our studies have revealed a profound defect in the normal proliferation and differentiation of haematopoietic precursors in absence of the HAT activity of MOZ. These results indicate a critical function of the HAT activity of MOZ during haematopoiesis. We are currently investigating the precise molecular and cellular mechanisms affected and evaluating the consequences of the HAT mutation during *in vivo* haematopoiesis.



Critical functions of *Runx1* and *Moz* in haematopoietic development. *Runx1* is essential for the generation of definitive haematopoietic precursors from definitive haemangioblasts. *Moz* is critical for the proliferation and differentiation of both primitive and definitive haematopoietic precursors.

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During development of the mouse embryo, blood cells are generated from mesodermal precursors at specific times and locations. 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 haemangioblast. To track the formation of mesodermal subpopulations and to study their specification toward blood lineages, we are using the *in vitro* differentiation of mouse Embryonic Stem (mES) cells as a model system.

Mesoderm formation and specification to the blood lineages

In the mouse embryo, mesoderm is generated from the epiblast through the process of gastrulation that is initiated at approximately day 6.5 of gestation. At the onset of gastrulation, the epiblast cells in the region that defines the posterior part of the embryo undergo an epithelial to mesenchymal transition and form a transient structure known as the primitive streak from which the mesoderm emerges. The newly formed mesoderm migrates away from the primitive streak and is patterned into various populations with distinct developmental fates. *Brachyury* is expressed in all nascent mesoderm and down regulated as these cells undergo patterning and specification into derivative tissues including skeletal muscle, cardiac muscle, kidney, blood and endothelium. The first mesodermal cells to develop within the embryo contribute predominantly to the extraembryonic tissues, giving rise to the haematopoietic and vascular cells of the yolk sac. Haematopoietic progenitors are found in the developing yolk sac as early as day 7.0 of gestation, approximately 12 hours following the beginning of gastrulation. Flk1, the receptor 2 for VEGF, is expressed in the yolk sac at this stage and is essential for the establishment of the blood cell and vascular lineages.

The first haematopoietic and endothelial precursors arise from extra-embryonic mesoderm and differentiate to form the blood islands in the yolk sac of the early embryo. The close spatial and temporal development of these lineages within the blood islands provided the basis for the hypothesis that they arise from a common progenitor, the haemangioblast. Direct demonstration for the existence of a progenitor with haemangioblast properties has been provided by experiments using a model system based on the *in vitro* differentiation potential of ES cells. Recent studies have now demonstrated the existence of haemangioblast precursors in gastrulating embryos.

In vitro differentiation of ES cells as a model system to study lineage specification

Following the initiation of differentiation in culture, ES cells form colonies known as embryoid bodies (EBs) that generate haematopoietic and endothelial progeny in a temporal pattern recapitulating the development of these populations in the yolk sac. Analysis of early EBs, prior to the haematopoietic and endothelial commitment stages, revealed the presence of a progenitor with haemangioblast potential. In response to VEGF, these progenitors which express Flk1, generate blast colonies that display both haematopoietic and endothelial potential. The characteristics of the BL-CFC (Blast colonies forming cell), namely its early development and its potential to generate haematopoietic as well as endothelial progeny, suggests that it represents the *in vitro* equivalent of the yolk sac haemangioblast.

To track mesoderm formation and enable us to isolate this population from developing EBs, we targeted the green fluorescence protein (GFP) gene to the brachyury locus in ES cells (referred as GFP-Bry). EBs generated from this ES cell line expressed readily detectable levels of GFP when observed under a fluorescence microscope. The haematopoietic potential of the heterozygous GFP-Bry ES cells was normal, indicating that this aspect of mesoderm development was intact.

Importantly, GFP expression faithfully recapitulated brachyury expression in differentiating EBs and as such, provides a unique marker for the identification and isolation of cells expressing this gene. Co-expression of GFP with Flk1 revealed the emergence of 3 distinct cell populations, $GFP^{-}Flk1^{-}$, $GFP^{+}Flk1^{-}$ and $GFP^{+}Flk1^{+}$ cells, which represent a developmental progression ranging from pre-mesoderm to pre-haemangioblast mesoderm to the haemangioblast.

Dissecting the molecular cascades leading to haematopoiesis specification

Using both DNA microarray chip and proteomic approaches, we have initiated a research program allowing us to dissect the molecular events culminating in the formation of the first blood precursor, the haemangioblast. As depicted in figure 1, four populations have been analyzed along the path of ES cell differentiation: pluripotent ES cells, epiblast-like cells (GFP-Flk1-), mesodermal cells (GFP+Flk1-) and haemangioblast committed cells (GFP+Flk1+). Figure 2 illustrates how patterns of gene expression can be clustered during the differentiation process. The expression of genes, such as Rex1 or sox2, is strongly downregulated during the differentiation toward mesoderm and blood (figure 2a). The expression of a different set of genes, such as Fgf5 or follistatin, is transiently upregulated during the first phase of mesoderm formation (figure 2b). Finally, the expression of a unique set of genes, such as scl or Runx1, is strongly upregulated upon commitment to the blood lineage (figure 2c). Results from these experiments have also lead to the identification of several new genes which are distinctively upregulated in the specific subpopulations. These genes are now the focus of intense studies in the lab.

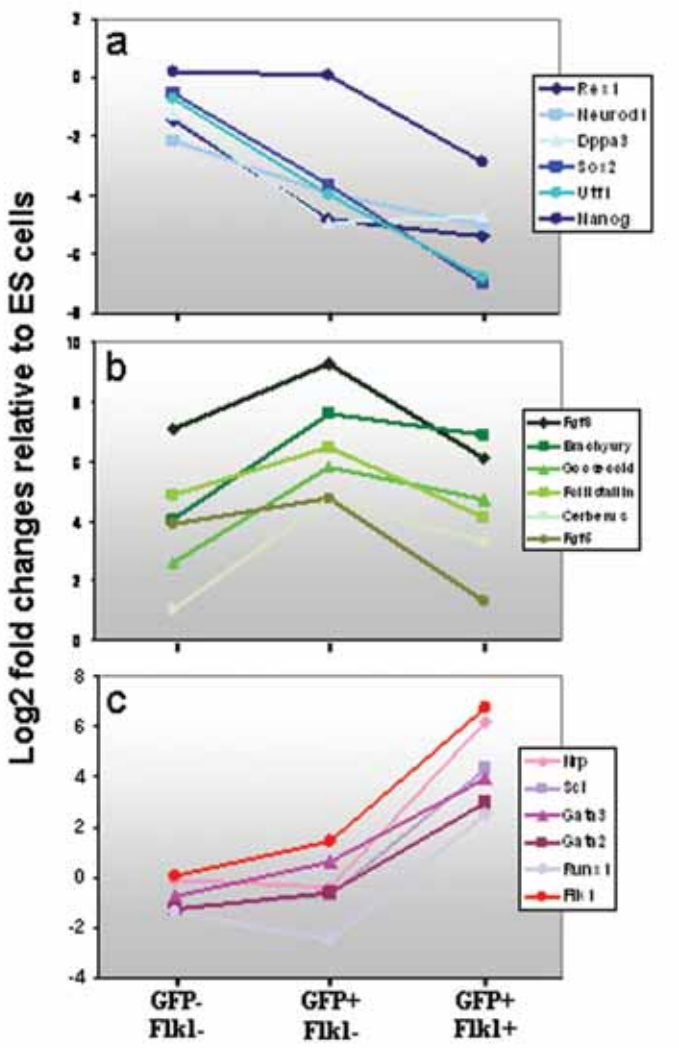

Figure 2: Affymetrix data analysis representing the relative expression of various sets of genes following identical pattern during differentiation. The data are presented for the 3 GFP/Flk1 subpopulations as log2 fold changes relative to ES cells.

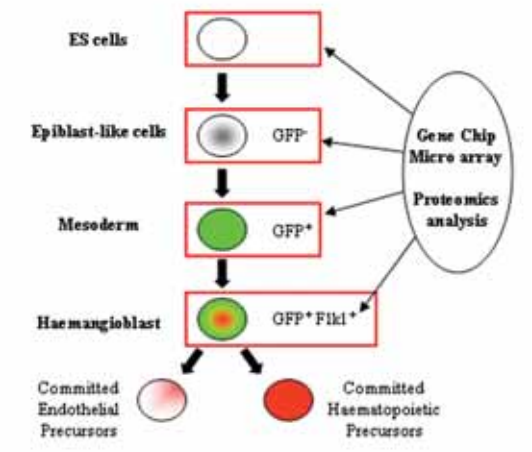

Figure 1: Schematic representation of the differentiation steps leading to blood lineage formation.

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Compartmentalisation of the eukaryote cell during evolution has brought about unique mechanisms to control multiple activities. The Nuclear Envelope (NE) restricts replication and transcription to the nucleus, and translation to the cytoplasm. Communication is mediated by nuclear pore complexes (NPCs). Despite the involved molecular architecture of the NPCs, the nuclear membranes and nuclear lamina, all these components are dismantled at mitosis. Reassembly begins at metaphase, with the binding of a Nucleoporin protein complex (106-170) at the chromosome surface. We have shown that two of the members of this complex, (Nup37 and Nup43), localize to kinetochores at metaphase, and also that NPC formation at mitosis differs significantly from interphase NPC assembly. A new project investigates the roles of condensin and cohesin in the formation and separation of chromosomes.

Nuclear pore formation differs in 'open' mitosis, 'closed' mitosis and during interphase.

The Nuclear Envelope (NE) is the defining feature of eukaryotic cells, with high evolutionary conservation. During division however, there are significant differences between higher and lower eukaryotes in the fate of the NE and NPCs. In lower eukaryotes mitotic spindles arise from the spindle pole body, a structure embedded in the NE that duplicates and migrates to organize a mitotic spindle within a persistent NE - termed closed mitosis. In higher eukaryotes the centrosome duplicates and migrates in the cytoplasm to opposite poles of the cell, at which point (prophase) the NE and NPCs are broken down to allow spindle microtubules to access the chromosomes at kinetochores (open mitosis). New NE and NPCs are reassembled at anaphase and telophase. During interphase, NPCs increase from 2000 to 5000 in vertebrates. Consequently there are three potential scenarios for NPC formation, namely open and closed mitosis, and throughout interphase. Closed mitosis and interphase NPC formation share an assembly mechanism in which NPCs are inserted into the NE membranes at sites of fusion between the inner and outer NE membranes as observed during *in vitro* nuclear formation. (Goldberg *et al.*, J Cell Sci 1997; 110: 409) and also *in vivo* in the syncytial phase of *Drosophila* embryonic development. (Kiseleva *et al.*, J Cell Sci 2001; 114: 3607). This mechanism can be simplified as 'membrane first, NPCs second'. In open mitosis in HeLa cells however, we have observed advanced NPC formation directly on the surface of the anaphase/telophase chromosomes, clearly in advance of membrane recruitment i.e., 'NPCs first, membrane second'. This demonstrates a novel and distinct route to NPC formation, found only in open mitosis, and a possible evolutionary change restricted to higher eukaryotes.

The nup107/160 complex redistributes to kinetochores upon nuclear lamina disassembly and prior to completion of nuclear envelope dispersal.

The nup107/160 complex is essential for nuclear pore complex (NPC) assembly after mitosis in mammalian cells. This complex contains the recently identified tryptophan-aspartic acid (WD) repeat proteins nup37 and nup43. To examine the dynamics of the nup107/160 complex during mitosis, HeLa cells were transfected for expression of nup37-EGFP and visualized in real-time by confocal microscopy. Figure 1 shows images from transfected HeLa cells along-side a schematic representation of a mammalian nucleus proceeding through division. During interphase nup37-EGFP labels mature NPCs at the nuclear periphery (Figure 1A). The nuclear lamina (brown layer) underlies the inner nuclear membrane (INM) and associates with chromatin binding proteins. The chromatin is decondensed and NPCs are present at sites devoid of peripheral heterochromatin. Upon initiation of chromatin condensation, but prior to disassembly of the NE, the nuclear lamina depolymerises and nup37 and the nup107/160 complex redistributes to kinetochores (Figure 1B-paired red arrows). The

NE disperses into the endoplasmic reticulum (ER) and the NPCs further disassemble liberating protein complexes (green and circled dots) into the cytoplasm. Another nucleoporin, nup358/RanBP2 (bound to RanGAP1) (pink dots) also relocates to kinetochores although the timing of its recruitment relative to the nup107/160 complex remains undefined. (Figure 1C). Metaphase is characterized by the alignment of chromosomes at the metaphase plate and the polymerisation of microtubules that bind to kinetochores to separate and transport "sister" chromatids to opposite poles of the cell. During metaphase nup37-EGFP is clearly visible at kinetochores facing the cell poles (inset micrograph – red arrows; diagram – red dots). Present work aims to determine the contribution of nup107/160 complex to microtubule-kinetochore association and mitotic progression.

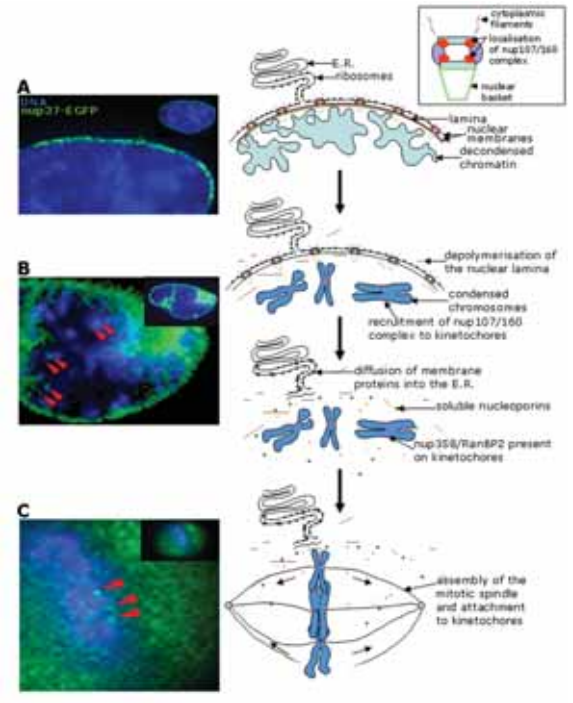

Figure 1: Stages in Nuclear Envelope breakdown

Direct visualization of the interactions of condesin and cohesin complexes with chromatin and chromosomes

Cell division requires accurate condensation and partitioning of chromatin to allow the flawless duplication of genetic material. Chromatin replication generates sister chromatids; chromosomal arms held together by a mechanism involving the cohesin protein complex. Cohesin subunits are proposed to form a huge ring structure which 'embraces' DNA duplexes from each chromatid. Cohesin is cleaved by the cysteine protease separase in response to the passage through the spindle checkpoint. The major cohesin proteins, hSMC1 and hSMC3 are highly related, structurally conserved proteins with an extended coiled coil domain and ATPase head region. We are investigating the predicted ring structure of cohesin using FEISEM immunogold labelling of specific cohesin subunits *in situ*. Studies to date have identified cohesin subunits in conserved structures associated with mitotic chromatin.

The condensin complex is directly related to cohesin but is involved in the condensation of replicated chromatin by an uncharacterised mechanism. Condensin exists as condensin I and condensin II complexes, which have hSMC2 and hSMC4 core subunits and a variation of the three non-SMC regulatory subunits, thus forming distinct complexes which access chromatin at specific stages of condensation. Although chromatin still condenses in condensin mutants, the structural integrity of chromosomes is however impaired, leading to defects in chromosome segregation. Immuno-FEISEM of mitotic chromatin during condensation and de-condensation should provide insight into the mechanism of this complex (Figure 2). Our current data support a model for condensin acting as a molecular hinge, contacting two chromatin fibres in its open conformation and bringing them into close proximity upon closure.

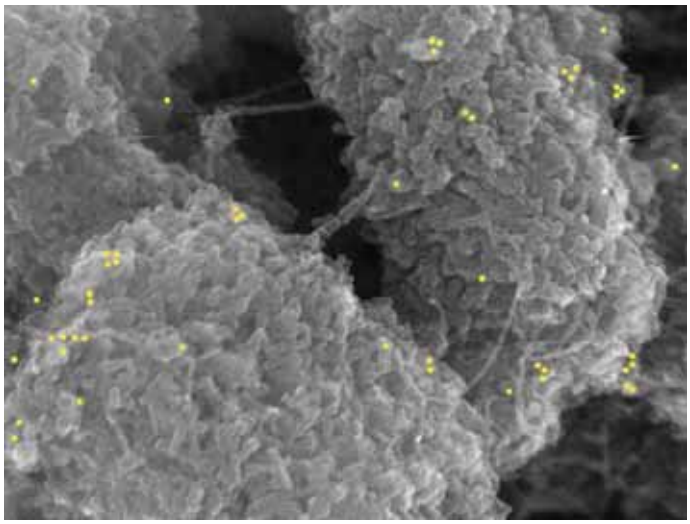

Figure 2: Immuno-FEISEM image of chromosome surfaces in metaphase HeLa cells. Antibodies to the Kleisin –β subunit of condensin are imaged with secondary 10nm immunogold, shown in yellow. Field width = 1.5 microns.

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Academic Radiation Oncology: Translational Radiogenomics Group

The Academic Department of Radiation Oncology (ADRO) was established at the Christie Hospital by Pat Price in 2000 to innovate advances in technical radiotherapy, radiobiology and imaging for translation into clinical practice. The translational radiogenomics group, based in part at the Paterson Institute, explores and develops methods for the individualisation of radiation therapy. With the advent of high-throughput techniques, the group is interested in the characterisation of molecular profiles that reflect relevant biological phenotypes and predict tumour and normal tissue response to radiation.

Development of a hypoxia transcriptome in head and neck cancer

The knowledge that hypoxic cells are resistant to the biological effects of sparsley ionising radiation has dominated radiotherapy-associated radiobiology research for fifty years. Only in recent years, however, has hypoxia been widely recognised as a key factor driving malignant progression, metastasis formation and cancer treatment resistance. The ability to measure the hypoxic status of a tumour in a routine clinical setting would be a major advance. Towards this goal, a prospective study was established in patients with head and neck cancer to investigate the potential of RNA microarrays (Priy Silva). Samples are collected for profiling using Affymetrix U133A GeneChips. The project involves collaboration with Professor Adrian Harris at the Weatherall Institute of Molecular Medicine in Oxford, Dr Francesca Buffa at the Gray Cancer Institute and Dr Crispin Miller (Bioinformatics Group), and is carried out in the CR-UK Affymetrix Microarray Facility in the Paterson. Clinicians involved in the local development of the project are Mr Jarrod Homer, Professor Philip Sloan and Dr Nick Slevin. A recent analysis of microarray data from the first 59 patients (26 recruited in Manchester) used a knowledge-based approach to define an *in vivo* hypoxia transcriptome (Fig 1). The work identified several novel genes not previously recognised as hypoxia regulated. The hypoxia transcriptome was an independent prognostic factor for recurrence-free survival in an unrelated dataset, outperforming the original intrinsic classification.

Is hypoxia important in gastric cancer?

Evidence for the presence and prognostic significant of hypoxia is available for many solid tumours but is lacking in gastric cancer. The latter, along with the need to develop approaches for selecting patients likely to benefit from receiving radiotherapy as part of their treatment, led to the initiation of a project looking at the role of hypoxia in the disease. This work involves collaboration with Mr Ian Welch and Dr Sue Pritchard at Wythenshaw Hospital. Work over the past year (Ewen Griffiths) showed that hypoxia inducible factor-1α (HIF-1α) appears to have a role in gastric carcinogenesis (Fig 2). HIF-1α expression was not observed in normal gastric mucosa. Low and weak HIF-1α expression was observed in mucosa infected with *H. pylori* and increased in percentage and intensity with the sequence of *H. pylori* associated gastritis, intestinal metaplasia, dysplasia and intestinal type adenocarcinoma. A retrospective study showed HIF-1α expression had no prognostic value in gastric oesophageal junction tumours, but was associated with improved survival in distal gastric cancers. Its good prognostic effect may be related to induction of apoptosis and reduced cellular proliferation which override the transcription of pro-angiogenic and metastatic molecules.

Measurements of hypoxia in cervix cancer

As part of studies investigating approaches for assessing tumour hypoxia, we have given pimonidazole to 64 patients with carcinoma of the cervix (collaboration with Drs Susan Davidson and Robin Hunter). Expression of adducts of the hypoxia specific marker in tumours is measured using

immunohistochemistry. This work is part of an international collaboration involving Professor Jens Overgaard's group in Aarhus, Dr Jim Raleigh in North Carolina and a group in Vancouver. Staining and scoring of the sections was recently completed and over the past year we have finished updating the clinical database. The relationship between pimonidazole staining and radiotherapy outcome is currently being explored.

Changes in plasma TGFß1 are prognostic for radiation morbidity in patients with head and neck cancer

Another biological feature important in determining how a patient responds to radiotherapy is their intrinsic sensitivity to radiation. Some patients are inherently radiosensitive and likely to develop severe long-term complications, which may not become apparent for several months or even years following treatment. The small numbers of patients who develop severe late radiation toxicity limit the total curative doses that can be safely administered to all patients. The ability to predict patients predisposed to developing toxicity early has potential for allowing safe dose-escalation in others to increase their chance of a cure. The Translational Radiogenomics group is interested in not only hypoxia but also methods for measuring normal tissue radiosensitivity. Transforming growth factor-β1 (TGFβ1) is implicated in the development of radiation-induced fibrosis and a study in patients with lung cancer showed changes in TGFβ1 predicted the likelihood of acute toxicity following radiotherapy. A prospective study was initiated, therefore, in 1999 to measure changes in plasma TGFβ1 during radiotherapy in patients with head and neck cancer. Analysis of the results in the past year (Christine Clinton) showed that changes in TGFβ1 – end to pre-treatment ratio of less than one, with an elevated end of treatment TGFβ1 level - correlated with both acute and late radiation toxicity.

Radiogenomics: assessment of polymorphisms for predicting the effects of radiotherapy (RAPPER)

Current thinking is that an individual's intrinsic radiosensitivity is determined by polymorphic sequence variation in a large number of genes. The availability of a reference sequence of the human genome is raising the possibility of genotyping studies looking at variations in a large number of genes, i.e. those involved in DNA damage recognition and repair, proliferation, apoptosis and cytokine response to cellular damage. In order to have sufficient statistical power to find meaningful correlations, these studies require large numbers of patients. Following a successful grant application to the Cancer Research UK Translational Research in Clinical Trials Committee, we obtained funding for RAPPER. The study is being co-ordinated by our group (Rebecca Elliot) and involves collaboration with Drs Neil Burnet and Alison Dunning in Cambridge, along with numerous Clinical Oncologists locally and around the UK. The study has a planned accrual of up to 3000 patients with breast, prostate or gynaecological cancer. Patients will have received radiotherapy with curative intent and have detailed late effect data. Blood samples will be taken and analysed for single nucleotide polymorphisms in candidate genes in Cambridge at the end of the project. With the appointment of Teresa Bailey in October we are now looking forward to banking our first blood samples.

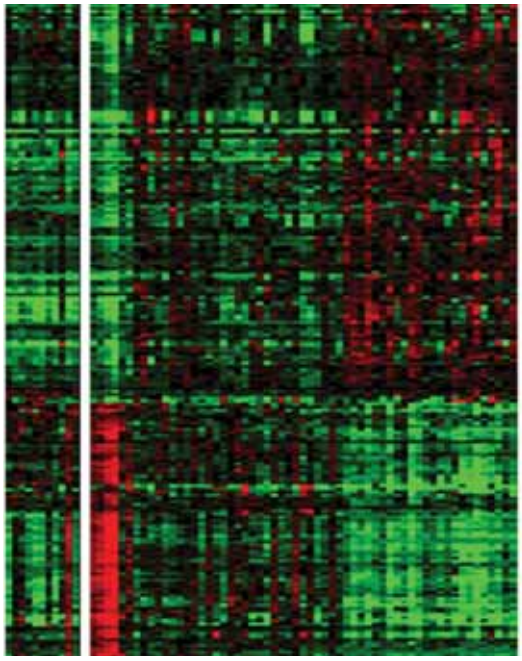

Figure 1: Dendogram of 59 head and neck cancers clustered using a knowledge-based derived hypoxia

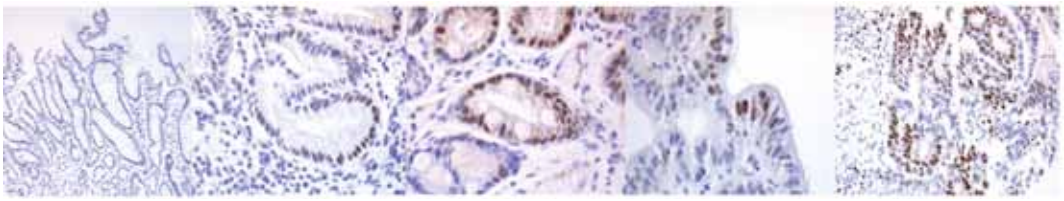

Figure 2: HIF-1α expression was not observed in normal gastric mucosa. Low and weak HIF-1α expression was observed in mucosa infected with *H. pylori* and increased in percentage and intensity with the sequence of *H. pylori* associated gastritis, intestinal metaplasia, dysplasia and intestinal type adenocarcinoma (left to right).

Publications listed on page 62

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GRADUATE STUDENTS
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Medical Oncology: Breast Biology Group

Normal tissues are maintained by the self-renewal capacity of a rare population of stem cells, which divide asymmetrically both to replace themselves and to generate progenitors. After limited cell division, progenitor cells produce the non-dividing differentiated cells specific for each tissue. An emerging concept is that in leukaemia as well as in neural and epithelial cancers, including breast cancer, only a minority of cells, i.e. the "cancer stem cells", have the capacity to initiate tumours; the others are committed to differentiation pathways and senescence. Thus, characterising the cancer stem cell and understanding the molecular basis for dysregulated self-renewal will be crucial for identification of targets for effective therapeutic intervention.

Stem cells and differentiated lineages in the human breast

Development of the mammary gland involves the formation of collecting ducts and lobules, both of which are bilayered epithelia made up of contractile myo-epithelial and milk-producing luminal cells. We have recently developed new methods for the isolation and characterisation of putative breast epithelial stem cells that give rise to these 2 cell types. Our results suggest a steroid receptor-negative long-term stem cell that gives rise to a short term stem cell that expresses steroid receptors; from this precursor two lineages of differentiated myo- and luminal epithelial cells are formed (see figure). The stem cells express markers such as $p21^{WAF1/CIP1}$, musashi-1 (Msi1) and keratin 19 and lose these as they differentiate.

Stem cell self-renewal pathways

In future studies, identification of stem cell self-renewal pathways will be important for cancer prevention, and therapy of the cancer stem cells that have the capacity to initiate tumours. Although some cell-specific markers and signal transduction pathways are similar in normal and cancer stem cells, the tight regulation of self-renewal that is operative in the normal stem cell may well be disrupted in cancer. Thus, understanding the regulation of self-renewal will be crucial for improving therapeutic intervention by targeting the cancer stem cell that is predicted to initiate tumour recurrence.

Our current aim is to exploit culture and *in vivo* techniques to address the regulation of normal and cancer stem cells in the breast. We have already established methods to isolate human mammary epithelial stem cells from normal tissue, using Hoechst dye-efflux to obtain the mammary cell 'side population'; this method can be coupled with the use of mammary stem cell surface markers. To identify the factors that regulate breast stem cell phenotype, we are currently collecting material from consenting patients undergoing surgery for either breast reduction, removal of a benign or primary tumour, DCIS or pleural effusions (from advanced breast cancer patients). Where appropriate, our approach is being validated by investigating these factors in human breast cancer cell lines such as MCF-7 for which a 'side population' has also been reported, and by immortalising primary stem cells to create cell lines useful for modelling mammary stem cell biology (using a tri-cistronic retroviral vector containing hTERT and HPV16 E6/E7 cDNA).

One current interest is which of the Notch, Hedgehog, Wnt, TGFbeta, ErbB and other relevant (eg. Prl, GH and ovarian hormones) signalling pathways regulate stem cell self-renewal. In order to investigate these pathways, we have developed

methods for non-adherent mammosphere suspension culture, analogous to neurosphere culture that enriches for brain stem cells. Our recent unpublished results indicate that in this culture system self-renewing stem cells can be enriched and passaged.

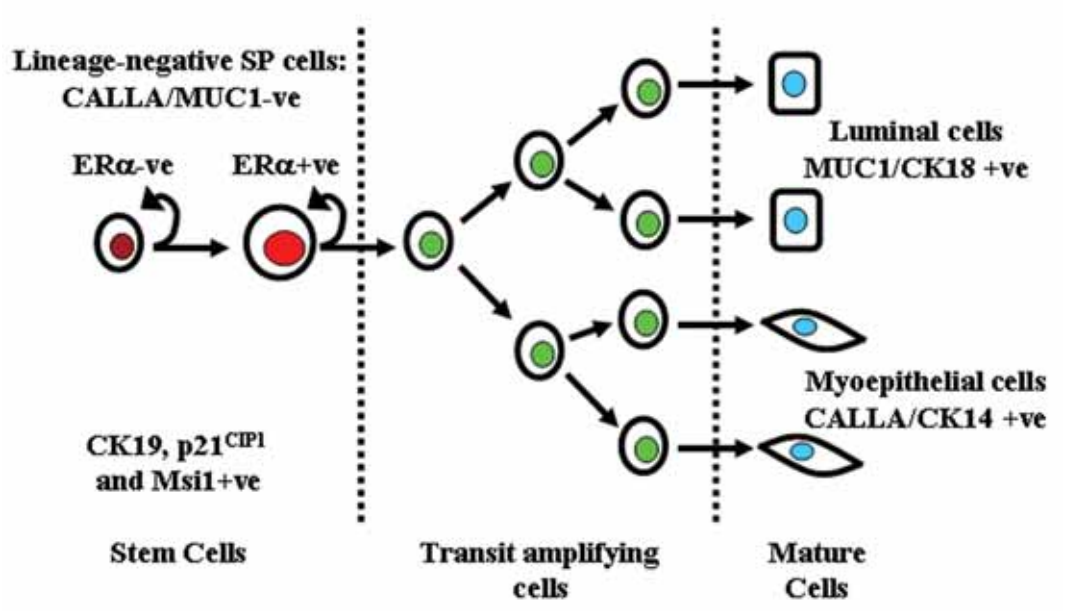
Gene expression and functional genomics

Gene expression arrays and functional genomics methods are being employed to identify novel pathways that participate in stem cell regulation and to identify early changes that occur in normal and premalignant tissues and predict the emergence of a tumour.

For functional genomics studies, we are collaborating with a group at the NKI, Amsterdam to use a retroviral short hairpin (sh) RNA library that targets approximately 8,000 genes (Berns *et al.,* Nature 2004; 428: 431). We use the library to screen for genes that function in stem cell self-renewal by using several rounds of mammosphere culture to enrich for integrated shRNA sequences that increase production of non-adherent sphere colonies. This involves large-scale non-adherent culture as between a quarter and a half million mammospheres need to be infected with shRNA to achieve good representation of the library. DNA must be collected at each passage and 'bar-coded' by amplification, labelling and hybridisation to custom arrays of the shRNA sequences. The enriched sequences light up on the array and the most important ones will be increased by each passage of mammospheres.

Another area of focus is gene expression and risk of breast cancer where we compare tissues from women at high or normal risk of breast cancer, for example, parous versus nulliparous women or those with a family history of breast cancer versus population controls. If these changes can be detected and prevented, then it may be possible to reduce the increasing incidence of breast cancer. We have therefore initiated a collaboration with Professors Howell and Evans at the South Manchester Family History Clinic to examine the effects of preventative strategies to see whether gene expression returns to 'normal risk' following a clinical intervention.

It is hoped that the results of these investigations should lead to an increased understanding of the biology of the normal human breast which, in turn, could lead to the development of new strategies or new targets for breast cancer prevention and therapy.



A model of the cellular hierarchies in the epithelium of the human breast lobule. The putative stem cells are negative for lineage markers and can be isolated as the Hoechst dye-effluxing side population (SP). These cells are a mix of steroid receptor-positive (ERα+ve) and negative (ERα-ve) cells and express $p21^{CIP1}$, Msi1 and CK19, markers of putative stem cells. After a small number of cell divisions, transit amplifying cells exit from the cell cycle, switch on expression of the CDKI $p27^{KIP1}$ and differentiate into myoepithelial or luminal cells characterized by the lineage-specific markers CALLA and CK14 (myo) or MUC1 and CK18 (luminal).

Publications listed on page 62

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Medical Oncology: Gene-Immunotherapy Group

This is an integrated clinical-laboratory group undertaking early phase clinical trials and translational research. We focus on the development of novel biological therapies, particularly immunotherapy. Clinically we are undertaking several trials of genetic vaccines, trials of antibody and cell based therapy. The laboratory research uses gene delivery to develop novel therapies utilising understanding of the basic immunobiology of cancer. The major focus is on developing retroviral delivery of chimeric receptors to T cells to allow optimal recognition and destruction of cancer cells – clinical trials of this approach are about to start. Together with the Immunology group and external collaborators we are also developing improved cancer vaccines. We are also part of the UK Centre for Tissue Engineering investigating gene therapy approaches to cartilage repair, wound healing and vascular disease.

Clinical Trials

We are developing a number of novel biological therapies and several significant major trials of cancer vaccines have been completed. A focus of clinical trials remain 5T4 targeting (see also Immunology Group). Phase II trials of Trovax (Modified Vaccinia virus Ankara expressing 5T4) vaccination in combination with chemotherapy indicates that immune responses are still produced and thus combined therapy is possible. The potential for larger scale trials is being investigated. We have also recently complete recruitment to a Cancer Research UK trial in which we are (jointly with the Immunology Group and the Hepatobiliary Surgery Unit in the North Manchester General Hospital) investigating the intra-tumoural, as well as, the systemic immune responses in patients undergoing vaccination prior to resection of liver metastases. Other important completed studies include a phase I prime-boost study in melanoma which produced interesting immune and clinical responses. A Phase I trial of 5T4 antibody targeted superantigen is also underway along with a mechanistic PET imaging study using the same agent. In addition we are lead centre in a number of major multi-centre trials of renal call cancer.

Cellular therapy is a major focus of future trials and trials are expected to start in the near future. The first trial will examine the effect of depleting regulatory T cells from renal cancer patients and is based on studies by Richard Griffiths showing increased numbers of regulatory T cells in patients with advanced renal cancer. There are two trials evaluating Engineered T cells which now have approval of the Gene Therapy Advisory Committee (GTAC) and will commence once manufacture of the virus is completed and once all the processes for cellular handling to GMP are fully implemented. A two bedded unit to undertake such work is now open and fully staffed – establishment of the operational policies has been facilitated by a visit of Fiona Thistlethwaite and Susan Neeson to the NCI in Washington. Pioneering work on cell therapy is being developed there using tumour infiltrating lymphocytes and this provides an excellent model for our approach to engineered cell therapy. Fundraising and preparations for a larger unit are underway. In addition plans are well advanced for a larger GMP unit being built adjacent to the Paterson Institute.

Engineered T cells

We have previously demonstrated effective vaccination of against B-cell idiotypes in lymphoma models and identified epitopes recognised by T cells. Utilising these true tumour specific T cells we have now shown that we can cure lymphoma in animal

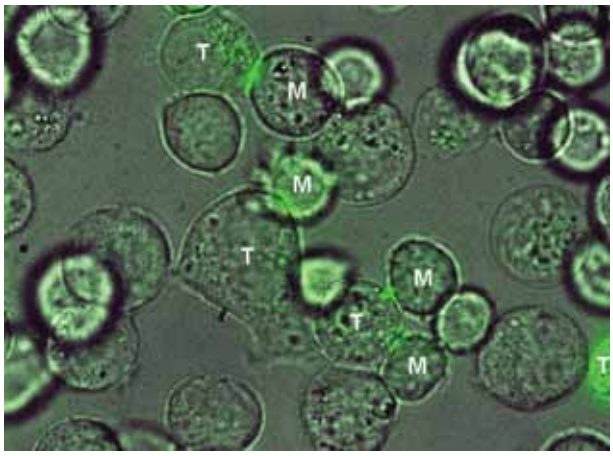
models. However, this is not a feasible approach to the treatment of patients and we are developing engineered T cells to target tumour associated antigens. Tumour associated surface antigens have been shown to be a good target for antibody recognition and chimeric T cell receptors which link a single chain antibody to a signal transducing domain can potentially bypass defects in antigen presentation. This chimeric T cell receptor approach, combined with the ability to efficiently transduce primary T cells with retroviral vectors provides a potentially powerful new method of cancer treatment. Utilising a single chain antibody to carcinoembryonic antigen (made from a phage library) we are testing this system to target gastrointestinal and many other common cancers. The chimeric receptor construct has been optimised and transduces human T cells efficiently. These engineered T cells efficiently (and selectively) kill a variety of CEA positive tumour cell lines *in vitro*. In therapeutic models transduced murine T cells can be used to treat tumours and in local therapy models human cells are also effective. In preparation for trials important questions have been addressed such as the potential for soluble antigen to inhibit T cell mediated killing or T cell migration in response to chemokines. Encouragingly, there is no detrimental effect of soluble CEA in either case. This work has also led us to examine the chemokines produced by natural tumours. Working with David Sherlock in the North Manchester Hepatobiliary Unit we are exploring the expression of chemokines from resected liver metastases. We have also undertaken similar work on targeting CD19 (a target on B cell malignancies). We plan trials to target both CEA and CD19 positive malignancies as soon as GMP virus and validated assays are available and these are both due to start in 2006. We have also completed an initial evaluation of 5T4 as a target on renal cancer for this type of approach. We are also investigating the combination of engineered T cells with Trovax. The comparison of the optimal construct for various different molecular targets has provided some insights into the appropriate design of chimeric receptors.

The year also saw the success of an application to the European Union for a five year grant to study scientific aspects of engineered T cells. The ATTACK project (www.ATTACK-cancer.org) brings together 14 of the leading academic centres in Europe and Israel along with two companies to address the key issues surrounding this novel form of therapy. The project is co-ordinated from Manchester by Robert Hawkins and David Gilham leads one of the seven work packages which focuses on methods of T cell transduction, selection and expansion.

Other aspects of our work involve the investigation of relevant patient immunobiology and target identification/validation. Patients with advanced cancer are often relatively immunosuppressed and we are investigating aspects of this -understanding the biological basis of this and investigating techniques to reverse it may be critical to successful immunotherapy. A current focus is the examination of regulatory T cells in patients with renal and gastrointestinal malignancies. We are also investigating targets in renal cell cancer and have demonstrated strong expression of 5T4 which is thus a potentially attractive target.

Tissue Engineering

The major focus of this programme remains the genetic modification of chondrocytes and dermal fibroblasts. Cultured chondrocytes lose their chondrocytic phenotype and fail to produce extracellular matrix and collagen type II. We have shown that this de-differentiation can be reversed by transduction with the Sox9 gene using retroviruses. The expanded, transduced chondrocytes are potentially suitable for clinical use – appropriate pre-clinical models are being developed to test this. Other targets include wound repair. Based on experiments that show reduced scarring when recombinant TGFβ3 is applied to the wound we are investigating gene therapy based approaches. Cultured dermal fibroblasts transduced with TGFβ3 can produce appropriate levels of growth factor - evaluation of ways to control expression and limit survival of these cells are being explored to ensure safety of the approach.



Jurkat T-cells (T) expressing GFP-tagged chimeric receptors forming a "raft" on binding MKN45k tumour targets (M). T cell in bottom right has yet to bind tumour target and shows even distribution of GFP (John Bridgeman, Steve Bagley).

Publications listed on page 63

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Medical Oncology: Glycoangiogenesis Group

Angiogenesis has been proven as a clinically valid target for anti-cancer treatment. While phase III trials have focused on VEGF as a target, it is clear that other angiogenic cytokines should be inhibited and we have focused on FGF2 because of its obligate dependency on heparan sulphate (HS). We have shown that heparin octasaccharides inhibit angiogenesis in a number of *in vivo* models of angiogenesis and have developed an organic chemistry programme in which we are making octasaccharides. These will be evaluated *in vitro* and *in vivo* over the next year.

Angiogenesis, the formation of new blood vessels, has been validated as a target for anti-cancer treatment in several phase III trials in which patients with non-squamous non-small cell lung cancer, recurrent breast cancer or colorectal cancer were treated with a combination of cytotoxic chemotherapy and anti-VEGF antibodies. All trials showed that the combination was associated with an improved response rate. However, while the median survival of the patients was superior to that of patients who received conventional chemotherapy regimens, the overall survival was largely unchanged suggesting that the impact of VEGF inhibition should be augmented by inhibiting other targets.

Development of anti-angiogenic oligosaccharides

We have focused on the HS-dependent growth factor family, the prototype of which is FGF2. The importance of this molecule has been highlighted recently in a study that showed that FGF2 is responsible for the development of resistance to VEGF inhibitors *in vivo* (Casanovas *et al.*, Cancer Cell 2005; 8: 299). If borne out clinically the implication would be that the benefit of VEGF inhibitors could be increased if co-administered with antagonists of FGF function.

We have previously shown that HS-proteoglycans are of prognostic significance in ovarian cancer (Davies *et al.*, Clin Cancer Res 2004; 10: 5178) in that stromal syndecan 1 was associated with a worse prognosis while syndecan 3 was aberrantly expressed in the tumour endothelium. This year we showed that the entire extra-cellular signalling mechanism for FGF2 is present in the ovarian cancer endothelium. In particular, using a novel molecular probe we showed that endothelial HS is able to activate FGF2 (Whitworth *et al.*, J Cancer Res Clin Oncol 2005; epub Aug 05). In view of this we were keen to develop inhibitors of FGF2 based on the molecule's dependency on HS.

Using size fractionated heparin oligosaccharides we investigated the anti-angiogenic potential of these species in models that were sequentially less dependent on FGF2. These data showed that heparin octasaccharides inhibited FGF2-induced angiogenesis but were also effective in models that were less dependent on FGF2 (Hasan *et al.*, 2005; Clin Cancer Res; 11: 8172).

One of the impediments to the clinical development of saccharides as anti-angiogenic agents is the availability and purity of potential drugs. We have therefore developed an organic chemistry programme in which we are making a defined octasaccharide that we have shown completely abrogates FGF2-induced HUVEC erk-1 phosphorylation *in vitro* (unpublished). The synthetic chemistry programme has led to the generation of tetrasaccharides and will develop octasaccharides and more complex species subsequently; the aim being to explore structure-function and structure-inhibition relationships using defined oligosaccharides.

The heparan sulphate-FGF axis in ovarian cancer

The above *ex vivo* tissue-based studies suggested

MEDICAL ONCOLOGY: GLYCOANGIOGENESIS GROUP

that the ovarian tumour endothelium was the most active site of HS synthesis and we therefore carried out a detailed RNA in situ hybridisation (ISH) study to investigate the distribution of HS-synthetic enzymes in ovarian cancer. These data have shown that in fact the tumour cells express the synthetic enzymes at the RNA level to the greatest extent but that they also express heparanase and 2-O-sulphatase; enzymes that cleave and edit cell surface HS. The implication is that HS synthesis is most tightly controlled in non-malignant lineages but that in cancer cells HS is made but then cleaved from the cell surface.

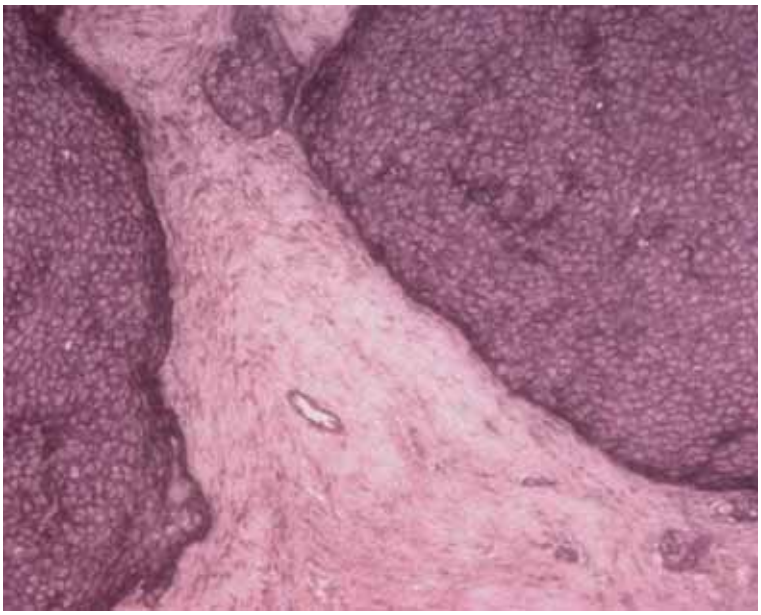
In a further arm of our programme to develop saccharide based inhibitors of FGF we have initiated a detailed study of the expression of FGFs and FGF receptors in ovarian cancer and normal ovaries. The aim is to identify the FGFs and FGF receptors that are most abundantly expressed by cancer tissue and to investigate and target them. To date these studies have narrowed the number of FGFs that are differentially expressed in ovarian cancer and we will focus on these FGFs in forthcoming studies.

Phase I PK/PD anti-angiogenic trial programme

We have completed first-into-man phase I evaluations of an anti-α_V integrin antibody and a di-Fab anti-KDR molecule. The anti-α_V integrin antibody was well tolerated and we observed a prolonged partial response in a patient with cutaneous angiosarcoma. Biopsies showed that the antibody was bound and associated with the α_V integrin. Interestingly we also observed down-regulation of phosphorylated MAP kinase and BCL-2 after treatment, potentially identifying intracellular mechanisms of action.

In this study we also carried out dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) of the patients receiving anti-α_V integrin antibody. While these studies did not demonstrate a drug-related effect we were able to identify patients who were destined to develop progressive disease by examining the vascular characteristics of the tumours on DCE-MRI. These data raise the possibility that we can identify patients who will most benefit from anti-angiogenic agents through a radiological investigation.

As a result of these programmes we are now coordinating the translational research programme for two forthcoming MRC/NCRN ovarian cancer trials that investigate the benefit of anti-angiogenic agent- cytotoxic chemotherapy combinations in the disease. This will allow us to test and therefore also to validate our angiogenesis biomarkers at the phase III level.



Ovarian adenocarcinoma stained using RNA *in situ* hybridisation for 6-O-sulfotransferase 1 showing staining in the endothelium and tumour cells.

Publications listed on page 64

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Rebecca Baldwin (joint with Stem Cell Biology)
Eun-Ang Raiber (with Salford University)

Medical Oncology: Proteoglycan Group

It was over 30 years ago that heparan sulphate (HS) was first identified on cell surfaces, but it is only in the past decade or so that evidence has emerged to reveal its key roles in regulating cell growth and differentiation. Mechanistically, the mode of action of HSPGs is still unclear, although specific sequence motifs in the HS polysaccharide chain are known to be essential for binding growth factors and morphogens and for efficient engagement of their specific tyrosine kinase receptors. We are investigating the molecular design of HS in different cell types, including embryonal stem (ES) cells, and examining the proposition that its unique domain structure and conformational flexibility enable it to function as a template for the assembly of ligand-receptor signalling complexes on the plasma membrane.

Recognition of hepatocyte growth factor/scatter factor (HGF/SF) by glycosaminoglycans (GAGs)

Our previous understanding of GAG recognition by HGF/SF, based upon its selective binding to the iduronate-containing HS/heparin and dermatan sulphates (DS), but not the glucuronate-containing chondroitin sulphate (CS), was that the iduronate residue is a critical element of the binding sequence. Defining the additional importance of individual sulphate groups has been a more complex task. Binding experiments using an array of native and selectively desulphated GAGs now suggest that, in the presence of iduronate, one sulphate/disaccharide in a sequence of three disaccharides is sufficient for binding (as in mammalian DS). However, the presence of glucuronate is permissible, but only if a higher density of sulphation is present than is the case in most CS species. A combination of both iduronate and high sulphate density (as in HS, heparin) further strengthens the interaction. This reflects the capacity of the binding site to adapt to different structural motifs in GAG chains. In parallel, we are collaborating with Drs Jim Wilkinson & Sylvie Ducki (Centre for Molecular Drug Design, Salford University) in the design of synthetic, sulphated GAG mimetics that may inhibit GAG-mediated activation of HGF/SF. The antagonistic properties of some initial compounds are showing promise for further development.

A new method for analysing the conformation of GAG oligosaccharides

The limited information available on the conformation of GAG oligosaccharides is based on fully sulphated heparin oligosaccharides in a few co-crystals with protein. In collaboration with Drs Perdita Barran and Dušan Uhrín (Edinburgh University) we have used ion-mobility mass spectrometry, in combination with molecular modelling, as a novel approach to probe GAG oligosaccharide conformations (Jin *et al.*, Phys Chem Chem Phys 2005; 7: 3464). This technique measures the retardation, due to collisions, of oligosaccharide ions propelled by a weak electrostatic field through low pressure gas. Experimentally-determined, rotationally-averaged, collision cross-sections of the ions can then be compared to theoretical cross-sections of candidate oligosaccharide conformations obtained by molecular modelling. Analyses of a number of purified HS/heparin oligosaccharides gave good agreement between experimental and theoretical cross-sections. Interestingly, relatively small changes in the primary sequence of oligosaccharides lead to measurable changes in gas-phase conformation.

HS and the activation of fibroblast growth factors

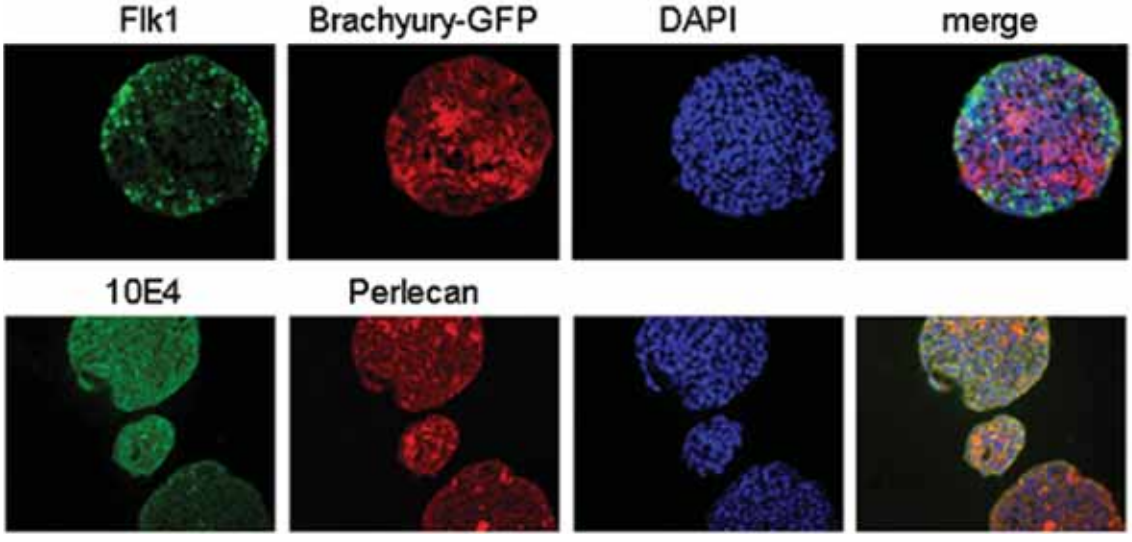
We have previously described a novel size-exclusion chromatography technique for studying the role of heparin in facilitating the interaction between fibroblast growth factor 1 (FGF1) and FGF receptors (FGFR2c). A model has been developed showing how co-operative trans-dimerisation of FGF1, mediated through a conformational change in heparin, drives the formation of FGF1-FGFR2c-

heparin ternary complexes with a 2:2:1 stoichiometry. We have recently extended our studies to FGF2, a potent stimulator of tumour angiogenesis. This revealed some similarities in the way that heparin promotes the activity FGF ligands. In common with FGF1, heparin dp6 was the shortest FGF2-binding saccharide, and dp8 the minimum needed for FGF2 dimerisation. Moreover, like FGF1, dimerisation appeared to be highly cooperative, because in the presence of excess saccharide a strong preference for the formation of 2:1 FGF2-heparin complexes was observed. Ternary complex formation in the presence of FGF-receptor revealed a 2:2:1 FGF2-FGFR2c-dp12 assembly exactly as observed with FGF1. However FGF2 also gave rise to smaller "half" complexes (1:1:1) that were not observed with FGF1 Previous studies showed that in contrast FGF1 heparin dp6-dp12 species failed to stimulate FGF2 mitogenesis through the FGFR2c receptor. Our binding data suggests that these saccharide species may form some unproductive FGF2-FGFR combinations that may be more abundant on cell surfaces and compromise transmembrane FGFR signalling. Overall, these findings suggest that although FGF ligands engage heparin by a co-operative process different mechanisms of heparin-mediated formation of signalling complexes may operate in the FGF family.

Embryonic stem cells as a model system for studying proteoglycan biology

We have established the novel approach of using murine ES cells as potential *in vitro* model systems for studying HS during development. ES cells undergo self-renewal in culture whilst retaining the ability to differentiate into all cell lineages. Cell fate decisions are controlled by a complex and subtle interplay of secreted factors, cell-autonomous factors and cell-cell and cell-matrix interactions. Many of the signalling molecules are HS-dependent growth factors and morphogens. ES cells therefore present a tractable experimental system for assessing the role of HS in signalling processes in differentiation. This enables us to study the relationships between developmentally-regulated expression of HS-polymerase and sulphotransferase enzymes, and the structural and functional attributes of the resulting HS. This has become a hotly debated issue as evidence has emerged of the deleterious effects of enzyme mutations on both embryogenesis and HS function in the adult.

Structural analysis of radiolabelled HS from mES cells grown in the presence of serum and LIF, to maintain pluripotency, demonstrated a very low level of sulphation (reflecting weak sulphotransferase activities), compared to HS from other mouse tissues. Using GFP reporter lines (i.e. Sox-1, a specific marker of neuroectodermal precursors, and brachyury, an early marker of mesoderm, in combination with Flk1 to identify haemangioblast specification) we were able to isolate discrete populations. Uniquely, in this study, we could directly compare HS structure with expression of a wide panel of relevant genes for FACS sorted populations. Biochemical analyses, and the use of ScFv antibodies (from Prof. Toin van Kuppevelt, Nijmegen) that recognise distinct HS epitopes, uncovered significant differences in HS structure between mES cells and their differentiated progeny. Neural differentiation is dependent on an initial response to FGF4 and, at a later stage, to other FGF family members. In contrast, initial mesodermal differentiation is critically dependent on FGF5, Wnt 3a/8a and BMP2/4, with VEGF and FGF-2 becoming more significant later. We therefore suggest that the observed changes in HS sulphation pattern and distribution may regulate responses to these HS-dependent exogenous factors and thus play key roles in determining cell fate.



Mesodermal differentiation from embryoid bodies of brachyury-GFP ES cells at day 3.25. Top panels: haemangioblasts marked by brachyury-GFP and Flk1 staining. Bottom panel: staining for HS chains (10E4 Ab) and the HSPG perlecan. Nuclei visualised using DAPI. Magnification: x400

Publications listed on page 66

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Cancer Studies: Targeted Therapy Group

The aim of the laboratory programme is to increase our understanding of the interactions between monoclonal antibodies (mAb) and irradiation in the treatment of cancer. There are two research domains which interrelate to form a cohesive programme of work under the title of radioimmunotherapy (RIT) of cancer, which include optimisation of RIT and irradiation and immunoregulation. In the first project, the roles of mAb as both vectors to deliver irradiation to tumours and in direct cancer cell killing are further explored. The specific aims are to further understand the mechanisms of action RIT and define the relative contributions of targeted radiation and mAb effector mechanisms to clearance of tumour. In the second project, mAb are used to augment T-cell responses to tumour by blocking or stimulating co-receptors in the immune system. This project aims to further define the factors which are important in combining irradiation and immunoregulatory mAb.

Mechanisms of action of radioimmunotherapy

The group began the process of relocation from Southampton to the Paterson Institute nearly a year ago. Over the last few months considerable progress has been made in rebuilding the group with the appointment of a senior research fellow (Dr Jamie Honeychurch) who is due to relocate in 2006 and two new post-doctoral fellows who are shortly to be appointed to commence work in 2006. Over the last four 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. We have demonstrated for the first time in a variety of different syngeneic models of lymphoma that successful RIT leading to long-term tumour protection consists of both targeted irradiation and mAb effector mechanisms. This work has provided us with new insights into how RIT may be working in the clinic. Our recent work has focused on the importance of the micro-distribution of radiolabelled mAb to the delivery of targeted radiotherapy to tumour.

A recent interesting finding to emerge is that the biodistribution of a single radiolabelled mAb is unable to predict tumour response when a significant biological contribution is derived from a signalling mAb. Furthermore, we have shown that successful eradication of lymphoma in RIT consists of targeted irradiation and mAb signalling and these two components can be provided by two different mAbs. As such, a therapeutically active signalling mAb that is itself poor at delivering radiation can be combined with a therapeutically inactive mAb that is effective at targeting radiation to tumour to provide long term clearance of tumour *in vivo*. Our results confirm that RIT is much more than targeted radiation and provide a scientific rationale to support the use of selecting combinations of mAb in RIT rather than using a single mAb. We have demonstrated for the first time *in vivo* that this type of combination mAb approach is effective in RIT and are now aiming that this type of approach can be readily translated to the clinic.

Another line of investigation has been the intracellular signalling pathways underlying the combined treatment of anti-CD20 antibodies and irradiation in non-Hodgkin lymphoma cells. We are investigating whether the combined treatment results in a downstream signal transduction which differs from that obtained after treatment with anti-CD20 antibodies or irradiation (IRR) alone. The activation of ERK/MAPK cascade is a critical factor involved in cell death induced by combined treatment with anti-CD20 antibodies and irradiation. Our initial work has revealed that some anti-CD20 mAb induce cytoplasmic or paraptotic cell death and we are currently characterising this further.

Irradiation and immunoregulation

In this work we aim to exploit the therapeutic potential of combining immuno-modulatory mAb such as anti-CD40 with irradiation or cytotoxic chemotherapy. CD40 is a member of the tumour

necrosis factor (TNF) receptor family, and has a broad pattern of distribution including B-cells and antigen presenting cells (APC) such as macrophages and dendritic cells (DC). Ligation of CD40 on APCs results in their activation, "licensing" them to prime an antigen specific cytotoxic T-lymphocyte (CTL) response. CTL responses against a range of CD40+ and CD40- syngeneic lymphomas have been observed, providing long-term clearance of tumour and immunisation. However, responses are dependent on large doses of mAb treating an optimal tumour burden, with rapidly growing tumours able to overwhelm the CTL response. We have been able to demonstrate that irradiation and anti-CD40 mAb can act in concert to eradicate lymphoma and induce long-term survival under conditions whereby either treatment alone is ineffective. The much smaller doses of anti-CD40 mAb that provide long-term tumour clearance with EBRT may have very significant implications in translating this work into the clinic given the potential toxicity and risk of triggering unregulated immune responses and autoimmune disorders associated with the use of larger doses of anti-CD40 mAb. We believe that this type of combination approach using tumour cytoreduction with standard anti-cancer approaches such cytotoxic chemotherapy and irradiation followed by host immunostimulation may provide an excellent therapeutic opportunity for future clinical testing.

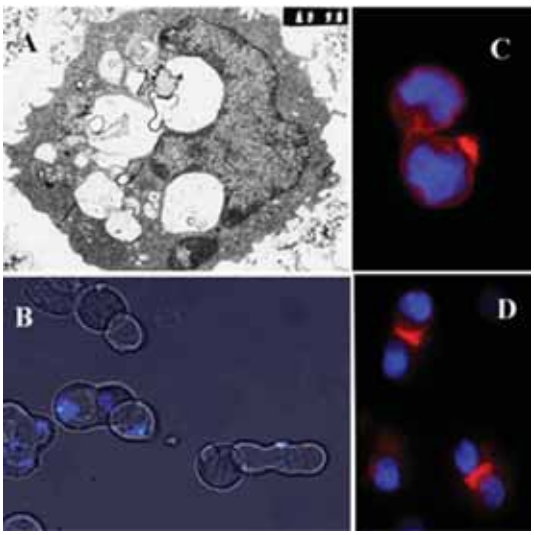
The combination therapy appears to be priming an anti-tumour CTL response and long-term survivors treated with this combination therapy were protected against subsequent tumour challenge, suggesting that they were actively immunised. *In vivo* administration of anti-CD8 mAb prior to and during treatment, to deplete CTL, results in a complete abrogation of the survival obtained with 5 Gy EBRT plus anti-CD40 mAb. In contrast, depletion of CD4 T-cells has no effect on therapeutic efficacy. Furthermore the specificity of the expanding CTL population was confirmed with the CTL able to lyse syngeneic but not other tumour targets and this could be blocked by anti-MHC class I mAb. Splenic CTL taken from control animals, or those treated with either EBRT or anti-CD40 alone, showed no cytolytic activity. Our data suggest that if sufficient antigen is liberated by EBRT and subsequently processed and cross-presented by APCs to CTL, a specific anti-tumour immune response is primed. Within this scenario it is possible that the anti-CD40 mAb acts to replace T-help and license the APC to perform this function.

Clinical translational research

A major focus of the group is to work alongside and to interact with the clinical trial programme. One of the major successes from the laboratory programme which we have translated from the clinic to the laboratory has been the use of fractionated RIT and a novel anti-Idiotype against Rituximab (an anti-CD20 mAb). We have now completed a Phase I dose escalation study of fractionated ^{131}I Rituximab and are now embarking on a Phase II study. The anti-Idiotype against Rituximab is now being in used in clinical trials to measure serum Rituximab levels. There are two further novel fractionated RIT studies in aggressive and indolent lymphoma respectively which involve collaborations with colleagues in medical oncology and nuclear medicine at the Christie hospital site and other sites across the UK.

Collaborators

Sue Owens Nuclear Medicine, Christie hospital; John Radford Medical Oncology, Richard Cowan Clinical Oncology and members of Manchester Lymphoma Group; Richard Myers Pathology, MRI Martin Glennie, Peter Johnson, Cancer Sciences Division, Southampton University



The mode of cell death in Raji cell line. A) Involvement of cytoplasm (Cy) in cell death induced by anti CD20 antibodies. Gross vacuolazation (V) of cytoplasm resembling autophagy is apparent following treatment. B) Monodansylcadaverine, a specific marker for autophagic cell death, is included into cytoplasm of Raji cells treated with anti-CD20 antibody. Homotypic aggregation of cells (C; D) followed by polarization of F-actin (D) are important factors contributing to anti CD20 induced cell death. (C) and (D): F-actin is detected with phalloidin (red) and DNA is counterstained with DAPI (blue).

Publications listed on page 67

HEAD OF RESEARCH SERVICES
Jenny Varley

DEPUTY
Caroline Chadwick

Research Services

http://www.paterson.man.ac.uk/facilities/scifacs.jsp

The Scientific Research Services continue to underpin all the research activities within the Paterson Institute as well as providing one CR-UK wide service (the Affymetrix Microarray service). It is essential that the services provide comprehensive state-of-the-art facilities which dovetail with the requirements of the users, and to this end each service has an associated user group which meets at regular intervals. These user groups carry significant weight, and can advise on the introduction of new services or cessation of redundant ones as well as equipment needs. We have continued to invest to ensure that all our services are optimally supported.

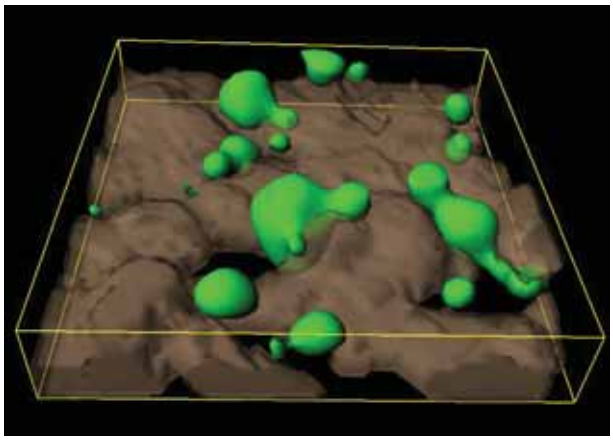
Advanced Imaging Facility

Head: Steve Bagley

http://www.paterson.man.ac.uk/facilities/advimg.jsp

Since 1985 Laser Scanning Confocal Microscopy has facilitated the digitisation of data in three dimensions. This has been achieved by the use of a high intensity monochromatic light source (generally a laser) and an imaging aperture placed just before the detector leading to the imaging of those structures that were in-focus and excluding data that were above or below the plane of focus. The system was originally developed to assist imaging at depths inside embryos that normal microscopy could not resolve because of interference from information above and below the plane of focus, however, the main advance for the broader biological research community was that 3D imaging became a routine process available to most laboratories. Over the last ten years there have been major advances in optical design, detection sensitivity and labelling technology which have allowed live cell imaging via widefield optics to become a viable possibility.

Widefield imaging requires less light to form an image hence it is less damaging to cellular processes. In the last four years the Advanced Imaging Facility has been investigating, designing and developing techniques for the visualisation of live cellular processes on the micro- and macro-scale where the very act of describing real world, four dimensional objects in a digital manner disrupts biological processes as little as possible. This has led to a move away from laser scanning systems to less harmful and more sensitive techniques utilising standard optics and image processing algorithms.



Time-lapse of PC3-GFP cells interacting with the BMEC-1 cell layer over 30 minute time increments. The image shows both the invading PC3-GFP cells (green) pushing through the mono-layer of Bodipy Ceramide labelled BMEC-1. Magnification x400

Over the last year we have been increasing the sensitivity of the available equipment as a matter of priority. The facility recently purchased a bespoke multi-position four dimensional microscope, whilst another has been developed by assembly of optimal component parts for long term time-lapse; both allow the collection of several fields of view over time. This permits questions to be addressed at levels of temporal and spatial control that were not possible with previous systems. A considerable amount of research and development pushed the system towards two primary goals; 1) to increase the 'imaging window' - the available time when biologically relevant data can be collected whilst reducing photo-toxicity and 2) to push the sensitivity of the equipment to image proteins which are present in low concentration

To achieve these goals we have purchased new cameras, light sources and more specific fluorescent filters and mirrors to minimise light loss in transmission. In parallel we have been fine-tuning the cell environment on the microscope stage. The result has been a widespread increase in live cell imaging within the Institute and a decline in imaging fixed samples. In short, emphasis can now be given to the temporal dynamics of structural changes rather than simply cataloguing snapshots of the variety of forms within a population.

Multi-position time-lapse over the last year has become the all more important. Many events occur fairly infrequently, for example cell division can occur in a 40 minute window every 24 hours, and waiting for these events to occur in one field of view would be prohibitively slow. Visiting multiple points greatly enhances the return such that questions can be asked with multi-point visiting that would be impractical in single fields of view. We have therefore added a high resolution XYZ stage to our imaging platform. Several research groups within the institute are involved in these forms of data collection on a weekly, if not daily basis.

In the previous year we started developing systems for controlled spot-limited photo-bleaching. These techniques are now in routine use and have been employed with great effect to study polymer dynamics and the turnover of proteins on spindle poles by Iain Hagan's laboratory. These time-lapse investigations are ongoing and are yielding information about dynamics within bundles of microtubule polymers that are simply not possible to obtain from looking at the dynamics of an unbleached bundle.

We are currently reviewing new fluorescent proteins and testing particular filter/dichroic mirror combinations to optimise transmission. We are also trying to exploit the potential of quantum dots as a labelling technology for both long term tissue sections to support target validation and clinical trials and for use in live cell imaging. It is hoped that quantum dot technology will be in routine use in both clinical and laboratory applications within a year.

The increase in capabilities of the systems, in particular the multi-point visiting and photo-bleaching studies, are highlighting the need to keep the software and archiving in step with the developments in microscopes and cell culture. We are therefore planning measures to deal with this increase in dependence upon large scale computing platforms and high-end software over the next year and anticipate an increasing need for mathematical modelling of the biological phenomena we are documenting.

Biological Resources

http://www.paterson.man.ac.uk/facilities/bru.jsp

The Biological Resources unit continues to provide a modern integrated service, supporting scientific research programmes whilst ensuring that the highest quality of health and welfare are maintained at all times. The use of animals in medical research remains a controversial issue and although alternative methods such as tissue culture are used wherever possible, there is still a need for some research involving animals in the understanding of cancer and the development of better treatments for patients. All the work at the Paterson Institute that involves the use of rodents is covered by licences issued by the Home Office and reviewed by a local ethical committee.

Since 2004 we have brought in several new genetically altered strains of mice which are vital to keeping us at the forefront of the scientific research. Once they arrive within the BRU all the mice are housed in individually ventilated cages (IVCs); these cages prevent the spread of potential disease from one cage to another, with each cage having an individual Hepa filtered air supply and exhaust. In addition to protecting the mice from disease these cages also help to protect the staff from exposure to allergens from the mice and bedding. Exposure of staff to laboratory animal allergens (LAA) is a current hot topic with the Health and Safety Executive (HSE). Long term exposure to LAA can induce asthma and skin or eye allergies in staff handling the animals. The use of IVC caging dramatically reduces this risk. All the cages are provided with environmental enrichment, in the form of nesting material and wooden chew blocks or play tunnels. The addition of these items to the cage provides environmental stimulation for the mice and reduces aggression amongst males of some strains. We undertake routine health screening from our colonies to ensure that the mice are free from a list of specific pathogens (SPF) and any new strains brought into the unit are health-screened before introduction into the facility.

Two thirds of the space in the unit has been given over to the development and breeding of genetical-

ly altered mice, which are important in providing mouse models of human disease or understanding the function of a particular gene in the living organism. The transgenic service has provision for both pronuclear and ES cell injection, and over the last year we have successfully developed several novel strains. In addition we offer cryo-preservation and re-derivation of embryos, sperm freezing and in conjunction with the Molecular Biology Core Facility (page 54), a full genotyping service is provided for all genetically altered mice. The remaining third of the unit is dedicated to the care and housing of experimental mice and a dedicated staff of highly skilled technicians can undertake a range of procedural studies. There is close liaison between the BRU staff and the scientific groups within the Institute.

Cancer Research UK GeneChip Microarray Service.

Head: Stuart Pepper.

http://bioinf.picr.man.ac.uk/mbcf/index.jsp

Last year saw the GeneChip microarray service based at the Paterson reach a throughput of over 800 arrays, and as this year draws to a close we are reaching similar numbers again. To help in tracking the projects we work on, we have started using our on-line database, MIAME VICE, as a primary means for storing data and experimental annotation. This database has been designed and written by the Bioinformatics group headed by Crispin Miller, and is now coming to be a central part of the operation of the service. For users who are ready to publish results we can offer a MIAME compliant repository which will satisfy the demands of journals for suitable annotation of all microarray data.

During this year we have been working with Epistem who are world leaders in developing RNA amplification protocols for microarray analysis. We have been able to generate reproducible data from samples of 10 - 100pg of total RNA and are now evaluating the feasibility of transcription profiling on populations of fewer than 100 FACs-sorted cells. This should lead to some very exciting opportunities for studying small cell populations.

The Affymetrix platform has had a series of developments this year, with the release of new, higher density SNP arrays, an 'all exon' array, and tiling arrays which will allow 35bp resolution interrogation of the entire human and mouse genomes. To develop services supporting these new arrays will require a considerable amount of training, both in the laboratory protocols required, and in the data handling and analysis. As training in the various applications progresses we will roll out services when we are ready to provide the level of support users will need.

Following a successful service review this summer, the service has started to integrate more closely with CR-UK Research Services. This integration has lead to closer interactions with other CR-UK service units, particularly the Genotyping Service run by Mike Churchman. Over the next few months we will be working together to develop a comprehensive genotyping service based on the high density Affymetrix SNP arrays.

Central Services

Head: Martin Chadwick

http://www.paterson.man.ac.uk/facilities/censerv.jsp

A modern and efficient central services facility provides a vital role in supporting the research carried out in the Institute. The roles carried out by the group include media and sterile services, central stores, porters and domestics. The services provided can often go un-noticed by staff if it runs smoothly and efficiently – a sure sign that the facility works well. During the year there have been a few changes with new members of staff arriving and old leaving. There has been the retirement of the ever-cheerful Ken McGivern from the porters. We have also progressed with the integration of our service, with a few members of the existing staff being able to transfer their skills between roles.

The service has continued its modernisation with a new intranet 'online shopping style' ordering system for the stores being used successfully by the whole institute. The bar code system to be used to monitor stores of glassware and improve efficiency is being developed and although there are still teething problems the system should be up and running in 2006, to help with the increased usage of the supplies provided by central stores.

Stores continues to grow at a rapid rate; there are over 100 different products available in stores for the end users, and with over 1500 individual products being delivered by the porters a month. The institute freezer program has again grown to now encompass six institute freezers providing a comprehensive stock of enzymes, reagents and media required within the institute.

The media and plate pouring service is proving more popular than ever now with four members of staff having to work hard to keep up with increasing demand in an attempt to provide a next day delivery service, and the service still continues to grow each month. All the lab aides are assigned labs in which they spend a few hours a week in helping with basic technical duties. Along side the Media Services the Sterile Services provide an important role ensuring the labs are fully stocked with sterile glassware, simple buffers and boxes of tips. All lab aides are responsible for ensuring the smooth running of the labs to which they are assigned.

During the evenings the domestic staff arrive and clean the institute from top to basement ensuring the office areas are clean and ready for the next days work. They do an exceptional job and ensure there is minimal disruption to staff.

The porters have been running smoothly and still provide an essential service and remove all rubbish from the institute as well as moving some equipment. They also not only deliver the normal deliveries but also the goods ordered from the 'intranet ordering system' to the labs during the day.

Flow Cytometry Unit

Head: Mike Hughes

http://www.paterson.man.ac.uk/facilities/facs.jsp

The Flow Cytometry service unit has two cell sorters, the FACSVantage and the FACSAria, run by two full time staff. There are also two cytometers for analysis, the FACSCalibur and the FACScan which is located in the Kay Kendall laboratory. All institute members are given adequate training enabling them to analyse their own samples. This year has been the first full year we have had the FACSAria digital flow cytometer. We had initial problems with the degradation of the nozzles as did most other facilities and also some stream stability problems. We have recently had a software upgrade and the promised change to ceramic nozzles which has solved most of our problems and helped us to sort cells more efficiently.

Amongst the new projects that the FACSAria has helped us with is the four-way sorting of hypoxic cells from 3D cell culture (spheroids) and xenografts for the Cellular Molecular Pharmacology group. The aim of the group is to determine the factors responsible for the increased drug resistance observed in hypoxic cells in solid tumours. Höechst 33342 is used as a perfusion marker with well oxygenated cells being brightly stained and those with decreasing oxygen exhibiting lower levels of fluorescence correlating with oxygen levels. The spheroids are then disrupted and the mixed cell population sorted based on Höechst 33342 fluorescence on the FACSAria. The four-way sorting of the FACSAria enables them to rapidly sort the cells into populations from different regions of the spheroid before the Höechst 33342 becomes equilibrated over the entire cell population and reduces the time the cells are in suspension prior to returning to culture conditions and analysis. Cells are normally isolated from drug treated spheroids and analysed by clonogenic assay so the reduced time taken on the FACSAria to sort into well differentiated populations is critical to maintain viability of the cells.

The Mass Spectrometry group (Professor Tony Whetton) has used the FACSAria to sort long term reconstituting haematopoietic stem cells ($Lin^-/Sca^+/Kit^+$) and more mature cells ($Lin^-/Sca^+/Kit^-$) prepared from bone marrow. To prepare cells for FACS sorting bone marrow was depleted using immunomagnetic beads. Cells were then stained with anti-Sca-1 and anti-c-kit antibodies directly conjugated to FITC and APC respectively. Goat anti-rat PE was applied to obtain lineage marked cells. Propidium iodide was added to gate out debris and dead cells. These sorted cells were used in a novel mass spectrometry method using an isobaric covalent modification of peptides for relative quantification (iTRAQ) to compare the proteomes of the two populations.

Histology

Head: Garry Ashton

http://www.paterson.man.ac.uk/facilities/histology.jsp

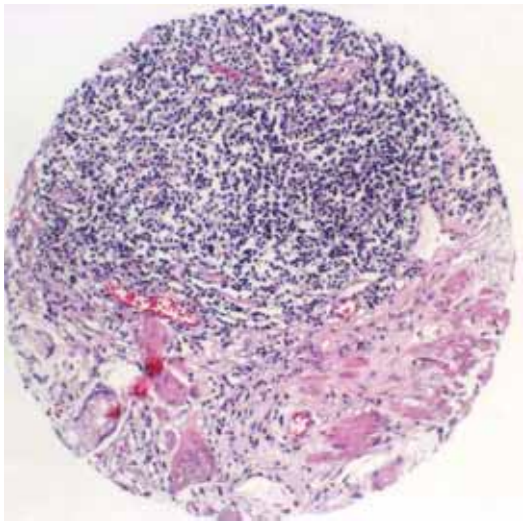
All the key services have seen heavy demand over the last twelve months. New equipment such as the cryostat, tissue processor and automated stainer have all performed reliably, allowing the workload within the unit to be managed more efficiently.

Immunohistochemical analyses have been designed to further characterise specific developmental defects observed in ATF2 knockout mice. Protocols for a number of tissue specific lineage markers have been established, including cardiomyocyte-, foetal liver- and haematopoietic-specific markers. In addition, protocols have been opti-

mised to detect stress activation in mouse embryos including phosphorylated p38 kinase or hypoxia specific marker HIF1α.

The unit, together with the Clinical and experimental pharmacology group, has also been involved in the optimisation of immunohistochemical (IHC) methodologies for cleaved caspase 3, PARP and cytokeratin 18 for use in pre-clinical pharmacodynamic IHC assays. The assays focus on detecting and quantifying levels of apoptosis in cells and tissue which will eventually be used in the clinical trial of agents which modulate/inhibit apoptotic pathways.

A new sucrose/gelatin method for the preparation and freezing of embryoid bodies and cell preparations has been implemented. The method both cryoprotects and immobilises specimens allowing for much improved morphology and numbers.



Single 0.6mm core of a gastric tumour from a tissue microarray

The unit recently purchased a tissue microarray (TMA) platform, allowing for the generation of multiple specimen slides. The platform is an essential tool in the shift towards translational research, conserving valuable material whilst allowing standardisation of methodology. TMAs of both gastric tumours and normal breast tissue have already been constructed and this service will continue to be expanded with the construction of TMAs from mouse models.

Advances in the isolation and extraction of quality RNA based on immunophenotype have been achieved. In parallel with this, work on the possible recovery of material from archival formalin-fixed paraffin-embedded specimens will be assessed over the coming months. This technique has the possibility of enabling the gene expression analysis of huge numbers of previously inaccessible samples. Another area of interest to the unit is quantum dot technology. Methodologies for direct antibody conjugation and the use of Q dot bioconjugates will be assessed and optimised, allowing for multi-labelling and accurate signal quantification of tissue sections.

Molecular Biology Core Facility.

Head: Stuart Pepper.

http://www.paterson.man.ac.uk/facilities/mbcf.jsp

The molecular biology core facility provides access for Paterson staff to a range of state-of-the-art technologies for analysis of DNA, RNA and protein. Routine plasmid extraction and sequencing is offered as a complete service where facility staff carry out all the processing, whereas quantitative PCR is offered as a technology platform for which we offer technical support and advice. Over the last year our capability to support qPCR has improved significantly; we now have a 384-well pipetting system available to users which allows larger experiments to be tackled in a time effective manner. Purchase of the Exiqon probe library has also streamlined the design phase of qPCR projects, meaning that users are able to start generating useful results very quickly. As 2005 draws to a close we are starting to offer an assay validation service in which we will design and validate assays to specific genes for users so that the first experiment a user performs will be to generate the results they want.

Last year the facility expanded into the area of protein analysis by mass spectrometry. Developments in this area have benefited immeasurably from the support and expertise of the Stem Cell Leukaemia and Proteomics Lab, University of Manchester.

The facility operates two machines that are routinely delivering excellent results for our users: a Q Star which offers excellent mass accuracy and resolution, and is well suited to 'routine' identification of sequencing of peptides, and a Q Trap which is a unique hybrid instrument that is amenable to studies on post-translational modifications such as phosphorylation. In combination with 3 LC Packings Ultimate Nano LC systems, two of which are able to perform 2-dimensional chromatography, these mass specs offer a very flexible system for the comprehensive analysis of protein mixtures. Last year these machines were used in a number of

successful pilot studies, this year they have been in routine use for protein identification, either from gel spots or from complex LC fractionation, as well mapping post translational modifications such as phosphorylation.

The facility has also undertaken a pilot study using the Isotope Tag Relative Absolute Quantification (iTRAQ) technique. This project generated relative quantitation data between two cell lines for several hundred proteins; in collaboration with the Bioinformatics Group we then cross mapped these results with existing microarray data and related the results to pathway mapping information. The pilot study has demonstrated the utility of this approach and we would be happy to support similar projects in the future.

Kostoris Medical Library

Head: Steve Glover

http://www.christie.nhs.uk/profinfo/departments/library/default.htm

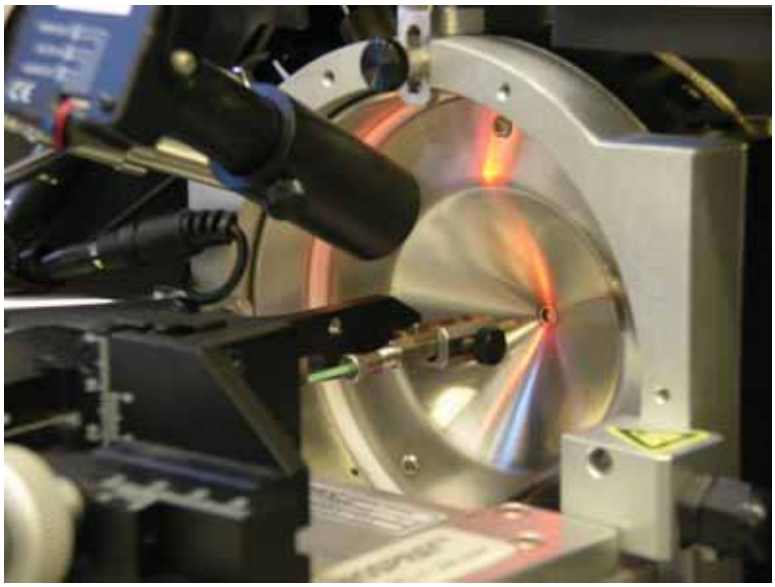
Collaboration has been the underlying theme of 2005. The announcement regarding the Manchester Cancer Research Centre should further formalise the relationship between the Kostoris Medical Library and the University of Manchester's John Rylands Library. Indeed, the last 10 years have seen the two libraries converging as the product has moved towards a predominately online environment.

Medical library staff members have acquired university status as clinical academic support staff whilst members of the NHS have benefited from walk-in access to the resources on the main John Rylands site. The Paterson Institute has also benefited from this association with access to the academic network (JANET) enabling the libraries to provide IP authenticated resources including databases and online journals. An added post-merger bonus will be the eligibility of all Paterson Institute staff to ATHENS authenticated resources such as BIOSIS, EDINA and MIMAS.

The medical library also has a close collaborative relationship with the other CR-UK libraries at the London Research Institute and the Beatson Institute in Glasgow. In 2005 the library supplied over 200 documents to scientific staff at the other CR-UK locations and received 80 for Paterson staff.

In February 2005 the library was accredited by an external NHS body under the Health Libraries and Information Confederation (HELICON) scheme. The library achieved a Stage 3 accreditation with areas of significant excellence. 33 out of 36 categories were awarded an 'excellent' level and all 11 must-have criteria were reached. In 2005 the library also provided the Chair of the Libraries and Information for Health Network Northwest (LIHNN), a body that represents over 80 NHS libraries in Cheshire and Mersey, Cumbria and Lancashire, and Greater Manchester Strategic Health Authorities.

2006 will see a move to a more customised Current Awareness Service (CAS). The CAS service will combine data mining strategies from the published literature with the profiled e-toc services aimed at group level. This enhanced CAS service will maximise the information opportunities arising from the Paterson/University merger and give greater access to published science.



Close up view of the 4000 Q Star showing the electrospray ion source.

Publications

BIOINFORMATICS GROUP *(page 8)*
Crispin Miller

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CARCINOGENESIS GROUP *(page 10)*
Geoff Margison

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Plummer, E.R., Middleton, M.R., Jones, C., Olsen, A., Hickson, I., McHugh, P., Margison, G.P., McGown, G., Thorncroft, M., Watson, A.J., Boddy, A.V., Calvert, A.H., Harris, A.L., Newell, D.R. and Curtin, N.J. (2005) Temozolomide pharmacodynamics in patients with metastatic melanoma: DNA damage and activity of repair enzymes O^6-alkylguanine alkyltransferase and poly(ADP-ribose) polymerase-1. *Clin Cancer Res*, **11**, 3402-3409.

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Karim Labib

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Iain Hagan

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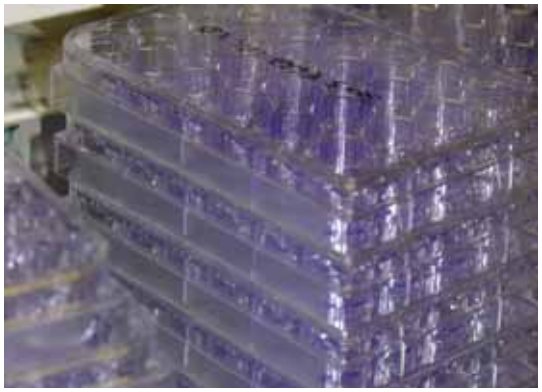

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Angeliki Malliri

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Nic Jones

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Caroline Dive

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Caroline Dive and Malcolm Ranson

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Lez Fairbairn

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Peter L Stern

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Elmar Schiebel

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Jamal Zweit

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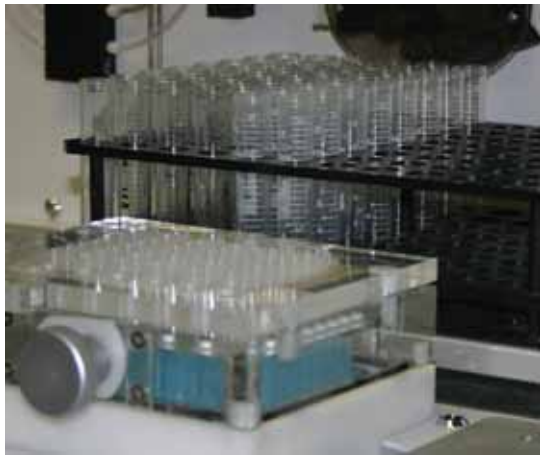

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Georges Lacaud

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Valerie Kouskoff

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Terence D Allen

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Catharine West

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Rob Clarke

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Robert Hawkins

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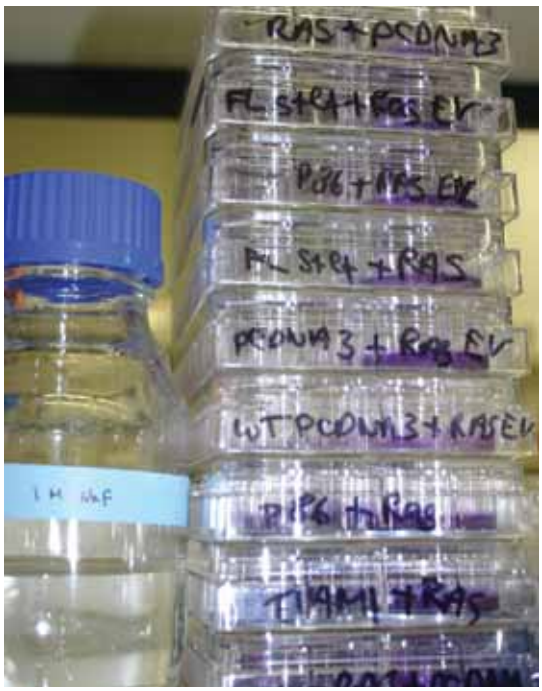

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CANCER STUDIES – MEDICAL ONCOLOGY: GLYCOANGIOGENESIS GROUP

(page 44)

Gordon Jayson

Refereed Research Papers

Dark, G.G., Calvert, A.H., Grimshaw, R., Poole, C., Swenerton, K., Kaye, S., Coleman, R., Jayson, G., Le, T., Ellard, S., Trudeau, M., Vasey, P., Hamilton, M., Cameron, T., Barrett, E., Walsh, W., McIntosh, L. and Eisenhauer, E.A. (2005) Randomized trial of two intravenous schedules of the topoisomerase I inhibitor liposomal lurtotecan in women with relapsed epithelial ovarian cancer: a trial of the national cancer institute of Canada clinical trials group. *J Clin Oncol*, **23**, 1859-1866.

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Duff, S.E., Saunders, M., McCredie, V., Kumar, S., O'Dwyer, S.T. and Jayson, G.C. (2005) Pre-operative plasma levels of vascular endothelial growth factor A, C and D in patients with colorectal cancer. *Clin Oncol (R Coll Radiol)*, **17**, 367-371.

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Shnyder, S.D., Hasan, J., Cooper, P.A., Pilarinou, E., Jubb, E., Jayson, G.C. and Bibby, M.C. (2005) Development of a modified hollow fibre assay for studying agents targeting the tumour neovasculature. *Anticancer Res*, **25**, 1889-1894.

Ward, T.H., Danson, S., McGown, A.T., Ranson, M., Coe, N.A., Jayson, G.C., Cummings, J., Hargreaves, R.H. and Butler, J. (2005) Preclinical evaluation of the pharmacodynamic properties of 2,5-diaziridinyl-3-hydroxymethyl-6-methyl-1,4-benzoquinone. *Clin Cancer Res*, **11**, 2695-2701.

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CANCER STUDIES – MEDICAL ONCOLOGY: PROTEOGLYCAN GROUP *(page 46)*

John Gallagher

Refereed Research Papers

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CANCER STUDIES: TARGETED THERAPY GROUP *(page 48)*

Tim Illidge

Refereed Research Papers

Erenpreisa, J., Kalejs, M., Ianzini, F., Kosmacek, E.A., Mackey, M.A., Emzinsh, D., Cragg, M.S., Ivanov, A. and Illidge, T.M. (2005) Segregation of genomes in polyploid tumour cells following mitotic catastrophe. *Cell Biol Int*, **29**, 1005-1011.

Honeychurch, J., Glennie, M.J. and Illidge, T.M. (2005) Cyclophosphamide inhibition of anti-CD40 monoclonal antibody-based therapy of B cell lymphoma is dependent on CD11b+ cells. *Cancer Res*, **65**, 7493-7501.

Other Publications

Geldart, T. and Illidge, T. (2005) Anti-CD 40 monoclonal antibody. *Leuk Lymphoma*, **46**, 1105-1113.

Illidge, T. (2005) Anti-CD40: Janus or gatekeeper? *Blood*, **106**, 2595-2596.

Illidge, T.M. and Johnson, P.W.M. (eds.) (2005) *Lymphoma Methods and Protocols*. Methods in Molecular Medicine volume 115. Humana Press, Totowa, NJ.

McFarlane, V., Friedmann, P.S. and Illidge, T.M. (2005) What's new in the management of cutaneous T-cell lymphoma? *Clin Oncol (R Coll Radiol)*, **17**, 174-184.

ADDITIONAL PUBLICATIONS

Refereed Research Papers

Hart, C.A., Brown, M., Bagley, S., Sharrard, M. and Clarke, N.W. (2005) Invasive characteristics of human prostatic epithelial cells: understanding the metastatic process. *Br J Cancer*, **92**, 503-512.

Lakhani, S.R., Reis-Filho, J.S., Fulford, L., Penault-Llorca, F., van der Vijver, M., Parry, S., Bishop, T., Benitez, J., Rivas, C., Bignon, Y.J., Chang-Claude, J., Hamann, U., Cornelisse, C.J., Devilee, P., Beckmann, M.W., Nestle-Kramling, C., Daly, P.A., Haites, N., Varley, J., Lalloo, F., Evans, G., Maugard, C., Meijers-Heijboer, H., Klijn, J.G., Olah, E., Gusterson, B.A., Pilotti, S., Radice, P., Scherneck, S., Sobol, H., Jacquemier, J., Wagner, T., Peto, J., Stratton, M.R., McGuffog, L. and Easton, D.F. (2005) Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. *Clin Cancer Res*, **11**, 5175-5180.

POSTGRADUATE TUTOR
Graham J Cowling

EDUCATION COORDINATOR
Julie Edwards

EDUCATION COMMITEE 2005

Iain Hagan – Chairman
Fiona Blackhall
Noel Clarke
Graham Cowling
Caroline Dive
Dave Gilham
Roger Green
(until September 2005)
Tim Illidge
Valerie Kouskoff
Catharine Merry
Crispin Miller
Mark Saunders
(from April 2005)
Jenny Varley
Catharine West
(from September 2005)

Postgraduate Education

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

Developing first rate cancer research and treatment in future decades depends on today's quality training of our clinical and science graduates. The Paterson Institute continues to act as the centre for postgraduate study in many aspects of molecular, cellular and translational cancer research by providing the major elements required of a training programme for postgraduate research students and clinical research fellows studying for MPhil, MD and PhD degrees by research. As the major centre for research and teaching in the Division of Cancer Studies, School of Medicine, University of Manchester, our active research environment serves all our students equally and they can draw on the wide expertise of our scientists and clinicians as well as gaining benefit from our modern laboratories and first-rate service units.

The Paterson PhD Programme

In 2005 we welcomed eight new Paterson 4-year PhD students from the UK, Poland, Greece, Hungary and India, who were selected after three application rounds from over thirty interviewed candidates who were chosen from a massive 400 applications received by the Institute during the year. The School of Medicine also awarded the CR-UK Medical Oncology Group a PhD studentship and funded three new clinical fellows who will be working between the Christie Hospital and the Institute. Another clinical fellow won the Robin Dodge Fellowship and started a PhD project that links the Department of Pathology, Christie Hospital and the Institute. The contributions of our fifty postgraduates to the scientific work of the Institute are described elsewhere in this report. The 4-year PhD students from previous years have mastered a remarkable range of skills in their three 10-week rotation periods and several have already published parts of their work. Our programme is still evolving and, in 2006, we are offering an even more flexible approach to PhD training with eight further CR-UK studentships on offer. Successful UK, European and International candidates can opt to work on one main project or three 10 week options in their first year.

Research Degree Structure

Postgraduate students entering the Institute to study for the degree of PhD are under the direct guidance of an appointed Supervisor(s) and are also allocated an Advisor, with whom they meet regularly to review and record progress, set new targets and identify any assistance required. 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 Manchester University. To enhance our training programme further, the monthly series of cancer biology master-classes has been continued, allowing lead researchers in the Paterson and Christie Hospital to explain the "what", the "how" and the "why" of their area of research. These relaxed evening sessions are proving extremely popular with all of our postgraduate students and many of the postdoctoral fellows also take part in the scientific discussions. Students also 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 (project dependent), bioinformatics (optional), pharmacology (optional), safety (compulsory), innovation (compulsory), written and oral presentations (compulsory), careers, animal usage (compulsory if using animal models) and ethics (compulsory if using human tissue). The Faculty Training programme has developed in two new and exciting directions during 2005. The introduction of a student personal development plan allows

students to go on line and to develop their own personal plan. The first introductory session of an academic skills programme explored the psychological skills required for a PhD. This taster session, led by the University Counselor and in which cancer studies students rubbed shoulders with social historians and engineers, proved a great success and discussed how to overcome the "bad" times that all PhD students go through during their studies. Further sessions lead on from this session for those students who feel that they need more help to reach their research goals.

In the Division of Cancer Studies 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 programme. This process often takes place before the candidate is chosen. The Education Committee, a body made up of senior scientists, postdoctoral fellows and student representatives, continues 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 is through written reports at three months (literature review) one and two years, and short talks to the Institute at similar times (6, 12, 24 months and as part of the final *viva voce*). All students have the opportunity to give written feedback on any aspect of their degree programme by means of an annual questionnaire, the results of which are published. Students can ask advice of any member of the Education Committee or use their student representatives. All Students are automatically members of the Cancer Studies Postgraduate Association, an independent student body at the Institute and Christie Hospital.

All work and no play?

When our students are not working, our student representatives have been busy organising a programme of activities outside of the laboratory. This begins in September when all our students join the Institute for the annual Colloquium. These three days of intensive science also includes two nights of relaxation that allow new students to find their feet for hard work and play. All students are invited to annual student social occasions to meet other new postgraduates and postdoctoral fellows and, so far in 2005, this has been an evening at Dog Races.

Paterson students out on a social evening

ASSOCIATE DIRECTOR - ADMINISTRATION
Pippa McNichol

DEPUTY
Margaret Lowe

Administrative Services

http://www.paterson.man.ac.uk/facilities/adminfacs.jsp

Administrative services provide the foundation upon which the Paterson Institute runs. It has been an exceptionally busy year for the team, as they have been responsible for undertaking the bulk of the work for the Institute's merger with the University of Manchester as well as contributing to Cancer Research UK's National Pay & Grading Project. This year, the Institute participated in an employment project for the long-term unemployed and those excluded from society (established by Stockport Job Centre, on behalf of all the Job Centres in Greater Manchester). To date, two staff have been successfully employed via this scheme.

Admin & Reception Services

Manager: Julie Hallett (on maternity leave)
Sharon Barnes, Trevor Haughton, Shirley Leonard (Acting Manager)

Shirley Leonard, PA to the Associate Director – Administration, has also been covering the Admin Manager's post, whilst Julie Hallett has been on maternity leave. Sharon Barnes joined the team to provide additional temporary cover and has settled in well. The team contributed to the production of this year's Colloquium booklet and assisted in its organisation.

Director's Office

Director's PA: Elaine Mercer

The newsletter continues to be undertaken by the office with a great deal of help from the Newsletter Editorial Board and the IT department. It has gone from strength to strength, with staff throughout the Institute contributing from both a scientific and social viewpoint. Printing is now done externally, resulting in a sleek and professional looking newsletter which is distributed not only internally, but also to colleagues at other Institutes/Universities and fund-raising bodies.

The Paterson Seminar Series has been reactivated and is now centralised through the office, with eminent speakers being invited from all over the world. Planning and correspondence is currently ongoing (in conjunction with Group Leaders) to ensure a full programme of exciting seminars is in place for the beginning of 2006.

Estates & Facilities

Manager: Steve Alcock
John Lord, Dennis O'Shea

It has been a demanding year for the Estates department with the majority of time devoted to capital schemes within the institute: the Translational Research Facility is ongoing and should be completed by Autumn 2006; the refurbishment of the Molecular Biology lab on the 2nd floor will be ready for occupancy in December 2005 and the new X-ray room in the basement has just been completed. There have been several engineering projects carried out which will be of benefit to the Institute.

The team has been pro-active throughout the year which has resulted in a reduction in the number of faults reported.

Finance & Purchasing

Manager: Margaret Lowe
Yana Anderton, Catherine Bentley, Liz Fletcher, Denise Owen, Debbie Suthern

The Finance and Purchasing team have continued to provide a comprehensive service to the Institute during 2005. Kate McCoy retired at the end of March and the decision was taken to leave the post vacant until the merger with the University was completed. The aim is to be able to incorporate the University systems into the Institute without any disruption to the service given to the groups and service units. The impending merger has involved input from the team in various information gathering exercises.

The team has also been involved in the Cancer Research UK National Pay and Grading Review. This has involved information gathering, co-ordinating local pay and grading fora and attending national meetings. Full implementation of the project will be in 2006.

Health & Safety

Manager: Colin Glesson

The preparations for the merger with the University; the Translational Research Facility and Molecular Biology refurbishment projects and the usual health and safety matters has meant it has been a busy and varied year.

Closer links have been developed between the Institute and the University Health and Safety Services Team. This has meant defining clear reporting lines for the Institute's Health and Safety, Genetic Modification and Radiation Protection Committees, as well as for accident and dangerous incident reports and safety inspection action plans. In more practical terms the Institute will be able to utilise the resources of the University Health & Safety Services and co-opt staff onto bespoke health and safety training courses. The merger will also result in a greater level of interest and involvement of outside agencies such as the HSE and the Environment Agency

The refurbishment of the Translational Research Facility, Molecular Biology laboratory and Kinnaird House has been a major focus and has meant some upheaval for groups within the Institute. The team has worked with the contractors and Institute staff to minimise disruption to the operations of the Institute. This has meant daily contact with the contractors troubleshooting problems.

The Health & Safety pages on the Institute's intranet have been developed further, with the addition of new guidance documents and training presentations. In the forthcoming year this information resource will be promoted. Lastly, favourable reports were received from the Environment Agency following an inspection of the Institute's storage, handling and disposal procedures for radioactivity.

Human Resources

HR Adviser: Anna Pearson
Laura Humes

The HR department has gained an extra member of staff this year in the form of an HR Assistant to support the HR Adviser in providing a professional and comprehensive service. The department provides professional advice and guidance to staff on all issues connected to the employment relationship including terms and conditions, policies and procedures and current employment legislation. Throughout 2005, a number of new tasks have been taken on by the department including administration for recruitment and pre-employment medical screening. New recruitment software, OnTrack, has recently been implemented to improve the efficiency and effectiveness of the recruitment and selection process and to aid in equal opportunity reporting. The team is currently working on a project for absence management together with the union and is also reviewing all of the HR policies to ensure they are relevant and comply with new legislation.

Margaret Lowe

The merger with the University of Manchester is inevitably producing a busy and diverse workload and will continue to challenge and stimulate the HR department for the foreseeable future.

IT

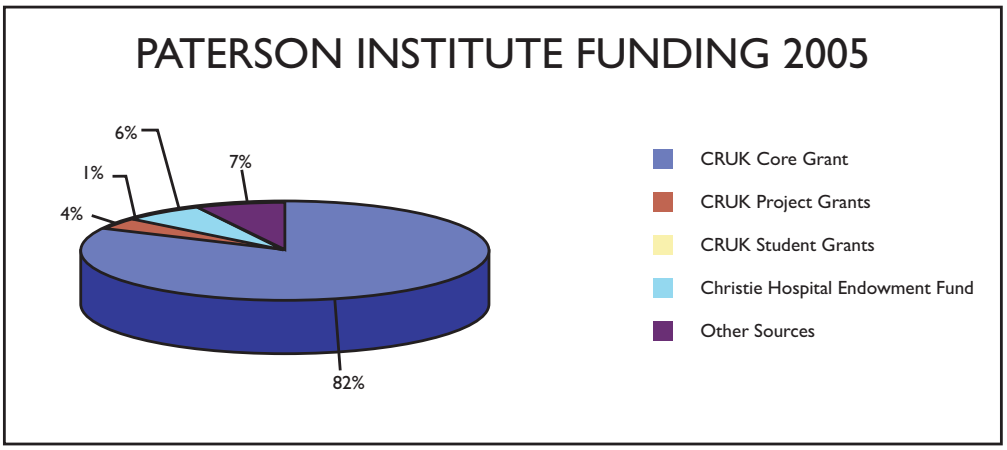
Manager: Malik Pervez
Brain Poole, Steve Royle, Mark Wadsworth, Zhi Cheng Wang, Matt Young

The team are constantly reviewing and applying new technological innovations to ensure that the Institute's technology is future-proofed and that the IT systems are secure, robust and dynamic. Continuous investment in IT services has enabled strong foundations to be built which have ensured that the service is available 24 hours a day, 7 days a week. In preparation for the merger with the University of Manchester additional systems have been integrated into the Paterson.

New developments include: an off-site disaster recovery plan has been developed which is currently being tested; additional storage space has been bought to house the data from the mass spectrometers and a trial of wireless working has been undertaken.

Acknowledgement for Funding of the Paterson Institute

The major source of funding (82%) of the Paterson Institute is through a core grant from Cancer Research UK (CR-UK). 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 5% of funding has been received from CR-UK for Project Grant Work and Studentships.



The infrastructure of the Institute is funded by the Christie Hospital Endowment Fund and together with specific project grants accounts for 6% of the total income.

The final 7% of the 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:

- National Translational Cancer Research Network (NTRAC)
- European Commission
- Medical Research Council
- Association of International Cancer Research
- Wellcome Trust
- Astra Zeneca
- European Molecular Biology Organisation
- Novartis
- Friends of Rosie

We are immensely grateful to all our sponsors.

Donations to the Institute in 2005

Legacies

- In memory of Olive Francis O'Connell
- In memory of Margaret Jean Parbrook
- In memory of Billie Simpson

Donations

- Mrs H M Emerson in memory of Gordon K Emerson
- Mr Neil Raghib
- All Global Ltd
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- Mrs S Cradden
- Mrs M Collier in memory of Bill Collier and Mrs C Baxter
- Mrs M K Sutcliffe
- Mr M Gannon and friends
- Staff and customers of the Three Legs of Man and the Platford Hotel in memory of Moya Connors
- Mrs Thomas and Mr & Mrs Horton in memory of Anthony Martin
- Mrs E Longden
- Mr Robert Longden
- Women's Trust Fund

Pictured is Stuart Pepper collecting a cheque from the Oldham branch of the Women's Trust Fund during their annual Strawberry Fair. During 2005 the group had raised £80 000 for the purchase of an ABI 7900 real time PCR system

Career Opportunities

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. New developments are in progress to create a Manchester Cancer Research Centre 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, 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 68). 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 compliment 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. Links to sources of potential funding for fellowships are provided on http://www.paterson.man.ac.uk/djs?jid=222.

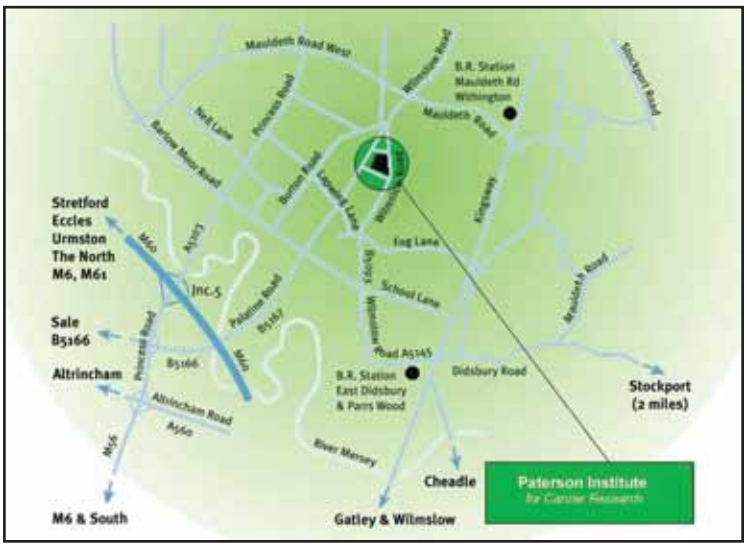
In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding 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/vacancies), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

The Manchester Wheel. *(Photograph courtesy of Adam Dangoor)*

How to Find 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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