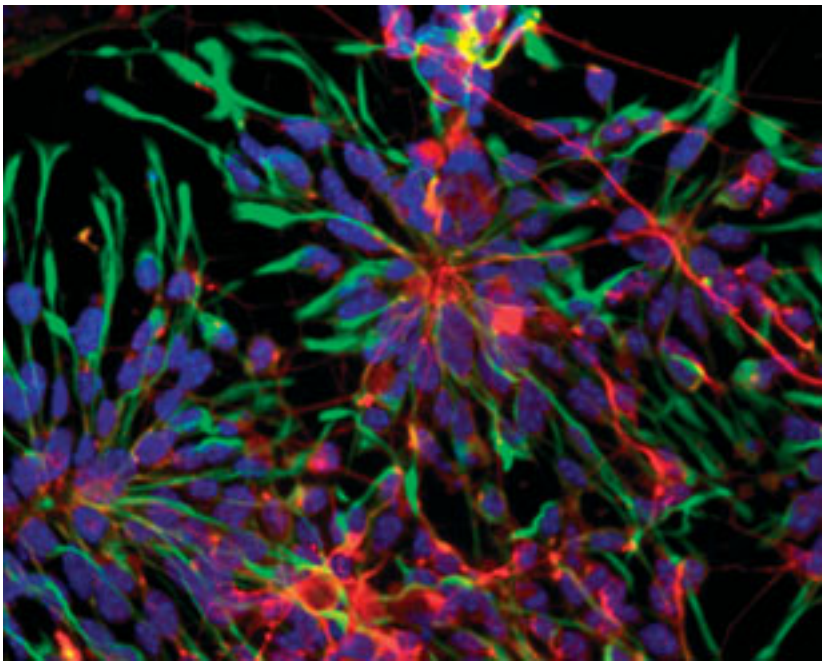
Paterson Institute for Cancer Research

SCIENTIFIC REPORT 2004

CANCER RESEARCH UK



Immunocytochemistry for neural cell markers in E14 ES cells at day 7 of neural differentiation in N2B27. Glial marker GFAP (red), neural marker nestin (green) and nuclei are marked using DAPI (blue).
Image supplied by Rebecca Baldwin, Medical Oncology

Illustration Credits

Many illustrations in this report were taken by Anne Murtagh of the Christie Hospital Medical Illustrations Department and by Jenny Varley.

Cancer Research UK

Paterson Institute for Cancer Research

SCIENTIFIC REPORT 2004

Cancer Research UK Paterson Institute for Cancer Research Scientific Report 2004

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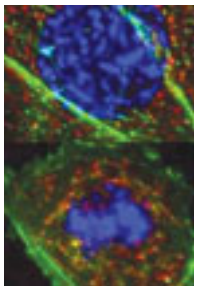
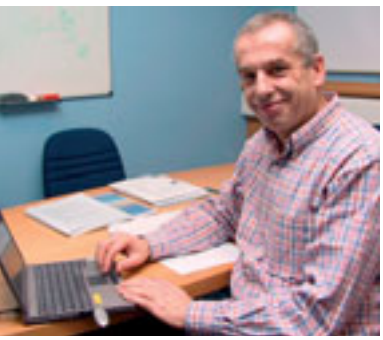


Director's Introduction

The big event of 2004 was the Paterson Review in July. The investment that Cancer Research UK makes to the Institute each year is very significant and clearly it is essential that mechanisms are in place to ensure that the research supported is of the highest quality. The main vehicle to ensure such quality is through the quinquennial review system where the research programme of individual groups is assessed by experts in the particular research field. The result of such assessment provides essential advice to the Director and to Cancer Research UK and influences the level of continuing support to the group. In addition to supporting world-class research groups, core-funded Institutes are also expected to provide added value in their research efforts through the development of a research strategy that maximises the stability and flexibility of core-research support. This added value could come from the development of focused research themes that promote interaction and synergy between different research programmes, from support to long-term and high-risk research as well as shorter to medium-term projects, from the provision of high quality research support that can benefit the research programmes in the Institute, but also in the wider research community, and from the provision of an optimal environment for the career development of young scientists. In addition, of particular relevance to the Paterson Institute, is the potential for fostering synergy and interactions between areas of basic and clinical research and developing the infrastructure and environment that promotes translational research. In order to assess the overall quality of the Institute, its research strategy, its future plans and the degree of added value that it provides, Cancer Research UK carries out an Institute review every five years through an international panel of scientific leaders who have experience in directing significant research centres. It is just such a review that took place in July.

The results of the review were very satisfying. The review party concluded that the Paterson was an internationally competitive Institute and fully endorsed the research strategy that had underpinned developments over the last five years and the strategic proposals for continuing development over the next five years. Such a resounding endorsement was crucially important to us since over the last few years we had progressed through a period of very significant change which was, at times, quite painful. However, we are now in a very strong position and will, over the coming years, continue to establish our strengths in key research areas and further develop meaningful and high quality translational research programmes. To help us reach our goals the review party recommended, and Cancer Research UK agreed, to increase our core support so that we can establish additional research groups and significantly increase the number of non-clinical and clinical research trainees.



Nic Jones, Director of the Paterson Institute

The review party also highlighted the enormous potential for integrated cancer research programmes in Manchester, particularly now that the new University of Manchester has been formed through the merger of the previous Victoria University of Manchester and UMIST. Closer interaction between the Paterson, the Christie Hospital and the University could lead to the development of common research goals and strategies. This would be of considerable benefit to further development of cancer research in Manchester and we look forward to strengthening these interactions over the coming year.

Angeliki Malliri joined the Institute as a new Junior Group Leader at the beginning of the year and has been busy building a research team to investigate the role of Rho-like GTPases in tumour initiation and progression. In particular, Angeliki's group is investigating how the Tiam1 protein, a

regulator of the Rho-like protein Rac1, promotes tumorigenesis. Angeliki's previous studies have shown that loss of Tiam1 reduces tumour formation in both a skin and intestinal tumorigenesis model. Elsewhere in the Institute, we were happy to welcome Colin Gleeson as our new Health and Safety Officer. Congratulations to Karim Labib who was an EMBO Young Investigator awardee and Elmar Schiebel who was awarded the British Society for Cell Biology Hooke Medal.

Work has now started on a major refurbishment of the north end of the Institute's laboratories. It is expected that the work will continue for all of 2005 and inevitably will be disruptive to the activities of the Institute. However, when complete it will provide much needed additional laboratory space that will house new Institute research groups, the relocation of the Clinical and Experimental Pharmacology group, and a new development in Molecular Pathology. In addition it will provide an upgrade to our seminar room and common room which are important facilities in ensuring that the Institute has an interactive and stimulating environment. We have continued to invest in our research services and during 2004 a mass spectrometry facility was established in the Institute that has state-of-the-art equipment to facilitate protein identification and the analysis of protein modifications. This facility is a joint venture with the Leukaemia Research Fund (LRF) and is embedded within the research activity of Tony Whetton, an LRF programme grant holder. It is running extremely smoothly and has already made a significant impact to research activities within the Institute. For example, in the Cell Division group the facility has been used to identify novel phosphorylation sites on a number of key mitotic regulators which has opened up new and exciting lines of research and new insights into the control of cell division. This success has reinforced our belief that the Institute needs first class services such as this, on site, to facilitate the work that we do. Our research services are available not only to the core-funded groups of the Institute, but also to other groups that occupy the Institute's laboratories. Therefore they benefit all research on site, a good example of the added value that a core-funded Institute such as the Paterson can provide.

In terms of research, significant advances in a number of areas were made during the year. Iain Hagan and Elmar Schiebel have provided new insights into the role of the spindle pole body in regulating mitosis and of microtubule binding proteins in spindle orientation. Caroline Dive continues to investigate factors that affect drug-induced apoptosis, a key event in drug therapy of cancer. They have identified the nature of the interplay between hypoxia and apoptosis which involves the down regulation of a key positive regulator of the apoptosis machinery. Lez Fairbairn and his group have shown that the inducible expression of the homeobox transcription factor HOXB4 can provide a major boost to the transduction of bone marrow cells and thus may significantly facilitate future gene therapy approaches. Peter Stern and colleagues have continued to investigate the potential for immunotherapy of 5T4, an oncofoetal antigen they discovered. During 2004 a 5T4-based vaccine clinical trial was initiated.

In summary, it has been a very significant year in the Paterson and the success of the Institute review means that we can look forward to a period of further development built on the progress we have made over the last five years. Manchester is an exciting and thriving city with a rapidly expanding academic and biotechnology research base. In addition, it has in the Christie NHS Trust one of the biggest specialist cancer hospitals in Europe and is at the heart of the biggest cancer research network in the country. All of these factors combined make the city ideally suited to the support of cancer research that encompasses basic through to clinical research. We look forward to contributing fully and being at the heart of further expansion of cancer research in the city.

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Bioinformatics Group

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

Bioinformatics is the application of Computer Science to the analysis of biological data. We are a research group that provides access to bioinformatics both within the Paterson Institute and beyond. Our own research focuses on developing novel techniques and software tools for microarray analysis, centred on the CR-UK Affymetrix system, housed at the Paterson Institute. We collaborate with bench scientists in the design and analysis of microarray experiments, and this work is providing the basis for much of our own research.

Microarrays measure the expression levels for many thousands of genes at once by determining, for each gene, how much mRNA is present in a sample. At one level, microarray experiments are easy – one simply extracts RNA, hybridises it to some chips and looks for the genes that change. In reality, the process of getting from a set of samples to a set of differentially expressed genes is a complex one that brings together biology, biochemistry, physics and statistics. Successful microarray analysis relies on understanding how each of these contributes to the data produced by an experiment. In addition, microarrays generate large amounts of data. Recent Affymetrix chips, for example, use ~500,000 features to probe for ~47,000 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

It is standard practice in microarray analysis to apply 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. Knowledge of the replicate structure of a microarray experiment is fundamental to its correct interpretation.

Searching or mining gene expression databases must also have access to this information, because replication introduces redundancy into the data that must be recognised and dealt with appropriately. We have been developing 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 (see Figure). 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 visualisation and gene-centred searches.

The majority of data analysis performed by our group uses BioConductor (www.bioconductor.org), a package of functions built with the statistical programming language R. We contribute code to BioConductor and have also been developing our own package, 'simpleaffy', which implements a variety of analysis algorithms for Affymetrix data, including Quality Control, signal detection, expression level generation, a set of graph plotting and visualisation functions, tools for fold-change comparisons and statistical tests, some of which have been re-implemented in C for speed.

The complex relationship between genes, probes and transcripts

Affymetrix microarrays record the presence of a transcript in solution by measuring the level of hybridisation 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. Hybridisation conditions are controlled with the aim of maximising the binding between a transcript and its PM probes, whilst minimising 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 hybridisation. One advantage of this approach is that the combination of short oligos and strict hybridisation 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. (*e.g.* 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.

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. Much of this work was done by Hui Sun Leong, an MSc project student who worked with us over the summer. At the time of writing, ADAPT stores data for ~250,000 probesets, mapping to ~178,000 different sequences.

The effects of experimental process on microarray data

A significant issue associated with microarray databases is a desire to combine the results from different experiments into larger *in silico* studies. Fundamental to this is the need to develop an understanding of how changes in experimental protocol can affect the data, and when these differences are large enough to thwart this kind of analysis. It also has a significant impact on what information it is necessary to capture within a database in order to allow such decisions to be taken. We collaborate closely with the Molecular Biology Core Facility (page 44) through an ongoing series of experiments designed to evaluate new protocols, to gain a better understanding of how they are likely to influence experimental data, and to use this information to develop analysis techniques that work effectively across heterogeneous data sets.

Screenshot of some of the software developed by the Paterson Institute Bioinformatics group. For more details see our website at http://bioinformatics.picr.man.ac.uk.

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The group's objectives are to establish the role of DNA damage and repair in the biological effects of specific genotoxic agents and to exploit these in the treatment and prevention of cancer. Our focus is on certain types of alkylating agents and ionising radiation which are used extensively in cancer therapy because of their cytotoxic effects. Current projects within the group are in the areas of therapeutic response modification and gene targeting. The former has resulted in the development the O^6-alkylguanine-DNA alkyltransferase (ATase) inactivating agent, PaTrin-2 and we previously reported that this drug has entered clinical trials.

Therapeutic response modification

Alkylating agents of various types are used in the treatment of many cancer patients. Their effectiveness is limited by adverse toxic side effects in normal tissues, which defines the maximum tolerated doses, and by inherent or treatment-induced tumour resistance. Until improved therapeutic agents that circumvent these problems are developed, the only option for better patient management is to establish the mechanisms of tumour resistance and normal tissue sensitivity of currently used agents and then to devise strategies to improve in their effectiveness. Understanding the mechanisms of action of these agents has come from studies of their DNA damaging, carcinogenic and toxic properties.

Chemotherapeutic alkylating agents generate a large number of 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 the CR-UK drug, Temozolomide produce O^6-methylguanine in DNA and this kills cells *via* the action of the post replication mismatch repair (MMR) system.

Repair pathways, which may have evolved to deal with low levels of damage, reduce the efficacy of such agents and there is increasing interest in developing strategies to attenuate the expression of such pathways in order to improve their efficacy. These, and especially that repairing damage at the O^6-position of guanine, *i.e.* O^6-alkylguanine-DNA alkyltransferase (ATase; also known as MGMT), but also the base excision repair (BER) system that processes 3- and 7-alkylpurines in DNA, have become targets for modulation in order both to enhance tumour response and to increase normal tissue resistance. 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) The 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 O^6-alkyalting agents is the use of pseudosubstrates that ablate ATase activity but are themselves non-toxic. In collaboration Prof Brian McMurry and Dr Stanley McElhinney (Chemistry Department, Trinity College, Dublin), we have developed PaTrin-2. This highly potent inactivator of ATase, which has been licensed by Cancer Research Technology to KuDOS Pharmaceuticals, has completed Phase I clinical trials that were carried out here at Christie Hospital and at University College, London. Under the auspices of KuDOS PaTrin-2 is now in Phase II trials and the results are awaited with interest.

One of the trials is addressing the effectiveness of PaTrin-2 in inactivating ATase in a number of tumour types in order to establish if the doses required for complete inactivation of ATase are different. This will lead on to Phase II studies in such patients. This has required us to develop and

validate to Good Clinical Laboratory Practice standards, a quantitative assay for total ATase protein.

We previously reported, in collaboration with Jim Heighway (Roy Castle International Centre for Lung Cancer Research) and Mauro Santibanez-Koref (Institute of Human Genetics, Newcastle), that the two alleles of ATase can be expressed at highly significantly different levels in the normal lung tissue of different lung cancer patients. This has been confirmed in a separate study and again suggests that there is indeed a genetic component of interindividual ATase expression levels and that at least some of this variation maps close to or within the ATase locus.

The possibility that there are polymorphisms in the human gene that can be used to predict the basal levels of expression of ATase is currently being examined.

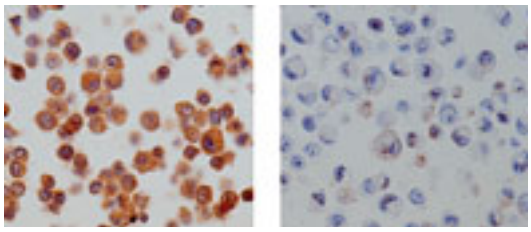
Preclinical studies

As mentioned above, MMR is essential for the toxic effects of O^6-methylguanine to be manifested and it is know that some colorectal, and less frequently, other types of cancers do not express MMR proteins because the gene is silenced by promoter methylation. Decitabine is an agent that reverses promoter methylation and in an EU-funded project, together with Prof Bob Brown of the CR-UK Beatson Institute, we have already shown in MMR down-regulated human ovarian cancer cells that combinations of Decitabine and PaTrin-2 can reverse both MMR and ATase-mediated resistance to Temozolomide. Depending on the outcome of Phase I clinical trials of Decitabine being carried out elsewhere, combinations of this with PaTrin-2 might be proposed for clinical studies. More recently we have shown that the toxic effects of Temozolomide are increased by inhibiting the BER pathway by means of an inhibitor of PARP (3-aminobenzamide, 3-AB) or by using an agent that prevents the processing of AP sites (methoxyamine. MX). Combinations of 3-AB and MX have no additive effects, but either or both agents combined with PaTrin-2 cause a substantial increase in cell killing. Such combinations are thus feasible propositions for clinical trials.

The expression of the anti-apoptosis protein Bcl-2 is frequently upregulated in tumours. In collaboration with Genta, we have now shown that, using an antisense RNA oligonucleotide (oblimersen) downregulation of Bcl-2 in a human ovarian tumour cell line increases its sensitivity to Temozolomide. In addition, oblimersen in combination with PaTrin-2 substantially increases the overall toxic effect of Temozolomide. Again, clinical trials of such combinations are warranted.

Gene targeting

Work has continued on the characterisation of murine models lacking specific DNA glycosylases, base excision repair (BER) enzymes involved principally in the release of chemically modified bases from DNA. Initial studies showed that BER of oxidised pyrimidines still occurs in the absence of mNTH1 and subsequent biochemical studies indicated that this residual activity is primarily due to NEIL1. In collaboration with Dr Will Bohr's group (NIH, Baltimore, USA) we have shown that mouse liver mitochondrial extracts lacking both NTH1 and OGG1 are also competent for the incision of oligonucleotides containing certain oxidatively damaged bases. In addition, work in collaboration with Dr Murat Saparbaev (Villejuif, France) has indicated that the major AP-endonuclease in mammalian cells (Ape1) can nick DNA 5´ to certain oxidised bases, providing an alternative repair pathway, termed nucleotide incision repair. The role of the compensatory DNA repair activities in protecting the genome from genotoxins is continuing using RNA interference in combination with insertional gene knockouts.



Downregulation of Bcl-2 using antisense oligoribonucleotides. Photomicrographs of anti-Bcl-2 antibody stained human ovarian cancer cells (A2780) before oblimersen (A) and after 5 days exposure to 33 nM oblimersen (B).

In collaboration with Dr Alain Barbin, (IARC, Lyon, France), we have measured the levels of 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine following treatment with vinyl carbamate (Vcar). As expected, there were higher levels and increased persistence of εA in hepatic DNA from mice lacking alkylpurine-DNA-*N*-glycosylase (APNG) than from wild-type animals. However, no increased susceptibility of APNG-/- mice to hepatocarcinogenesis was observed one year after Vcar treatment. Likewise, rates of cell proliferation and apoptosis were similar in both wild-type and APNG-/- strains immediately after Vcar treatment. Thus, although previous reports suggest a critical role for εA in Vcar induced carcinogenesis, under the conditions of extensive liver damage observed in this study, the differences in εA levels were not directly associated with a higher susceptibility of APNG-/- mice to hepatocarcinogenesis.

Publications listed on page 46

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Cell Division Group

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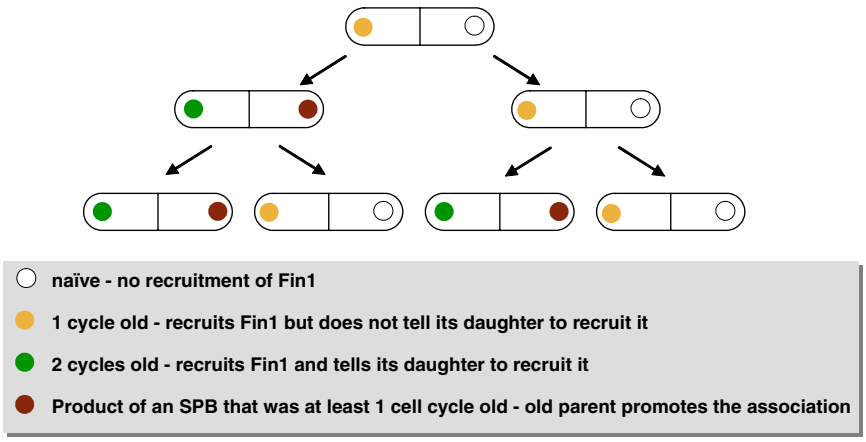
Errors in chromosome transmission alter the balance of tumour suppressor and tumour promoter genes. This imbalance favours changes in genome composition in the ensuing cell divisions that can lead to cancer. Chromosome segregation during mitosis is initiated by the attachment of the microtubules of the 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

Figure 1



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.

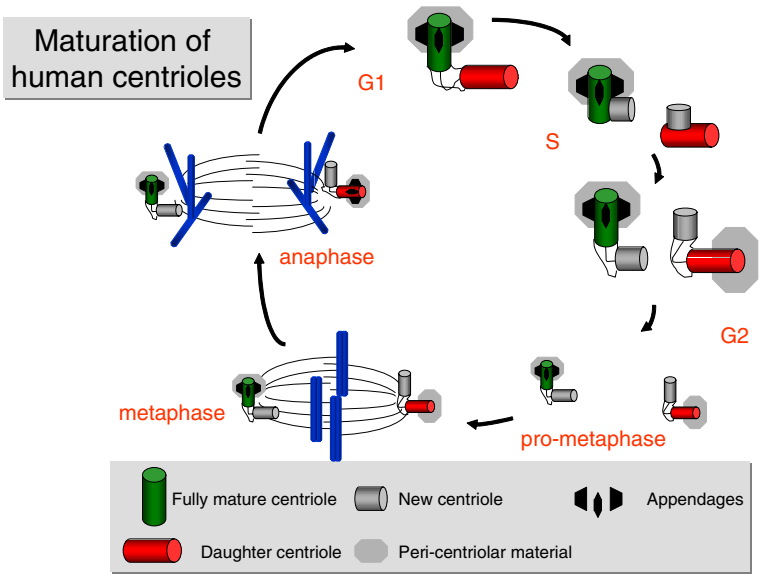
Recruitment of Fin1 kinase shows that the SPB takes two cell cycles to mature

Because we have previously shown that the fission yeast NIMA-related kinase Fin1 is involved in the MPF regulating Cut12/Polo feedback loop we were keen to learn more about this important cell cycle regulator. We were not surprised to find that Fin1 associated with the SPB, but were very surprised to see that in late anaphase cells (the stage in which the two genomes are pulled to opposite ends of the cell) Fin1 was found on both SPBs in one half of the population and on only one in the other half. Yeast spindle pole bodies divide by a conservative mechanism – a new one forms next to the old. As this seemed like a simple mechanism to differentiate between two SPBs, we asked whether the affinity of Fin1 for an SPB was related to its age. We exploited a trick developed by our colleagues in the Schiebel lab in which the slow folding properties of red fluorescent protein can be harnessed to specifically label the old SPBs. This showed that whenever Fin1 was asymmetric it was always on the old SPB and that an SPB could only specify that its daughter should recruit Fin1 (Fin1 on both late anaphase SPBs) after it had passed through at least two cell cycles. This inheritance pattern is strikingly reminiscent of the maturation of the centrioles in human centrosomes. Centrioles pass through one and a half cell cycles before they acquire the appendages that are characteristic of a mature centriole (see Figure 2). As centrosome amplification occurs in every cancer and cell cycle progression is controlled from the centrosome we are hopeful that understanding SPB maturation in yeast may lead to ways to restore centriole maturation to the tumours that have suppressed it to de-regulate growth. Such restoration of centrosome maturation would be expected to restore normal growth control.

Fin1 controls mitotic exit as well as entry

We found that the association of Fin1 with the SPB depended upon the Septum Initiation Network (SIN). This conserved regulatory network promotes cytokinesis. If SIN activity is absent cytokinesis is blocked, if it is hyperactive, multiple rounds of cytokinesis are induced. The G protein that is central to SIN function, Spg1, is anchored to the SPB via the scaffold proteins Cdc11 and Sid4. Active Spg1 recruits a protein kinase called Cdc7 that then recruits two more protein kinases to the SPB and septation is triggered. Spg1 activity is controlled by the Cdc16/Byr4 bipartite GAP protein complex that represses SIN activity on the old SPB of late anaphase cells. Fin1 associated with Byr4 and was required to repress the SIN on the old SPB in half of the cells in the culture. Fin1 is part of a SIN negative feedback loop because the recruitment of Fin1 to the SPB is dependent upon the positive effectors of the SIN as well as the repressors and the scaffold molecules. Activation of the SIN promotes Fin1 recruitment which then keeps the SIN shut down on the old SPB. The challenge now is to work out why this only happens on the old SPB and how the SIN is shut off in the half of the population in which the SIN remains repressed on the old SPB of *fin1* null cells.

Figure 2



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.

Publications listed on page 47

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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 and differentiation as well as apoptosis. On the organismal level AP-1 plays important roles in tissue stress responses such as inflammation and ischaemia, and is implicated in the onset and progression of tumours. The factor and its regulation are 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 stress-induced mitogen-activated protein (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.

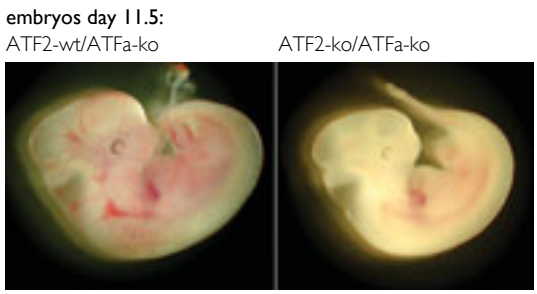

Figure 1: ATF2/ATFa double knockout embryos arrest development with severe defects in foetal heart and liver

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 c-Jun. ATF-2 is activated by the p38 or JNK kinases through phosphorylation of two N-terminal threonine residues T69 and T71. ATF-2 has been implicated in stress-induced cellular processes such as the activation of DNA repair genes in response to DNA damage, the activation of immune response genes after induction by inflammatory cytokines as well as the induction of pro-apoptotic mechanisms in response to cytotoxic drugs.

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 series of genetically modified mice expressing mutant ATF-2 proteins. Genetic inactivation of ATF-2 results in death shortly after birth due to a respiratory defect. A similar phenotype was observed in mice containing an ATF-2 allele where the phosphor-acceptor threonine residues (T69 and T71) are altered to alanines. An additional role for ATF-2 in development was revealed by the genetic

inactivation of ATF-2 together with ATFa, another member of the ATF family that is highly conserved. These double mutant mice die during mid-gestation with severe anaemia and significant disorganisation and hypotrophy of embryonic liver and heart. In the former case, massive apoptosis of foetal liver cells is observed. Cells derived from these double mutant mice are currently being characterised in more detail. Interestingly, while wild type and double knockout cells behave similarly during exponential growth, the knockouts have the ability to grow to significantly higher cell densities compared to wild types. Thus ATF-2 may have a role in cell-contact-dependent growth arrest and may explain the observation that oncogenic transformation of knock-out cells results in significantly increased tumour sizes in xenotransplant experiments compared to wild type cells.

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. The phenotypes of these mutant mice are currently under investigation. For example, inactivation of ATF-2 in endothelial cells using a tie-1-Cre line results in mice that are born at the expected rate but soon after develop a number of phenotypic abnormalities and as a result the majority of these animals die within two weeks of birth. The most striking defect is in the gut where extensive damage is evident resembling the 'GI syndrome' associated with radiation treatment. Increased apoptosis of endothelial and epithelial cells is observed possibly as a result of sensitisation to inflammatory signals that arise during colonisation of the gastrointestinal tract following birth. Mice containing specific inactivation of ATF-2 in a variety of other cell types are currently being investigated.

Stress response in fission yeast

We are using fission yeast as a model system for studying stress. The factors Atf1 and Pap1 coordinate most of the changes in gene expression following stress and we are currently trying to understand how these factors are regulated, what target genes they control and how they are differentially mobilised depending upon the nature and magnitude of the stress signal.

In collaboration with Jurg Bahler at the Sanger Centre in Cambridge, we have carried out comprehensive global microarray analysis of the transcriptional responses to a variety of different stress conditions. This analysis provides a comprehensive overview of cellular responses to environmental stress and mechanistic insights into how the cell integrates information concerning the state of the environment and as a result orchestrates the expression of the appropriate set of genes. Current studies focus on the regulation of the Atf1, Pap1 and other factors involved in such orchestration, their interaction with other proteins and protein complexes and the nature of the complexes that bind to promoter elements before and after stress conditions.

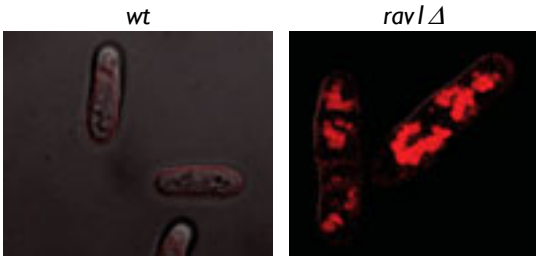

Figure 2: Doxorubicin uptake in wild-type and drug sensitive fission yeast cells (*rav1Δ*)

Characterisation of drug resistant mechanisms

Multidrug resistance is the main mechanism by which many cancers demonstrate resistance to chemotherapy drugs and is a major factor in failure of chemotherapy treatment. Significant progress has been made in understanding the molecular basis of some forms of drug resistance; many different mechanisms exist with most being poorly understood or not yet identified. In mammalian cells stress-activated protein kinase pathways are activated by treatment with a number of cancer chemotherapeutic drugs including cisplatin, adriamycin and etoposide. Moreover, numerous multidrug resistant cell lines have been reported to show increased JNK activation. We are using fission yeast as a model system to examine the role of stress-activated pathways in drug resistance and to identify new resistance mechanisms.

Using a genetic screen for multidrug sensitivity, novel genes have been identified that play a role in the innate resistance of fission yeast to a range of chemotherapeutic drugs. Characterisation of these genes has identified novel drug-resistance pathways. One gene encodes a ser/thr kinase that regulates the activity of a membrane-associated potassium transporter that plays a key role in ion homeostasis and membrane potential. A second gene encodes a regulator of vacuolar ATPase, a highly conserved enzyme complex found in all eukaryotes and associated with a number of important biological functions including vacuolar transport. How these activities give rise to drug resistance and the potential importance of similar pathways in human cells is under investigation.

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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 focuses 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. 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. Tiam1-deficient mice 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-dimethylbenzanthracene, 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. Ras has been shown to recruit Tiam1 and subsequently activate Rac in two ways: through a phosphatidylinositol 3-kinase-dependent mechanism; and through direct binding of active GTP-bound Ras to a Ras-binding domain located within the Tiam1 protein (Lambert *et al.,* Nature Cell Biol 2002; 4: 621).

Tiam1/Rac and canonical Wnt-signalling

Tiam1 is also a potent modifier of intestinal tumorigenesis. Min (multiple intestinal neoplasia) mice, which are predisposed to the formation of intestinal tumours due to a mutation in the *apc* tumour suppressor gene, develop far fewer tumours in the absence of Tiam1 (Malliri *et al.*, submitted). Tumour growth is also reduced. The Apc protein is a core component of the canonical Wnt signalling pathway. *Tiam1* appears to be, itself, a target of this pathway. It is expressed in the proliferative compartments (crypts) of the mouse adult intestine where the Wnt pathway is normally active. Further, Tiam1 is over-expressed in adenomatous polyps in Min mice where the Wnt pathway is hyperactivated. Significantly, Tiam1 is also up-regulated in adenomas from patients with either sporadic colorectal polyps or familial adenomatous polyposis (FAP). Colon cancer cells over-expressing an inhibitor of Wnt signalling rapidly lose *Tiam1* expression. Conversely, over-expression of activators of the pathway in non-transformed intestinal epithelial cells induces Tiam1 expression.

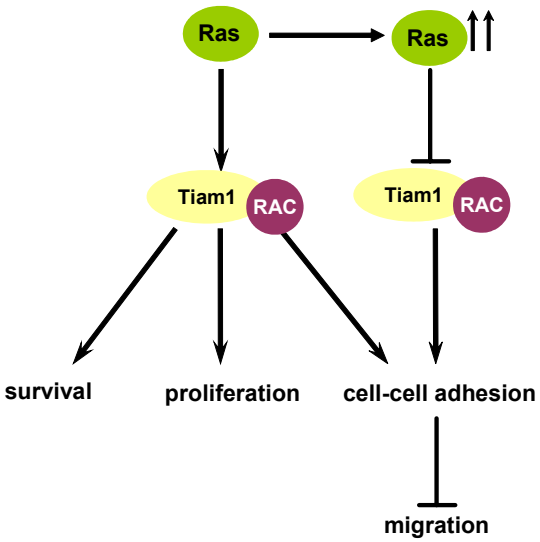
Thus, these two studies on tumorigenesis *in vivo* demonstrate that two independent oncogenic signalling pathways of major clinical significance (Ras and Wnt) recruit the Tiam1-Rac signalling pathway by specific, albeit distinct mechanisms. In the context of oncogenesis, activation of this signalling module promotes tumour initiation and growth.

Tiam1/Rac and malignant progression

The skin carcinogenesis model revealed an additional role for Tiam1 in tumorigenesis. 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. 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 antagonise Ras during tumour invasion (Figure).

One probable mechanism by which Tiam1/Rac antagonises malignant progression is through their positive effect on cell-cell adhesion. *In vitro* studies have shown that overexpression 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).

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 Rac GEFs. Clearly, 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. Our lab is using a combination of *in vitro* and *in vivo* approaches to identify signalling events downstream of the Tiam1/Rac module that can influence tumour susceptibility.



Interactions of Ras and Rac pathways in tumorigenesis based on chemical skin carcinogenesis of Tiam1 mutant mice. Tiam1-deficient mice develop fewer and smaller Ras-induced skin tumours than wild-type mice. This is related to the positive effects of Tiam1/Rac signalling on cell survival and proliferation. However, Tiam1-deficient tumours progressed more frequently to malignancy. This can be explained by *in vitro* and *in vivo* results showing that high levels of Ras signalling (Ras↑↑), as seen in advanced skin malignancies, down-regulates Tiam1 expression and thereby Rac activity which is required for cell-cell adhesion.

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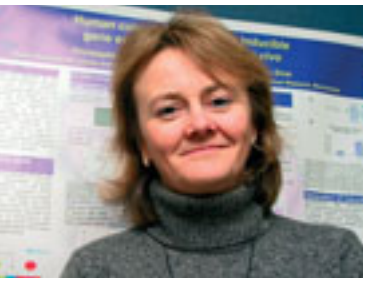

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With a focus on colorectal cancer (CRC), CMP study the impact of tumour hypoxia and the activity of non-receptor tyrosine kinase c-Src on tumour growth and drug-induced apoptosis. Our findings in 2004 include the discovery that Hypoxia Inducible factor 1 represses the transcription of the potent pro-apoptotic protein Bid *in vitro* and *in vivo*; that kinase dead mutants of c-Src sensitise CRC cells to oxaliplatin-induced apoptosis via a caspase-8 dependent pathway; and, using a unique single step system for inducible gene expression, that activated c-Src promotes delayed transit through G_2/M phases of the cell cycle.

Colon cancer and c-Src

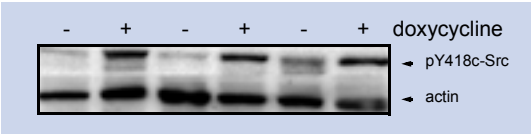
Almost 50% of the 35,000 patients diagnosed with colorectal cancer (CRC) each year in the UK will develop metastases and die of their disease. Without treatment, the median survival of patients with advanced CRC is 6 months, with few surviving beyond one year. For patients with advanced disease, 5-Fluorouracil (5FU) has been the mainstay of treatment for 40 years and its use increases median survival to 12 months. The development of new agents active in the treatment of advanced CRC has recently altered the management of this disease. Combinations of irinotecan and oxaliplatin with 5FU prolong survival and improve quality of life, whilst new oral fluoropyrimidines such as capecitabine, offer convenient alternatives to intravenous treatments. However, even after such treatments with newer agents such as Irinotecan and oxaliplatin, the median survival is in the order of 18 months and there is still a pressing need for improved therapies. The levels and/or activity of c-Src are 5-40 fold elevated in more than 70% human colon cancers relative to normal tissues. We are investigating the role of activated c-Src in CRC with respect to its impact on tumour growth and drug responsiveness by manipulating c-Src activity in a panel of human colon cancer cell lines grown as monolayers and as tumour xenografts.

To avoid the problems resulting from unspecific compensatory mechanisms associated with constitutive over-expression of signalling molecules we concentrated on the studies utilising doxycycline regulated gene expression. Based on the recently described two-step approach (Welman *et al.*, J Cell Biochem, epub Jan 24 2005), we have further developed a powerful single-step methodology (the super module vector system; SMV) designed for rapid generation of human cancer cell lines inducibly expressing a gene of interest. We applied this system to construct multiple human colon cancer cell lines inducibly over-expressing c-Src and diverse c-Src mutants. Following optimisation of doxycycline delivery to nude mice, c-Src mutants can now be robustly induced in CRC xenografts (Figure).

In vitro, induced expression of activated c-Src (Y527F) in HCT116 cells resulted in the phosphorylation of MAPK, PKB and STAT3 with minimal apoptosis observed. In addition, and counter to reports that activated c-Src promotes cell proliferation, there was pronounced delay in the G_2/M phase of the cell cycle. These events were prevented by treatment of induced cells with the Src inhibitor PP2. The acute cell cycle delay was overcome during prolonged c-Src activation but at no stage did increased c-Src activity hasten cell proliferation. The acute negative effects of activation of c-Src on CRC growth may go some way to explain lack of

activating mutations in c-Src in the vast majority of clinical CRC. Studies on c-Src activation in xenografts are under way together with its impact on drug responses *in vivo*. The molecular events that delay G_2/M transit are under investigation and may have ramifications regarding the combination of small molecule inhibitors of c-Src with conventional DNA damaging anticancer drugs.

With the arrival of two new post-doctoral fellows in 2004, the SMV system is now being exploited to investigate *in vitro* and *in vivo* the signalling crosstalk between c-Src and PI-3K, c-Src dependent STAT3 mediated regulation of anti-apoptotic Bcl-X_L, and the similarities and differences between c-Src and c-Yes signalling in CRC. Our overall objective is to dissect out pro- and anti-apoptotic functions of c-Src in CRC in order to optimise the use of c-Src inhibitors in the clinic.



Induction of activated c-Src in human colorectal cancer xenograft tumours. Lysates from HCT116 xenografts harvested without or after doxycycline treatment were blotted for activated c-Src and actin, and a clear doxycycline induction of activate c-Src is shown.

Tumour hypoxia and apoptosis

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 CRC? 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 (Erler *et al.*, Mol Cell Biol 2004; 24: 2875) 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 via induction of HIF-1 RNAi or dominant negative HIF-1 in human CRC xenografts.

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 Clinical Oncology, Christie Hospital NHS Trust).

Drug resistance in neuroblastoma

Neuroblastoma is the commonest extracranial solid tumour of childhood. These tumours often display adverse biological features and are highly resistant to current chemotherapy. Guy Makin and members of CMP are studying two elements of drug-resistance in these tumours; the contribution of hypoxia, and the mechanism of full activation of the pro-apoptotic protein Bax.

In colon carcinoma cells 16 hours of oxygen deprivation reduced etoposide-induced apoptosis and HIF-1-dependent down-regulation of Bid contributed to this drug resistance (Erler *et al.*, Mol Cell Biol 2004; 24: 2875 and see above). Areas of hypoxia are detectable in neuroblastomas. Neuroblastoma cell lines rapidly stabilise transcriptionally active HIF-1 after exposure to hypoxia. However, unlike colon cells, only prolonged hypoxia was able to protect against apoptosis induced by vincristine and etoposide. Prolonged hypoxia had no protective effect in p53 null cell lines. In neuroblastoma cells the mechanism of hypoxia-induced drug resistance does not seem to involve Bcl-2 family proteins, and may instead involve a novel p53 function.

We have previously shown that sequential steps in the activation of Bax occur regardless of cell fate. Since either Bak or Bax is essential for the release of apoptogenic factors from mitochondria, we have been investigating the roles of other Bcl-2 family proteins in this process. Bcl-X_L was unchanged both in level and in localisation and although Bcl-2 was up-regulated it did not change location or its phosphorylation status. Bim was up-regulated and translocated to mitochondria and Bid was also up-regulated, but did not relocalise. Bak levels were unchanged, but unusually, it relocated from cytosol to mitochondria. However, none of these changes predicted eventual cell fate. Recombinant Bid or Bim were able to release cytochrome c, Smac, and Omi from mitochondria from neuroblastoma cells, which have Bax but not Bak at their outer mitochondrial membrane, suggesting that given appropriate upstream signals, Bax can be fully functional. We are currently attempting to define these upstream signals.

Collaborators: Dr Eddy Estlin, Royal Manchester Children's Hospital. Funding from BBSRC and The Friends of Rosie.

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CEP develops, validates and implements pharmacokinetic (PK) and pharmacodynamic (PD) assays for Phase I clinical trials. The group is directly linked with the clinical research facilities of the Derek Crowther Trials Unit (DCU) at the Christie Hospital headed by Dr Malcolm Ranson. This year we conducted PK and PD analyses during ongoing, Cancer Research UK sponsored Phase I trials of the bioreductively activated alkylating agent RH-1 and of an antisense oligonucleotide to the inhibitor of apoptosis protein XIAP. PD assay development for anti-angiogenic and anti-vascular drugs became a focus with trials anticipated in 2005. DCU/CEP received the Christie Hospital Chief Executive's Award for 2004.

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 2003/2004, the DCU had 80 trials on its database involving 684 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 practise (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 progressed the development of its quality assurance (QA) system based on the

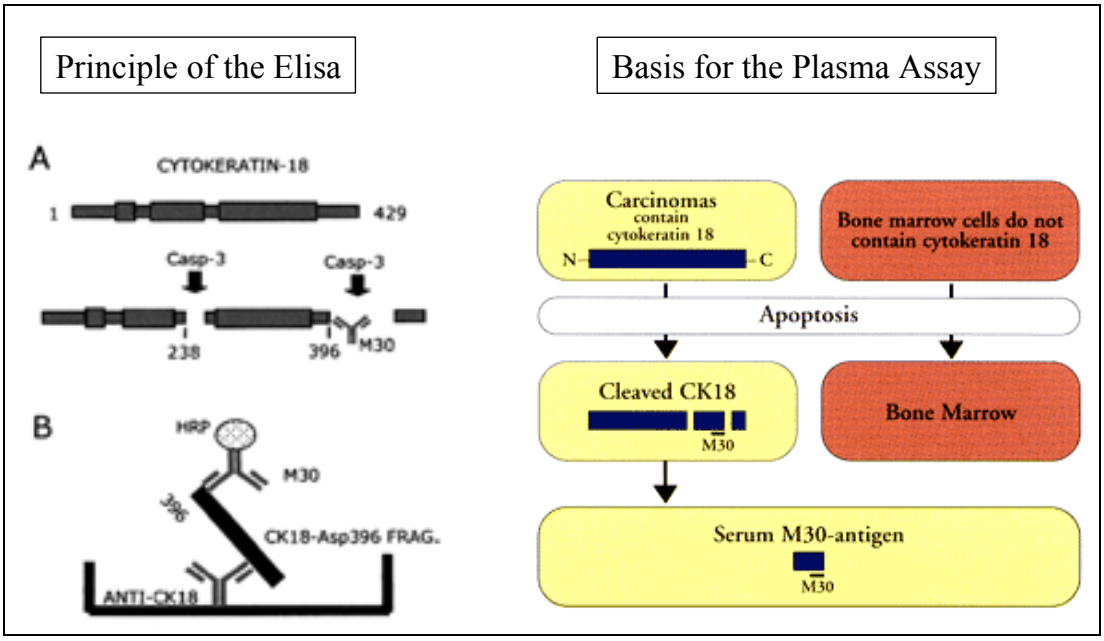

Figure 1:
The M30-Apoptosense™ Plasma Elisa Assay – a putative surrogate marker of tumour cell apoptosis based on the cleavage and shedding of cytokeratin 18 from apoptotic tumour cells, used by CEP in several ongoing clinical trials
(Figure reproduced with permission from PEVIVA AB, Bromma, Sweden).

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.

During the last year, CEP has put 11 projects through the QA system, based on Phase I clinical trials of RH-1 and XIAP antisense (see below) with an emphasis on the validation of PK and PD methods (Cummings *et al.*, Br J Cancer 2005; 92: 532). Eight new PK/PD methods were successfully validated to CR-UK and international quality standards (details on these methods are also available at the CEP QA web pages). All patient sample tracking and analysis are performed under full document and studies are now subject to QA auditing. With the move to a new suite of custom designed GLP compliant laboratories in the Translational Research Facility in 2005, this will further enable the group to achieve full compliance.

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

The inhibitor of apoptosis protein, XIAP is frequently over expressed in chemo-resistant tumours. Over the past year, CEP together with DCU has commenced evaluation of the PD effects of AEG 35156 during an ongoing Phase I trial. These studies were conducted in conjunction with the Canadian biotechnology company Aegera Therapeutics Inc. Method validation was completed on three PD assays (Cummings *et al.,* Br J Cancer 2005; 92: 532). Quantitative RT-PCR (Taqman) was confirmed to be specific for XIAP and assay linearity extended over four orders of magnitude. MDA-MB-231/U6-E1 cells and clone X-G4 stably expressing an RNAi vector against XIAP served as high and low XIAP expression quality controls (QCs). Within-day and between-day coefficients of variation (CVs) in precision for cycle threshold (CT) and delta CT values were always less than 10%. Western blotting was validated using a GST-XIAP fusion protein standard and HeLa cells and SF268 cells as high and low XIAP expression QCs. Specificity was demonstrated with a panel of cell lines including clone X-G4. The assay was linear over a 29-fold range of protein concentration and yielded acceptable precision. XIAP protein was also shown to be stable at -80°C for more than 60 days. M30-ApoptosenseTM plasma Elisa (Figure 1) detects a caspase-cleaved fragment of cytokeratin 18 in peripheral blood, believed to be a surrogate marker for tumour cell apoptosis. Generation of an independent QC was achieved through the treatment of cells with the apoptosis inducing agent staurosporine and collection of media. Measurements on assay precision and kit-to-kit QC were always less than 10%. The M30 antigen (CK18-Asp396) was stable for 3 months at -80°C while at 37°C it had a half life of 80–100 hr in healthy volunteer plasma. To-date these assays have been applied to the analysis of plasma and PBMCs collected from 10 subjects, including 7 accrued in Manchester.

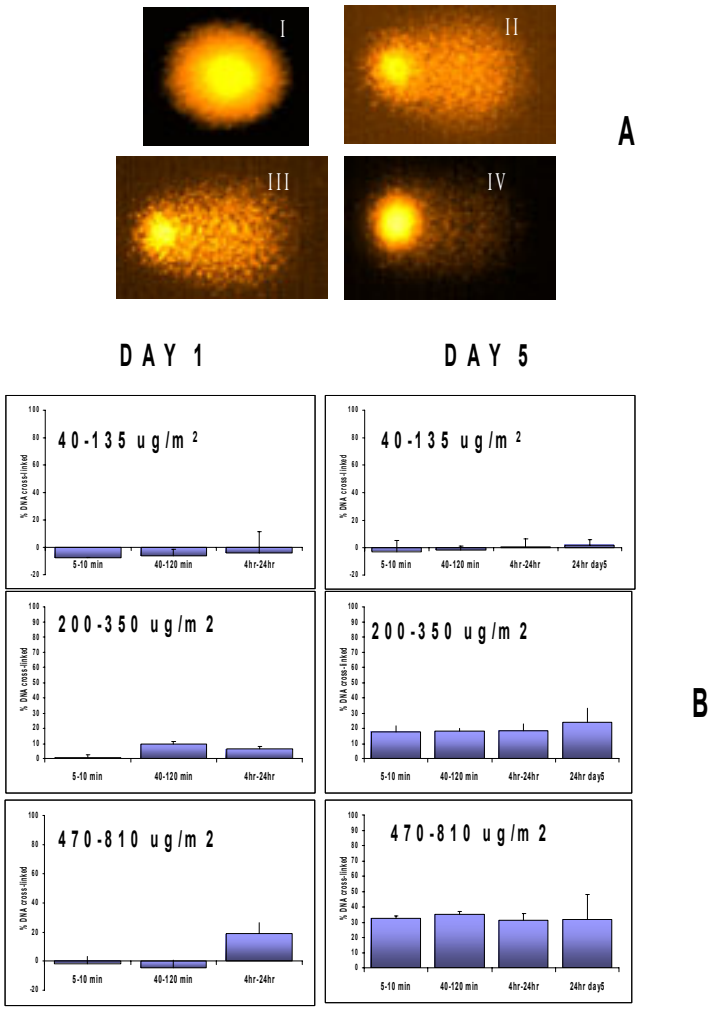

Figure 2:
A. Representative images of PBMC QC standards subjected to the Comet-X assay. (i) Control, (ii) Irradiated no drug, (iii) Irradiated+ low dose RH-1, (iv). Irradiated +high dose RH1. Cross-linking by RH1 reduces the extent of the radiation induced comet "tail".
B. DNA cross-linking observed in patients 1-9 following RH-1 treatment.

Publications listed on page 48

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Our group has used a novel form of "functional proteomics" to identify a number of new cell-cycle proteins. Four of these proteins are required for chromosome replication and, remarkably, they interact with each other to form a complex called "GINS". We have purified GINS from budding yeast cell extracts and found that it interacts both with the MCM helicase that is responsible for unwinding the DNA duplex at forks, and also with a number of proteins that regulate the function and progression of DNA replication forks. Understanding the structure and regulation of DNA replication forks has now become the major interest of our group. We are also studying the function of another protein found in our proteomics screen that is essential for cytokinesis.

The role of the GINS complex at DNA replication forks

Most eukaryotic cells make a single, near-perfect copy of their chromosomes during each cell-cycle. Chromosome replication is initiated from many origins on each chromosome, leading to the establishment of many DNA replication forks that move through the chromosome until they meet a fork from a neighbouring origin. Many other processes are co-ordinated with DNA synthesis at DNA replication forks, such as chromatin assembly and the establishment of cohesion between the newly formed sister-chromatids. In addition, cells monitor carefully the progression of DNA replication forks, and activate "checkpoint" mechanisms in response to problems in DNA synthesis, in order to somehow maintain the integrity of stalled DNA replication forks, and thereby preserve genomic stability. Recent work has suggested that individual forks carry with them proteins that can inhibit the progression of DNA replication forks in response to problems in DNA synthesis. The mode of action of such proteins is presently unclear, but they appear to interact with the MCM helicase that is responsible for unwinding the DNA duplex at the fork. Inhibiting the progression of the fork in response to problems in DNA synthesis may help to reduce the likelihood of generating mutations, and may also be important to prevent breakage of the fork and the generation of chromosomal translocations.

We have used chromatin immunoprecipitation (ChIP) experiments to show that the GINS complex is a novel component of DNA replication forks. By purifying GINS from yeast cell extracts, and then identifying associated proteins by mass spectrometry, we have found that GINS interacts with the MCM helicase during the process of chromosome replication. GINS also interacts with the above mentioned regulators of fork progression, and is essential for these proteins to associate with DNA replication forks. We are now using a variety of biochemical and genetic approaches to study the role of GINS in the establishment and regulation of eukaryotic DNA replication forks.

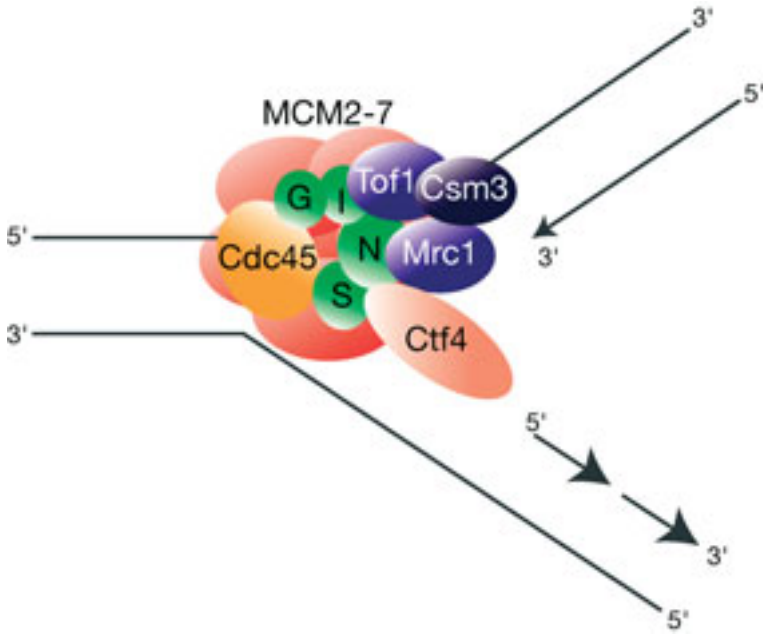
Studying stalled DNA replication forks at specific chromosomal loci

DNA replication forks move away from origins at an average speed of 3kb/minute. One challenge in studying forks, therefore, is knowing where to find them! The progression of forks can be arrested temporarily during chromosome replication either at naturally occurring "pause sites" in the chromosome, or else by the presence of certain kinds of DNA damage. The regulation of such stalled forks is poorly understood, though it's clear that some stalled forks activate checkpoint mechanisms that help stabilise the fork until the reason for the block has been dealt with, as well as preventing

unwanted events such as premature entry into mitosis. We have developed yeast strains in which we can stall individual DNA replication forks at specific loci, allowing us to study the regulation and structure of stalled forks in greater detail. We have exploited DNA sequences from the ribosomal RNA genes on chromosome 12, which act as "Replication Fork Barriers" (RFBs). These RFBs are bound by a protein, Fob1, that is essential for the RFB to stall the progression of replication forks, but is, however, not essential for cell viability. We have made yeast strains in which RFB sequences have been inserted between two active origins of DNA replication on chromosome 3 of budding yeast. By regulating expression of Fob1, we can synchronise cells in the G1-phase of the cell-cycle, activate the RFB by switching-on Fob1, and then allow cells to undergo chromosome replication. We have used ChIP to ask which replication proteins remain associated with the stalled fork, and we have also deleted genes encoding potential regulators of fork progression to ask which may be important for stalling of forks at the RFB. In this way we have shown that the stalling of forks at the RFB is an active process that is probably mediated by the forks themselves, using proteins that they carry with them.

Studying the role of Cdc104 in cytokinesis

We identified Cdc104 by "functional proteomics" as a protein that is essential for cytokinesis. We have found, in collaboration with Gislene Pereira in Elmar Schiebel's group, that Cdc104 is localised at the very end of mitosis at the site where cytokinesis will subsequently occur. We are currently investigating how the function of Cdc104 relates to that of other proteins that have previously been shown to be important for cytokinesis.



GINS allows a large complex of proteins to form around the MCM helicase at eukaryotic DNA replication forks. Cdc45 is probably an essential component of the helicase, and the other proteins may regulate the progression of DNA replication forks, or link the helicase to other components of the replisome.

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The group has continued with and expanded upon its chemoprotective/chemoselective studies this year. This has included development and characterisation of a tamoxifen-inducible form of HOXB4 which provides a pharmacologically controllable engraftment advantage to repopulating haemopoietic stem and progenitor cells. This inducible HOXB4 is also the focus for transcriptional profiling experiments aimed at elucidating the molecular consequences of HOXB4 expression in haemopoietic cells. Further work with Mn-dependent superoxide dismutase and XRCC1 has led to the development of strategies to provide protection to haemopoietic cells from radiation and topoisomerase I inhibitors.

O^6-methylguanine-DNA-methyltransferase (MGMT) and PaTrin2

Lorna Woolford has been investigating the $P_{140}K$ mutant version of MGMT, which we and others have previously shown to provide O^6-benzylguanine-insensitive protection of haemopoietic cells against the cytotoxic effects of a number of clinically relevant drugs including temozolomide and BCNU. She has shown that $MGMT_{P140K}$ is also resistant to inactivation by the alternative MGMT inhibitor, O^6-bromothenylguanine (PaTrin2). Moreover, she has shown that transduction of haemopoietic cells with the $P_{140}K$ mutant results in resistance to the cytotoxic effects of temozolomide in combination with PaTrin2. More importantly, she has also shown that haemopoietic stem and progenitor cells expressing $MGMT_{P140K}$ have an *in vivo* survival and selective advantage following challenge of recipient animals with temozolomide/PaTrin2. These data, along with previous data, form the basis of a clinical trial proposal which we submit for approval in April 2005.

Tamoxifen-inducible HOXB4

Last year we reported the results of our experiments exploring the potential of the homeobox transcription factor, HOXB4, to augment the selective potential of the DNA repair protein MGMT. In these experiments, a clear *in vivo* selective advantage was seen for cells expressing both HOXB4 and MGMT, over those expressing either selective marker alone. However, we also reported that overexpression of HOXB4 conferred a differentiation delay to myeloid progenitors. This latter effect may prove to be an unwanted long-term side effect of HOXB4 and we reasoned that it may be important to control the function of this protein in transduced cells. To this end Mick Milsom has developed a tamoxifen-inducible form of HOXB4, by fusion of a mutant (tamoxifen-responsive) version of the oestrogen receptor to the C-terminus of the homeobox protein (HOXB4-TxR). This has been incorporated into a retroviral vector and used to transduce primary murine bone marrow and the murine myeloid progenitor cell line, FDCP-mix. In the absence of tamoxifen, we saw no phenotypic effect of expression of this fusion protein. However, when tamoxifen was added, we saw clear evidence of a differentiation delay both in primary bone marrow and in the cell line. This was manifest as maintenance of colony-forming cells in the face of a differentiation pressure (GM-CSF).

We have since investigated the effects of our inducible HOXB4 *in vivo*. Following transduction, murine bone marrow cells were re-transplanted into recipient animals in competition with untransduced cells and the extent of reconstitution with transduced cells determined by FACS analysis of peripheral blood. In the absence of tamoxifen treatment, animals were reconstituted with HOXB4-TxR-transduced cells at a low level (5-9% GFP positive). In contrast, when mice received tamoxifen-citrate in their drinking water, the levels of transduced cells detected in peripheral blood were significantly higher (14-24% GFP positive). This is the first report of *in vivo*, pharmacologically regulated HOXB4 activity

Transcriptional profiling of HOXB4-expressing FDCP-mix

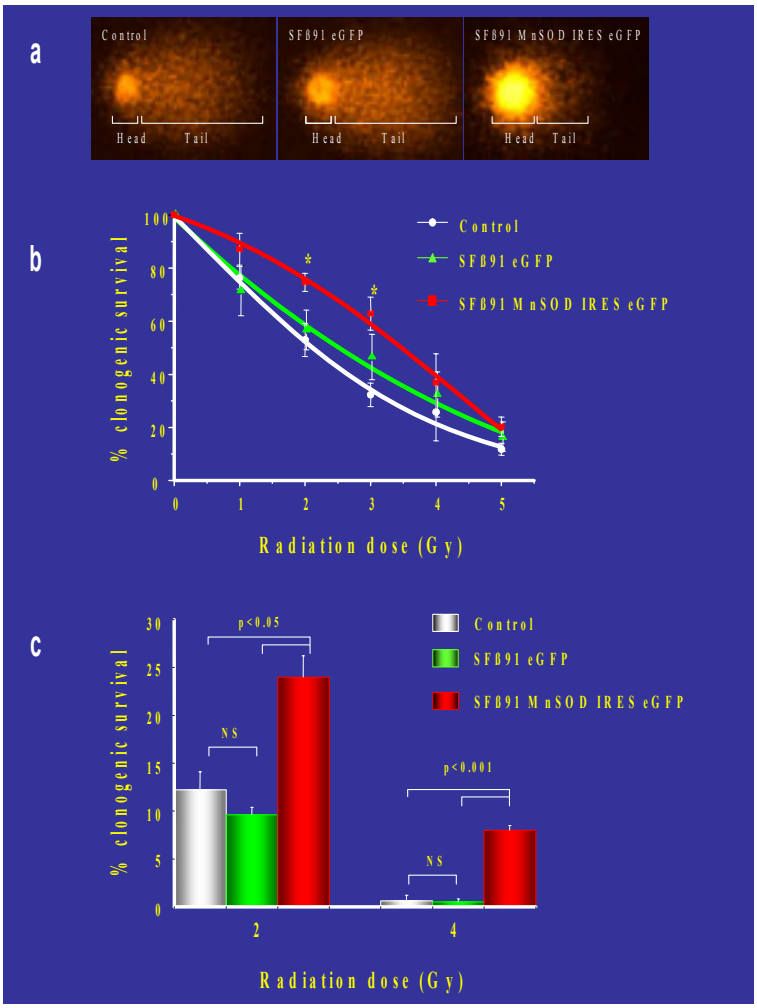
Whilst the biological effects of HOXB4-overexpression on the *in vitro* expansion and *in vivo* engraftment of haemopoietic stem and progenitor cells are well documented, the molecular events important in these effects are unknown. It is largely

assumed that HOXB4 overexpression alters the expression profile of cells, leading to the observed phenotypic changes. To determine the transcriptional consequences of HOXB4 activation in haemopoietic progenitors, we have expressed HOXB4-TxR in FDCP-mix cells. Laura Hollins, a PhD student, has begun investigating these cells. She has prepared mRNA from cells cultured in the absence of tamoxifen or at various times following addition of this activator and has used these to interrogate Affymetrix GeneChips. Data analysis is at an early stage, and we expect to begin identifying lead targets soon.

Chemoprotection/radioprotection studies

Myelotoxicity is a complication associated with the administration of both chemotherapeutic agents and radiopharmaceuticals. We reported last year the instigation of studies to investigate the utility of the manganese-dependent superoxide dismutase (MnSOD) to provide protection against the cytotoxic and genotoxic effects of ionising radiation. Retroviral vectors co-expressing MnSOD with eGFP have been derived and used to transduce the human erythroleukaemic cell line, K562. Following isolation of GFP^+ cells lines, western blot analysis showed effective expression of MnSOD in transduced cells. Moreover, K562-MnSOD cells were shown to have an approx two-fold increase in superoxide dismutase activity. Increased MnSOD activity in K562 led to a reduction in the extent of DNA damage following irradiation, as measured in a Comet assay (Figure a). In the absence of irradiation the comet tail contained 3.9±0.5% of total DNA. Following 10Gy irradiation percentage tail DNA content in the parental line was 44.9±2.1%, compared to 49.3±2.5% in cells transduced with SFß91-eGFP, (no significant difference). In cells transduced with SFß91-MnSOD-IRES-eGFP, however, tail DNA content was 37.3±1.9% which was significantly ($p<0.05$) different from either parental or GFP control cells. These results indicate that retroviral overexpression of MnSOD in K562 cells resulted in decreased DNA strand breaks following 10Gy irradiation. Radiation dose response studies, using a colony forming assay further determined that K562-MnSOD cells were significantly protected against killing following exposure to external beam radiation (Figure b).

Similar results were obtained in primary murine bone marrow following retroviral transfer and expression of MnSOD. In the Comet assay, after 10Gy irradiation, percentage tail DNA in the mock transduced cells was 48.0±0.9%, compared to 47.4±1.3% in cells transduced with SFß91-eGFP (no significant difference), or 42.8±1.2% in cells transduced with SFß91-MnSOD-IRES-eGFP ($p<0.05$ *versus* either mock-transduced or GFP-controls). Transduced primary bone marrow cells were also assessed using a soft agar colony forming assay. Following irradiation at either 2Gy or 4Gy, primary murine GM-CFC transduced with the SFß91-MnSOD-IRES-eGFP vector were significantly resistant compared to eGFP controls and untransduced cells (Figure c). These data point to MnSOD gene transfer as a means to achieve radioprotection of haemopoietic progenitor cells.



Radioprotection by MN-SOD gene transfer.
a) Representative comets from parental, eGFP control, or Mn-SOD-transduced K562 cells following 10Gy irradiation.
b) Clonogenic survival of control and MnSOD-expressing K562 following irradiation at various doses (* = $p<0.05$).
c) Clonogenic survival of control and MnSOD-expressing murine GM-CFC following irradiation at either 2Gy or 4Gy (ns = not significant).

Tom has also been investigating the potential of XRCC1 to confer resistance to various chemotherapeutic agents. Again using retroviral technology, he has transduced primary murine bone marrow and K562 cells and shown efficient expression of the protein. Early data indicate a protective role for XRCC1 gene transfer. Following exposure of cells to either camptothecin or 5-iodo-2'-deoxyuridine, he sees a significant (six-fold) increased survival of those overexpressing XRCC1 over untransduced and eGFP controls.

Publications listed on page 48

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We aim to translate knowledge of human papillomavirus (HPV) driven carcinogenesis or the expression and function of oncofoetal molecules into new cancer immunotherapies. In the past year, we have initiated new early phase clinical trials of different immunotherapies and are developing further optimisations in preclinical models. Our basic studies of the 5T4 oncotrophoblast antigen have focused on establishing the pattern of expression throughout development and we have extended the practical application in defining pluripotency of embryonic stem (ES) cells to human ES cells.

HPV associated neoplasia

Different types of therapeutic HPV vaccines including peptide, protein, DNA or viral vector based have proven safe and immunogenic in patients although there is often no simple correlation with clinical outcome (Stern, Expert Opin Investig Drugs 2004; 13: 959). Understanding the equilibrium between viral and immunological factors will be important in providing the appropriate tools to evoke therapeutic immunity. Drawing on our previous vaccination studies, we have now designed clinical trials to study local and systemic immune factors in high grade VIN (vulva intraepithelial neoplasia) HPV 16 positive patients who will receive treatment by either imiquimod followed by photodynamic therapy (PDT) or imiquimod plus vaccination. Since imiquimod acts at least partly through inducing a local inflammatory response, and PDT or vaccination may facilitate T cell mediated long term effects it raises the possibility that combinations may facilitate adaptive immunity overcoming HPV directed immunosuppression thereby leading to longer-term resolution of persistent VIN.

Cervix carcinogenesis is initiated with infection with high-risk HPV types. The subsequent progression from premalignant cervical intraepithelial neoplasia (CIN) to invasive cancer is driven by both genetic and epigenetic processes. In collaboration with Free University of Amsterdam, we assessed the role of the gene encoding the adhesion molecule tumour suppressor in lung cancer 1 (TSLC1) in this progression. It was shown that TSLC1 gene silencing via promoter hypermethylation is a frequent event in the progression from high-risk HPV-containing, high grade CIN lesions to invasive cervical cancer. (Steenbergen *et al.*, J Natl Cancer Inst 2004; 96: 294).

5T4 oncofoetal antigen and development

Over-expression of 5T4 oncofoetal antigen, an early marker of mouse ES cell differentiation, *in vitro* increases cellular motility and decreases adhesion, properties relevant to development and cancer. Embryonic expression of m5T4 antigen is first detected on trophectoderm at implantation and is restricted to extra-embryonic tissues to E11.5. In the embryo, significant m5T4 expression is detected at E12.5 in hindbrain roofplate and in various epithelia derived from all germ layers. In keratin 14-expressing epithelia, there is a congruent 5T4 expression pattern with many of these cells being Ki-67 positive. In brain, expression is observed in roofplate, ependymal layers, choroid plexus and subventricular zones of lateral ventricles at E14.5. By E17.5, expression is decreased in the subventricular zone with further restriction to choroid plexus in adult brain. Our data demonstrate a limited 5T4 expression profile during embryogenesis associated with actively cycling, undifferentiated epithelial progenitor cells which may contribute to their migration (Barrow *et al.*, submitted).

A key requirement of human ES cell applications is to identify and manipulate live pluripotent cells. We have now shown that surface expression of human 5T4 oncofoetal antigen is associated with loss of the pluripotent marker Oct-4 in two different human ES lines. 5T4 induction is correlated with a membraneous E- to N-cadherin switch, changes which are likely to facilitate increased motility. Importantly, human 5T4 specific antibody can be used to identify and sort ES cells to propagate homogeneous pluripotent cells or generate efficient differentiation of particular lineages. For example, low density 5T4-negative ES colony transfers cultured in serum generate homogeneous glial populations, while tyrosine hydroxylase-positive neurons are obtained with high yields in neurobasal medium. Thus 5T4 is a cross-species cell surface marker of ES cell differentiation, allowing isolation and maintenance of pluripotent populations *in vitro* (Ward *et al.*, submitted).

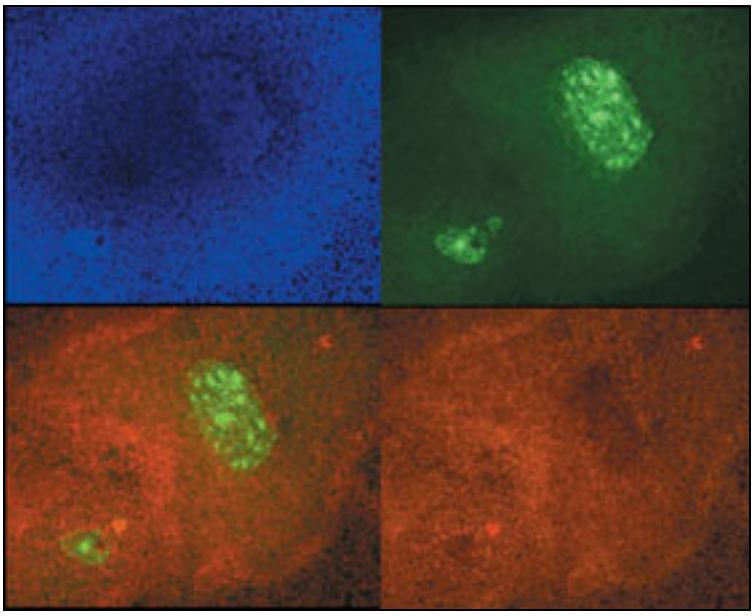
Preclinical studies of cancer vaccines

The restricted expression of 5T4 on tumour tissues as well as its implication in tumour progression and bad prognosis makes it a promising candidate for immunotherapy. We have shown human monocyte-derived dendritic cells infected with a replication defective adenovirus expressing 5T4 (5T4-Ad) can efficiently generate 5T4 specific CD8 T cell responses. In mice, we have used 5T4 retrovirally transduced DC lines in combination with the 5T4-Ad in heterologous prime boost regimens where they are highly efficient in inducing 5T4-specific CTL activity. However, the different heterologous prime-boost combinations provide different survival advantages to B16h5T4 tumour growth in mice which are h5T4 naïve (protection) or not (active therapy). The role of T regulatory cells in these outcomes is now being investigated.

Some B-cell lymphomas lack important costimulatory properties that could prevent them from being used as cell-based vaccines. We have shown that infection of A20 B lymphoma cells with a replication-defective adenovirus encoding murine (m) CD40L, but not mIL-2, produces an antigen presentation phenotype with up-regulation of MHC class I/II, induction of B7-1/2 molecules and production of murine IL-12 and MIP1-α. Direct therapy of pre-established tumours was achieved with the combination of Ad-mCD40L and Ad-mIL-2 given at days 4 and 8 at the tumour site with a significant long-term survival of 85% of tumour-bearing mice ($p=0.0001$). This work strongly supports the use of Ad-CD40L and Ad-IL-2 combination therapy for the treatment of patients with B cell lymphoma (Meziane *et al.*, Int J Cancer 2004; 111: 910). An idiotype-specific T cell line recognising a naturally processed idiotype derived epitope of A20 cells has been used to successfully treat mice with disseminated lymphoma supporting the clinical use of idiotype specific T cells (Armstrong *et al.*, J Immunother 2004; 27: 227).

Clinical trials of 5T4 immunotherapy

A CR-UK sponsored trial in collaboration with Oxford BioMedica evaluating 5T4-MVA vaccine in patients undergoing surgical resection of colorectal liver metastases has recruited ten patients. The unique aspect of this trial is the potential to investigate activity in the site of the tumour post vaccination. Our baseline studies of tumour infiltrating lymphocytes have shown a mixed representation of CD4 and CD8 cells which are recoverable and expandible.



Immunofluorescence staining of human ES cells for expression of 5T4 and loss of pluripotency. Detection of nuclei stained by DAPI (blue), 5T4 expression (green) and pluripotent marker Oct 4 (orange). Areas which express 5T4 are shown to be absent in areas which express Oct 4 demonstrating the loss of pluripotency in 5T4 positive cells.

In collaboration with the Radiochemical Targeting and Imaging Group, we have optimised, and validated to GMP standard, the methodology for indirect labelling with the positron emitting nuclide ^{124}I of 5T4 antibody targeted superantigen for use in a clinical positron emission imaging study. To accomplish this, a series of full-scale preparations were undertaken, during which the reproducible production of sterile, pyrogen-free, fully functional labelled antibody of high specific activity was demonstrated. Preclinical studies show that this labelled antibody specifically localises to 5T4 positive tumours and is retained for several days after administration.

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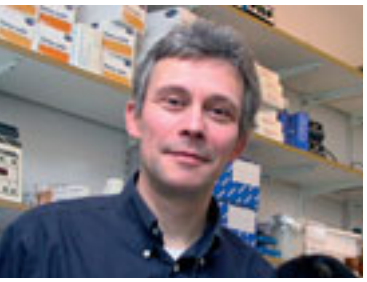

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The overall aim of our research is to understand the molecular mechanisms that achieve sister chromatid segregation and regulate cell cycle progression in mitosis. This is a very active and rapidly evolving research area that is driven by the parallel 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.

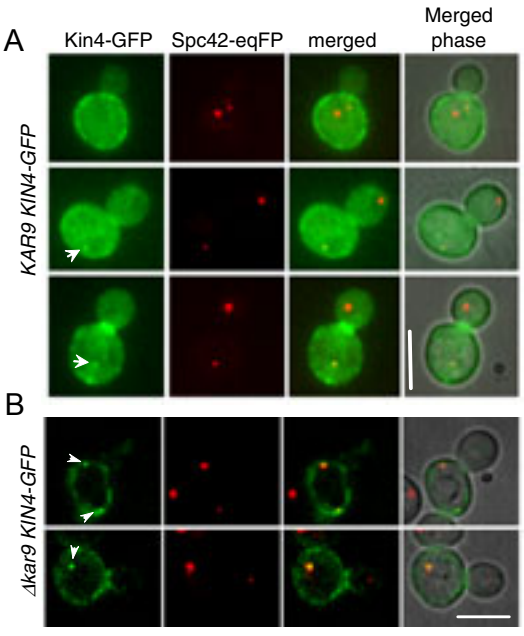
Spindle alignment

The orientation of the mitotic spindle is in many cells essential for the position of the division plane, the development of cell-type diversity, cell cycle progression and accurate chromosome segregation. As in many polarised animal cells, the yeast spindle becomes aligned according to a pre-defined cell polarity axis. The cytoplasmic microtubules organised by the spindle pole body (SPB), the functional equivalent of the mammalian centrosome, interact with the cell cortex and thereby align the mitotic spindle. Spindle alignment is an essential and cell cycle regulated process that immediately starts with the duplication of the SPB in G1/S of the cell cycle. The cortex of the daughter cell, the bud, captures the cytoplasmic microtubules organised by the pre-existing old SPB (SPB duplication is conservative resulting a pre-existing old and a newly formed SPB). Binding of cytoplasmic microtubules to the daughter cortex requires the conserved microtubule binding protein Bim1 and Kar9, which has limited homology to the tumour suppressor adenomatous polyposis coli (APC). We have recently shown that the Kar9-Bim1 complex first binds to the old SPB and then becomes transported along microtubules to microtubule ends. Kar9 then interacts with the myosin Myo2 that moves along actin cables into the growing bud. This mechanism positions the developing spindle early in the cell cycle along the mother-bud axis. In addition, the cyclin-dependent kinase Cdc28 in complex with the B-type cyclin Clb4 and the essential subunit Cks1 become transported to cytoplasmic microtubule ends through the Kar9-Bim1 complex where Cdc28-Clb4-Cks1 regulate the duration of microtubule interactions with the bud cortex.

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" (SPC). The distinct polar cellular distribution of MEN activators and inhibitors is only one element of the SPC. 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. We, in collaboration with Dr G Pereira (University of Manchester), have shown that the Kin4 kinase is an essential component of the SPC. In interphase and early mitosis Kin4 is associated with the cell cortex and binds in mid-anaphase predominantly to the SPB in the mother cell (Figure). In response to a misaligned anaphase spindle Kin4 localises to both SPBs and inhibits mitotic exit. This inhibition is important for the survival of cells with spindle alignment defects. Thus, in cells with misaligned spindle Kin4 inhibits the MEN in mid-anaphase to prevent premature mitotic exit.



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 (Δ*kar9* cells) Kin4 associated in 95% of the cells with both SPBs (top panel, arrow). However, when the spindle of Δ*kar9* 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).

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*. It is also unclear whether a MEN-like pathway regulates Cdc14A and Cdc14B in mammalian cells. 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.

Cell polarity proteins and mitotic exit

Cell polarity proteins such as the PAK kinases Ste20 and Cla4 are associated with the cortex of the bud and promote mitotic exit in yeast. In fact, the GEF Cdc24, the GTPase Cdc42 and Ste20 form a signal transduction cascade that promotes mitotic exit. We performed a genetic screen to identify components of this pathway. Two related bud cortex associated Cdc42 effectors, Gic1 and Gic2, were obtained as factors that promoted mitotic exit independently of Ste20. The mitotic exit function of Gic1 was dependent upon its activation by Cdc42 and upon the release of Gic1 from the bud cortex. Moreover, Gic1 bound directly to Bub2 and prevented binding of the GTPase Tem1 to Bub2. We propose that in anaphase the Cdc42-regulated Gic proteins trigger mitotic exit by interfering with Bfa1-Bub2 GAP function.

A role of the SPB component Cdc31 in regulating nuclear export

Centrins are calmodulin-like proteins that function in the duplication of microtubule-organising centres. In collaboration with the group of Dr Hurt at the University of Heidelberg we have described a new function of the yeast centrin Cdc31. We have shown that the nuclear export factor Sac3 recruits Cdc31 and Sus1 (a subunit of the SAGA transcription complex) to the Sac3-Thp1 complex, which functions in mRNA export together with specific nucleoporins at the nuclear basket. A previously reported *cdc31* temperature-sensitive allele, which is not defective in SPB duplication, induces mRNA export defects. Thus, Cdc31 has an unexpected link to the mRNA export machinery.

Publications listed on page 50

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Targeting and imaging of cellular and molecular pathways, with positron-emitting probes, continues to be the research focus of the Radiochemical Targeting and Imaging Group.

Over the last year we continued the development of [^{124}I]4IB-annexin V as a cell death probe, and compared the directly labelled ^{124}I-annexin V, with the indirectly labelled, [^{124}I]4IB-annexin V and found that the latter has a higher rate of binding to phosphatidylserine *in vitro*, lower uptake in apoptotic liver and preferential renal clearance compared to the directly labelled. Towards the development of a cell proliferation probe, we compared the thymidine phosphorylase cleavage activity and thymidine kinase activity in an *in vitro* and *in vivo* lung cancer model. The translational research has concentrated on the laboratory development of radiolabelled antibodies against the tumour-associated 5T4 antigen (immunotherapeutic), and against the $\alpha_V\beta_{3/5}$ endothelial integrins (antiangiogenic). The former has been developed into a clinical grade preparation in order to translate the laboratory development into a clinical pharmacokinetic study along side a Phase I trial in renal cancer at the Christie. We continue to underpin our laboratory and clinical research by technology-driven development in radiochemistry with a major focus on the development of nanotechnology platforms for radiochemical synthesis and analysis.

Targeting and imaging cell death pathways

We are interested in imaging cell death *in vivo* using annexin V radiolabelled with iodine-124. We made [^{124}I]4IB-annexin V and [^{124}I]4IB-ovalbumin using [^{124}I]*N*-succinimidyl-4-iodobenzoate and found that [^{124}I]4IB-annexin V binds to phosphatidylserine-coated microtitre plates and apoptotic Jurkat cells, and accumulates in hepatic apoptotic lesions in mice treated with anti-Fas antibody, while [^{124}I]4IB-ovalbumin does not. In comparison to directly labelled ^{124}I-annexin V, we found that [^{124}I]4IB-annexin V has a higher rate of binding to phosphatidylserine *in vitro*, lower uptake in apoptotic liver, higher kidney and urine uptake, lower thyroid and stomach content uptake, greater plasma stability, and a lower rate of plasma clearance.

Another feature of programmed cell death is the activation of caspases – cysteine proteases that cleave their substrates after aspartate residues. A successful MSc project showed 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, and experiments to see whether this molecule could be used to detect programmed cell death *in vivo* are in progress.

Cell proliferation PET probes

We have been working on the development of PET nucleoside analogues as potential probes of cell proliferation. In non-small cell lung cancer (NSCLC) models (H460 and H596), we have been investigating the thymidine analogues [^{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. We are currently investigating the relationship between regional tissue enzyme activity with the uptake of the radiolabelled nucleoside.

Drug targeting and pharmacokinetics

ABR-217620, an immunotherapeutic drug based on an antibody that recognises the tumour associated antigen 5T4, is a candidate for immunotherapy in solid tumours in man. In the forthcoming year we plan to conduct a clinical imaging study, in which positron emission tomography (PET) will be used to demonstrate proof of concept for the mechanism of action of the drug. To accomplish this, we have optimised an indirect labelling strategy, in which a precursor, N-succinimidyl-4-tributylstannylbenzoate, is oxidatively labelled with the positron emitting nuclide ^{124}I, purified then conjugated to the drug. The labelled drug retains the full native functionality of the unlabelled compound. In preclinical models the labelled drug exhibits specific localisation and retention in 5T4-positive tumours indicting an imaging window of 24-48 hours after administration is optimal.

In collaboration with Immunology, we have developed a PET analogue of the monoclonal antibody (MAb) specifically targeting the $\alpha_V\beta_{3/5}$ integrins. We have demonstrated that the PET analogue showed specific binding to A375.S2 human melanoma cells expressing the $\alpha_V\beta_{3/5}$ integrins. In a rat-bearing melanoma xenograft model, the radiolabelled protein localised predominantly to the tumour with minimum uptake in the rest of the tissues indicating specific binding to those cells over-expressing the integrin receptors. Work planned for 2005 include imaging of ^{124}I-labelled MAb in the xenograft model will allow detailed pharmacokinetics of the drug.

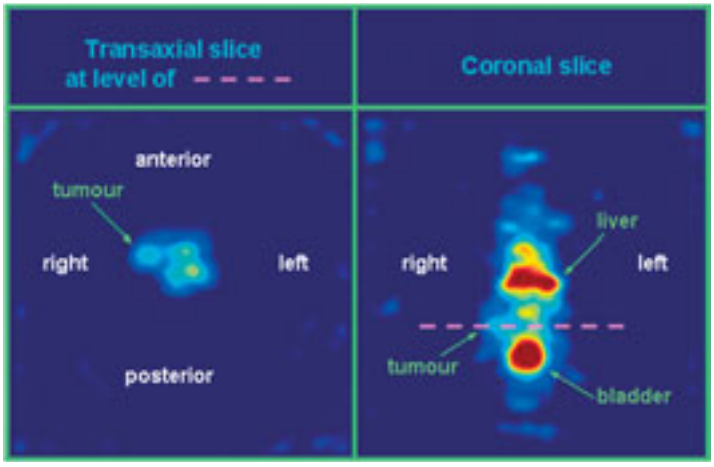
Pharmacodynamic probes for antiangiogenic therapy

We are developing, in collaboration with Drs JM Gardiner (Chemistry) and Gordon Jayson (Medical Oncology) at the University of Manchester, an oligosaccharide which inhibits bFGF binding to its receptor. The anti-angiogenic effect will be quantified indirectly by assessing the expression of a biomarker (v integrin receptors, uPAR or KDR) using ligands labelled with ^{124}I and ^{18}F (antibodies anti-uPAR, anti-KDR and RGD-peptide). These ligands will be investigated in a in a rat tumour xenograft model using radiotracer kinetic techniques and PET imaging. The three selected biomarkers are being assayed by measuring KDR, uPAR and integrin density on cell cultures (HUVEC) while varying the amount of bFGF (initially 5 to 15 nanomol/mL) in the cell culture medium. Measurements are made by ELISA and flow cytometry using the antibodies anti-KDR, anti-uPAR and anti-integrin family.

Microfluidics

Our current research involves a new application of microfluidic technology by combining miniaturisation and radiotracer techniques in biomedical research. The inherent characteristics of radiopharmaceutical research in terms of tracer quantities, short half-lives of biomedical isotopes and sensitivity of detection makes it compatible with the micro-scale nature of miniaturisation. In particular this work demonstrates, for the first time, the feasibility of applying this technology to PET radiochemistry. In the long term, the concept of an on-chip integrated system incorporating pharmaceutical manufacturing and analysis could be realised.

In this work, miniaturisation and PET radiochemistry has been married to produce a number of PET radiopharmaceuticals for molecular imaging research. The most important radiotracer used in PET, 2-[^{18}F]FDG, has been radiosynthesised using multiple micro-reactors functioning in sequence. This produced comparable radiochemical yields to current radiolabelling methods, with an overall reaction time of 4 seconds at ambient temperature as opposed to 15 minutes at 90^{o}C.



Imaging of ^{124}I-labelled anti-5T4 antibody in antigen positive tumours (B16F10 in C57BL6 mouse). Image capture using a dual HiDac camera and reconstructed in 1 mm slices

Publications listed on page 50

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The *Runx1* transcription factor is a frequent target of gene rearrangements and mutations in human acute myelogenous leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its initial implication in leukaemias, *Runx1* has been shown to be critical for normal haematopoietic development. *Moz*, a transcriptional coactivator, was initially identified in AML translocated to the CREB binding protein loci. Moz function remains largely unknown. Using the *in vitro* differentiation of mouse embryonic stem (ES) cells as a model system, our goals are to further define the role of *Runx1* and MOZ in early haematopoietic development and investigate how alterations of their function may lead 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.

Although the blood islands were identified as the earliest site of haematopoietic and endothelial development almost 100 years ago, attempts to identify, isolate and characterise 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 recently 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.

A critical function of *Runx1* in haemangioblast development

To investigate the role of *Runx1* at the earliest stage of haematopoietic commitment, we have analysed 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 targets of *Runx1*

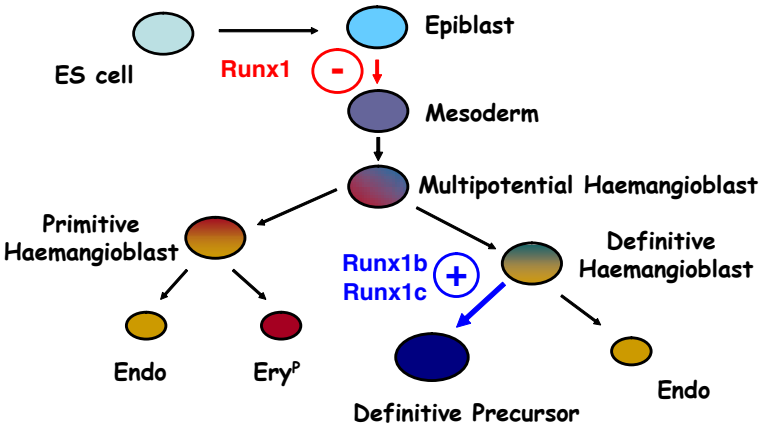
Runx1 has been shown to participate in the regulation of expression of a number of different genes including IL-3, myeloperoxidase, neutrophil elastase, M-CSF, GM-CSF and T cell receptors that are involved in later stages of haematopoietic development. Our results have indicated that *Runx1* is required 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 from haemangioblast-enriched-cell-populations derived from both *Runx1* deficient and *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 are now validating the differential expression of these candidates on samples generated from the ES/EB system.

An early role of *Runx1* in mesoderm development

Our studies have also indicated that although *Runx1*+/- EBs generated normal number of blast colonies, the kinetics of development of the BL-CFC precursors was accelerated relative to the pattern observed in wild type EBs. Gene expression analyses have indicated that the acceleration was already initiated at the level of mesoderm commitment. These results suggest that *Runx1* could play an important function during the development of mesoderm in addition to its role in commitment to the definitive haematopoietic program.

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 fulfil distinct functions at the different stages of the establishment of the haematopoietic system. We are currently testing this hypothesis by further defining the pattern of expression of the different *Runx1* isoforms and evaluating their respective functions.



Multiple functions of Runx1 during haematopoietic development

Moz and haematopoietic development

Moz was identified in AML at the breakpoint of translocation with CREB binding protein (CBP), the nuclear receptor TIF2 and 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 oncogenesis. We have created ES cells in which one or both allele of *Moz* are mutated for the HAT activity. We are currently evaluating the consequences of such alteration upon *in vitro* ES cell differentiation and *in vivo* in mice. In addition ES cells targeted for a deletion of the whole *Moz* gene or the closely related *Moz2* (MORF) gene are being generated.

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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 or embryonic ectoderm 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 is 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. Indirect evidence supporting the haemangioblast concept came from studies demonstrating that a large number of genes are expressed by both haematopoietic and endothelial lineages and that some of these genes are essential for both blood cell and vascular development. 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

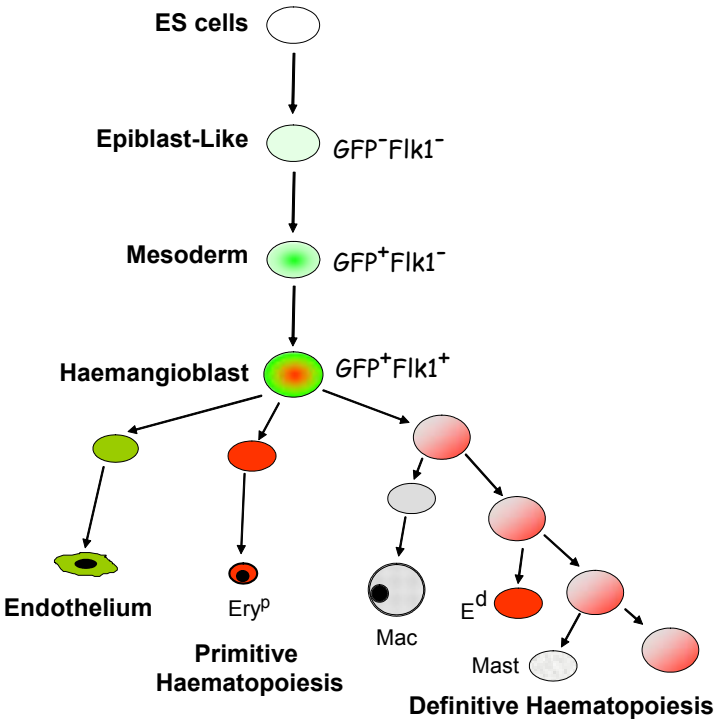
Following the initiation of differentiation in culture, ES cells will form colonies known as embryoid bodies (EBs) that generate haematopoietic and endothelial progeny in a temporal pattern recapitulating the development of these populations in the yolk sac. Analysis of early EBs, prior to the haematopoietic 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 three 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. In a first set of experiments, four populations are being analysed 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^+). Preliminary results from these experiments have lead to the identification of several genes which are distinctively up-regulated in the specific subpopulations. These genes are now the focus of intense studies in the lab.



Publications listed on page 51

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The nuclear envelope and associated structures provide the defining element of the eukaryotic cell. It is apparent that the 'nuclear periphery' has profound influence not only over transport between nucleus and cytoplasm, but in both nuclear/cytoplasmic interactions and the overall spatial organisation of the nuclear interior. Many of the elements of the nuclear periphery – including the nuclear lamina – have been implicated in a wide variety of disease states, as well as the established roles of nucleoporins in several oncogenic fusion proteins in human leukaemia, and a general overexpression of Nup88 in a high proportion of all human cancers. As cell division is fundamental in oncogenic transformation, a full understanding of the dynamics of breakdown and reassembly of the nuclear/cytoplasmic boundary is basic to the understanding of the disease.

Direct visualisation of the stages of Nuclear Pore Complex (NPC) reformation in human cells – a second route to NPC formation

We have previously identified the stages of NPC formation using a cell-free system of nuclear formation *in vitro*. We established a well-characterised series of intermediate stages, and were able to modulate these stages of formation with a variety of inhibitors. Briefly, NPC formation is initiated at sites of fusion between the inner and outer nuclear envelope membranes, followed by insertion of core structures and the progressive accumulation of structure from centre to periphery on both sides of the nuclear envelope. As NPCs are accumulated throughout interphase, the presence of a nuclear membrane as the starting point for NPC formation appears entirely reasonable. Furthermore, we found a virtually identical NPC formation *in vivo* in *Drosophila* embryos.

However, a recent collaboration indicated that this system might not be the case in dividing mammalian cells. Here we found that post mitotic NPC assembly initiates on chromatin via early recruitment of the Nup107-160 complex (Walther *et al.*, Cell 2003; 113: 195). To ascertain whether the sites of NPC initiation required the presence of new nuclear envelope membranes, we visualised stages of chromosome condensation, separation and decondensation through mitosis in HeLa cells using FEISEM with immunolabelling. This required accessing chromosomal surfaces for surface imaging (see next section). Chromosomes were imaged without making metaphase spread preparations, being exposed *in situ* in dividing cells through prophase, metaphase and early anaphase, and within paired daughter cells for late anaphase and telophase, often still connected by the midbody. Monoclonal Ab 414, which labels several nucleoporins, was compared with antibodies to Nup107 and Nup133, both of which are members of the 107-160 Nucleoporin complex. Although the extraction involved brief exposure to 0.1% Triton-X100, this was after a fixation step in 2% paraformaldehyde, identical to the prefixation standard for EM immunocytochemistry in general. Nuclear envelope membranes are retained in this protocol in interphase cells, but because of the possibility that reforming NE in mitotic cells might be more labile, we used a 'hypotonic only' pre-treatment of the cells, and obtained similar results.

The most striking finding was the clear appearance of several stages of NPC formation on the surface of anaphase chromosomes in the absence of any NE membranes. NPCs showing well developed cytoplasmic rings, with some development of cytoplasmic filaments were clearly imaged at the surface of the chromatin (see Figure 1). This indicates that NPCs can assemble directly on chromatin, without the need for pre-existing membranes (although this must be the case for

NPC increase during interphase), and appears to confirm the existence of 'prepores' as suggested from early experiments in the *Xenopus* cell free system (Sheehan *et al.*, J Cell Biol 1988; 106: 1). This is also indicative of two pathways to NPC formation, firstly in the presence of membrane (interphase nuclear envelope and annulate lamellae), and secondly directly on the surface of chromatin in the absence of NE membranes.

Accessing the surface of mitotic chromosomes *in situ* for FEISEM and immunocytochemistry

Mitotic cells were either released *in situ* from a nocodazole block, or shaken off and harvested by spinning onto poly-l-lysine coated silicon chips. The cells were then taken through the FEFF (Fix-Extract-Fix–Fracture) protocol (Allen *et al.*, Meth Cell Biol 1998; 53: 125). Mitotic cells fractured in a satisfactory manner, allowing direct secondary electron surface imaging of chromosomes and chromatin at most stages of division. Backscattered electron (BSE) imaging of the osmium fixed signal from the chromosomes gave an overall image of the chromosome complement which was similar to fluorescence staining in LM, allowing precise staging of division. At the end of the fix-extract stage, cells were stained with a variety of antibodies, reproducing the fixed and permeabilisation stage as used for conventional TEM immunocytochemistry. Antibody location was visualised with 10nm gold secondary antibodies by BSE imaging. In spite of the strong BSE image produced by the underlying chromatin, and the relatively small difference in Z number between osmium and gold, the 10nm colloidal particles were clearly apparent. SE and BSE images were collected simultaneously to maintain absolute register, and superimposed/transferred via a clear layer in *Photoshop* to display the combined information. In this way we were able to show Cenp-F labelling at the kinetochore by SEM for the first time, (in collaboration with S Taylor, University of Manchester)

Is gp210 an essential, functional protein of the mammalian NPC?

Gp210 is one of only two nucleoporins of the mammalian NPC that contains a transmembrane domain. Previous work involved investigation of the role of gp210 in NPC assembly in the amphibian *Xenopus* cell-free system. This work suggested that the cytoplasmic "tail" of gp210 (a short 60 amino acid domain extending beyond the pore membrane towards the centre of the NPC) is involved in the dilation of nascent nuclear pores.

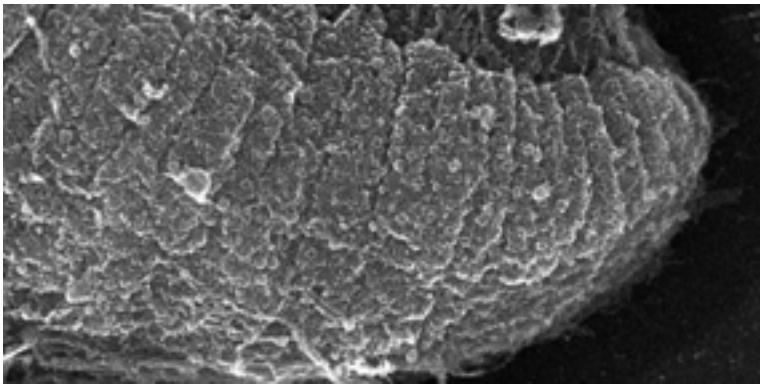

Figure 1: FEISEM image of anaphase chromosome surface, showing well defined stages of NPC formation in the absence of nuclear envelope membrane.

Recently our work has extended to looking at the function of gp210 in mammalian species. A panel of novel pSUPER vectors has been generated (in collaboration with Dr Helen Pickersgill, Netherlands Cancer Institute, Amsterdam) in order to "knock-down" and potentially "knock-out" gp210 protein expression within HeLa cells. We have developed the ability to significantly reduce the amount of gp210 protein in human cells (see Figure 2) and this down-regulation appears to reduce the number of viable cells in culture indicative of an essential function for this protein. Co-transfection with vectors that generate fluorescent protein markers for nuclear import (GFP-NLS) or both nuclear import and export (NES-GFP-NLS) has revealed no bulk inhibition of nuclear import capability, however preliminary work does suggest that nuclear export (of NES-GFP-NLS) may be reduced or inhibited. Cells with reduced amounts of gp210 will be visualised at higher resolution with the aim of determining the effects on NPC number, distribution, composition and association at defined time-points throughout the cell-cycle. To this end, cells will be transfected with a functional gp210-specific pSUPER vector (gp210-1pSUPER or gp210-3pSUPER) and, prior to lethality, cells will be visualised by FEISEM.

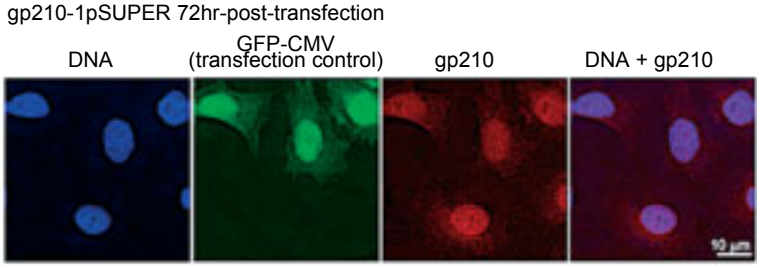

Figure 2: "Knock-down" of gp210 protein expression using a gp210-specific pSUPER vector (gp210-1pSUPER). GFP-CMV used as a fluorescent marker for positive transfectants.

Publications listed on page 51

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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 peptide 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. This approach is close to clinical trial. 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

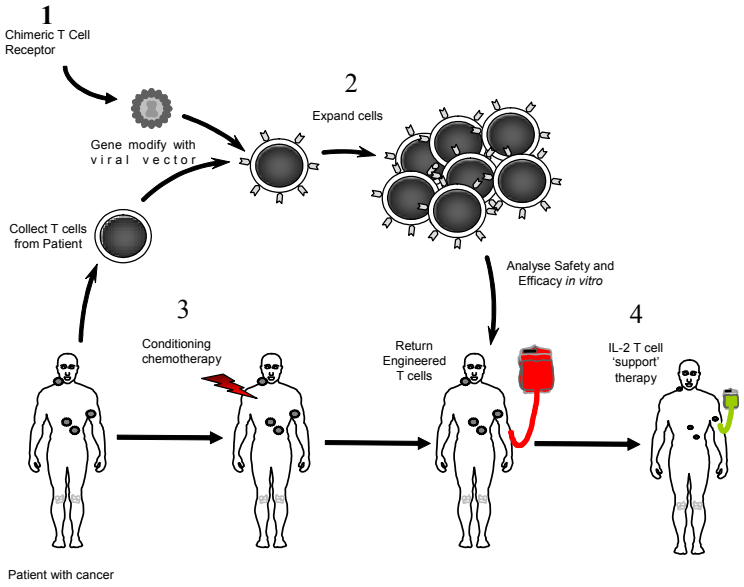
We are developing a number of novel biological therapies. These include a novel peptide (PCK3145) which induces apoptosis of prostate cancer. Pharmacodynamic monitoring of the trial also revealed that the peptide appears to decrease matrix metalloprotease-IX. Further investigation of the optimal dosing schedule in relation to pharmacodynamic effects continues. The major focus of clinical trials remain 5T4 targeting (see also Immunology Group, page 22). 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. In a Cancer Research UK trial 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. We are also the lead centre in a number of major multi-centre trials of renal call cancer.

A major focus of future work will be the evaluation of Engineered T cells in phase I / II clinical trials. These will require some inpatient care and both planning and fundraising for this innovative and important endeavour are under way. A highlight of the year was an international meeting hosted by our group and held in Manchester entitled "Cellular Therapy of Cancer". Leaders in the field from Europe and the UK together with the leading American group met to discuss the state of the art in this important area of cancer research. There was also a regulatory workshop (combining regulators, funders and industry representatives) to discuss ways of facilitating the development of this relatively complex form of therapy. Locally, we plan a joint development with the National Blood Service of larger GMP cell therapy facilities on the Christie site as part of the developments for the Cell and Gene Therapy Centre – this will facilitate a range of novel cell based therapies and in particular will focus on the use of Engineered T cells (see below). The development of these is facilitated by the appointment of a specific research fellow to manage these trials (Fiona Thistlethwaite).

Engineered T cells

We had 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 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 effectively (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 clinical 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 Mr 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. The CEA targeted trial is planned in to start in 2005 and the CD19 targeted trial in 2006. Assay validation and process development is ongoing at our GMP unit at the Manchester Blood Centre. An outline of the trial to target CEA is shown in the figure. We are also investigating 5T4 as a target for engineered T cells either alone or in combination with Trovax (see above).

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 immunosuppression and determining techniques which may 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.



Outline of Trial of Engineered T cell therapy. Particular areas where there are ongoing refinements under development are numbered:
1) Receptor design
2) *Ex vivo* expansion conditions
3) Chemotherapy conditioning regimes
4) IL-2 'support' therapy.

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. Culturing transduced cells, particularly as pellet culture, results in restored production of extracellular matrix. This can be achieved even with cultures from diseased joints. Biopsy material is expanded in a cocktail of growth factors to facilitate transduction and to expand the numbers of cells. 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.

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Melissa Whitworth

Medical Oncology: Glycoangiogenesis Group

Angiogenesis, the process of new blood vessel formation, has been identified as a target as it is required by tissues to grow beyond 2 mm diameter. Inhibition of angiogenesis is associated with tumour growth restraint in the laboratory and recently a randomised trial has shown a survival benefit for patients with metastatic cancer treated with chemotherapy and anti-angiogenic therapy. We are interested in the angiogenic cytokine, Fibroblast Growth Factor (FGF), which is dependent on the linear polysaccharide, heparan sulphate (HS), for its biological activity. Our programme focuses on the development of oligosaccharides which competitively inhibit heparan sulphate function and thereby inhibit growth factor activity. We plan to take a lead octasaccharide into Phase I clinical trial evaluation within two years in our Phase I anti-angiogenic programme.

Heparan sulphate proteoglycans (HSPG) in ovarian cancer

HS is found on the surface of all tissue forming cells, where it is covalently bound to a core protein in the extracellular matrix or the cell membrane, forming heparan sulphate proteoglycans (HSPG). We have completed a comprehensive analysis of HSPG in ovarian cancer, demonstrating that the expression of stromal syndecan 1 or endothelial syndecan 3 were poor prognostic factors.

FGF extracellular signalling apparatus

By combining a number of unique probes and different *in situ* techniques we have now shown that the ovarian cancer endothelium expresses FGF receptors, biologically active HS and is surrounded by FGF2. These data highlight FGF2 as a relevant target cytokine for anti-angiogenic agents and suggest that ovarian cancer might be a suitable disease to treat with FGF2 inhibitors and corroborate the findings seen in our analysis of HS synthetic enzymes.

HS synthetic enzymes

We have completed a semi-quantitative analysis of HS synthetic enzymes in human cancer and have shown that the most significant difference between normal and cancer tissue was seen with the enzyme 6-O-sulfotransferase in ovarian cancer. This is a key enzyme involved in the synthesis of biologically active HS and its presence in ovarian cancer endothelium suggests that the endothelial HS will be biologically active, that is, have the capacity to activate FGF2.

Heparanase

We have generated stable anti-sense cell lines that express different amounts of heparanase. Exposure of these lines to hypoxic or reducing conditions increased heparanase activity highlighting a novel and previously undescribed mechanism of heparanase activation. As the enzyme is involved in matrix and heparan sulphate degradation, invasion and metastasis, the potential to inhibit this enzyme with oligosaccharides is increasingly important.

Oligosaccharides *in vitro* and *in vivo*

We have tested size-fractionated oligosaccharides *in vitro* and *in vivo*. The data show that octa- and decasaccharides inhibit FGF2-induced mitogenesis and motogenesis *in vitro*. *In vivo* we have shown, using a double blind protocol, that the same size species (octa- and decasaccharides) inhibit FGF2-induced angiogenesis, H460 lung carcinoma induced angiogenesis and H460 lung carcinoma growth. Extensive testing of the anti-coagulant potential of these molecules suggested that the oligosaccharides do not affect factors X or II and

neither do they prolong the APTT; thus it should be possible to escalate doses without incurring significant toxicity.

Oligosaccharide organic synthesis

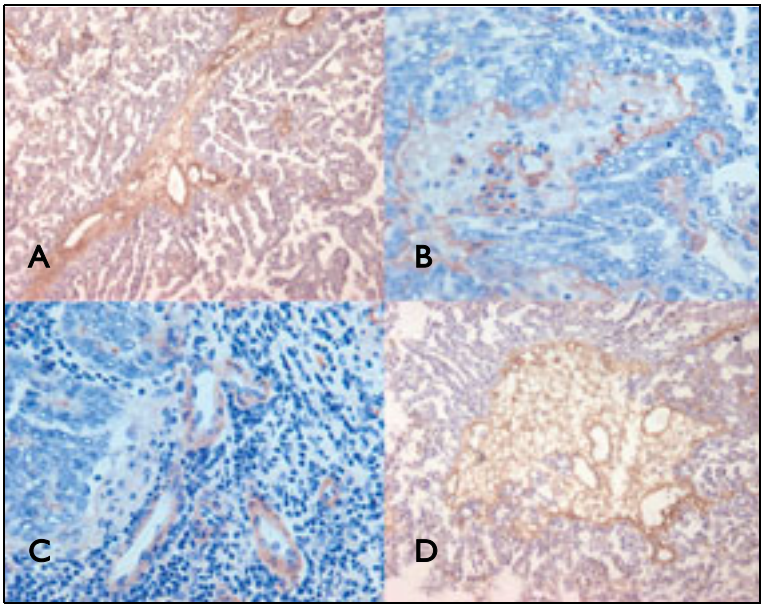
Our data suggest that octa- and decasaccharides have the capacity to inhibit angiogenesis *in vivo*. However, a clinical trial will require a kilogram of material. We have initiated a chemical organic synthesis programme to generate this material. One of the greatest synthetic hurdles is the generation of L-iduronate, the stereochemistry of which contrasts with most naturally occurring saccharides. We have now established a method to generate iduronate that can be readily achieved and scaled up using relatively cheap reagents. The rest of the chemistry is established in precedent.

Phase I programme

We plan to take the oligosaccharides into the clinic in the next two years. This will be performed through our Phase I anti-angiogenesis programme and we now have one of the largest phase I anti-angiogenic programmes in the world. Our portfolio currently includes drugs that inhibit VEGF, KDR, PDGF and the $\alpha_v\beta_{3/5}$ integrins, using antibodies and receptor tyrosine kinase inhibitors. We have established a number of pharmacodynamic endpoints that are included in our Phase I trials including dynamic contrast enhanced Magnetic Resonance Imaging, PET scanning and measurement of circulating endothelial cell precursors.

Resistance to anti-angiogenic agents

In the evaluation of the pure PDGF antagonist we detected unique changes in the vasculature of patients that were associated with the rapid onset of ascites. These findings explain the oedema observed in patients treated with mixed kinase inhibitors (*e.g.* imatinib) and caution against developing broad spectrum anti-angiogenic agents without a thorough understanding of the underlying biology. In a further study we have found considerable redundancy in the VEGF system in colon cancer, suggesting that although we have seen some benefit associated with VEGF inhibitors in colon cancer, a more comprehensive approach could improve the efficacy of the compounds.



FGF-2/FR1c-AP binding is limited to stromal and endothelial cells in ovarian serous adenocarcinoma.

Publications listed on page 52

GROUP LEADER
John T Gallagher

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Cathy Merry

POSTDOCTORAL FELLOW
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Christopher Robinson
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Claire Johnson
Rebecca Baldwin
Annie Wat

Medical Oncology: Proteoglycan Group

Cells of complex organisms contain heparan sulphate proteoglycans (HSPGs) on their surface; HSPGs play key roles in sensing and transmitting signals from the microenvironment to the cell interior. We are studying the mode of action of HS in the control of cell proliferation and differentiation.

Heparan sulphate proteoglycans (HSPGs)

Many growth factors and morphogens deliver signals to cells via a dual receptor mechanism composed of a high affinity transmembrane signal transducer and a lower affinity but more abundant HSPG. We are interested in the molecular recognition between HS and various classes of growth factor and in the mode of association, molecular architecture and stability of multiprotein signalling assemblies on the plasma membrane. We have an expanding programme of work on HS and its dependent growth factors in the regulation of self-renewal and commitment of mouse embryonic stem cells.

A new model for heparan sulphate

The HS chain is made up of two related disaccharide units in which the amino sugar is either N-acetylated (A) or N-sulphated (B) (Figure 1). These units are arranged in a non-random, mainly segregated manner creating a unique molecular design in which sequences of B units (called S-domains) are interspersed with extended regions of low sulphation in which A units predominate. Using a new enzyme called K5 lyase, which only attacks HS in non-sulphated regions, we have located a further type of structural motif, less sulphated than the S-domains, that forms an interface between low-sulphated and S-domain regions. These motifs of intermediate sulphation, termed transition (T) zones, are composed of alternating A and B units with variations in uronate isomers and C-6 sulphation (Figure 2). The fusion of T-zones and S-domains significantly extends and amplifies the sequence diversity in HS underpinning its near-universal role as a growth factor co-receptor.

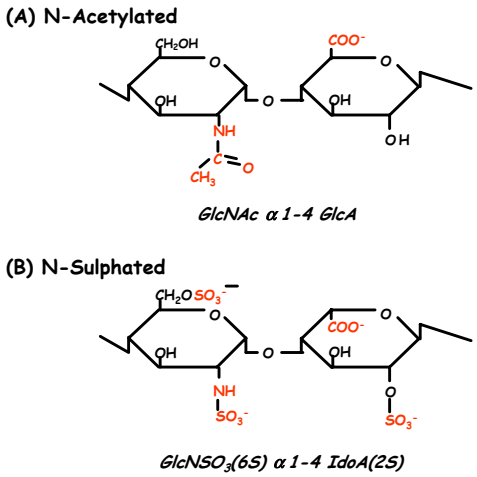


Figure 1: Disaccharides in heparan sulphate. Heparan sulphate is a linear, negatively-charged polymer made up of repeating disaccharide units in which the amino sugar (glucosamine) is *N-acetylated (A)* or *N-sulphated (B)* and the uronic acid is in the *glucuronate (GlcA)* or *iduronate (IdoA)* configuration. The major functional groups involved in growth factor recognition are shown in red. The B units occur in clusters called S-domains and form the active sites for binding growth factors. Variable sulphation of the B units confers specificity to these binding events.

Regulation of HGF/SF by glycosaminoglycans (GAG)

A full molecular description of the GAG co-receptor sequence required by HGF/SF is important for the design of inhibitors to regulate aberrant HGF/SF activity in tumours. Gel mobility shift assay (GMSA) is a new method for studying GAG-protein interactions in which binding events are detected by shifts in protein or GAG electrophoretic mobility. Using this method we have identified tetra- and hexa-saccharides from HS and dermatan sulphate (DS) respectively as the minimal sizes of binding sequences for HGF/SF. Selectivity of binding displayed within an unfractionated HS tetrasaccharide population is indicative of the structural specificity of this interaction. A purified, fully sulphated HS tetrasaccharide can be specifically desulphated under partial reaction conditions to allow the generation of all positional variants for N, 2-O- and 6-O-sulphates, independently or in combination. Similar, partial de-4-O-sulphation can be applied to a purified, HGF/SF-binding DS hexasaccharide, which contains sulphates only at C-4 of each GalNAc residue. Mixtures of partially sulphated HS or DS can be fractionated by SAX-HPLC, and each purified species structurally

identified by disaccharide analyses before being tested for retention, or loss, of HGF/SF–binding ability by GMSA. Results so far consolidate previous indications that neither N-sulphation nor iduronate 2-O-sulphation are necessary for HGF/SF binding to HS.

HS sulphation patterns in early development

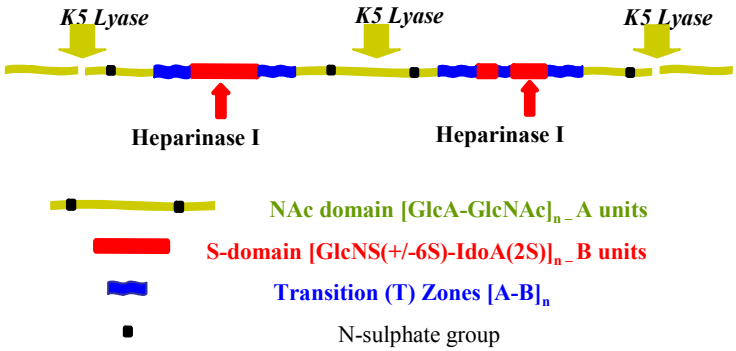
Embryonic stem (ES) cells are pluripotent cells from the inner cell mass/epiblast of pre-implantation embryos. Differentiation and retention of pluripotency require ES cells to respond to growth factors/morphogens, many of which are known to be regulated by HS. During differentiation along the neural pathway, which is dependent on FGF4, the sulphate content of the cellular HS increases, as does the total amount of HS expressed. We are in the process of correlating the changes in HS structure with expression patterns of the enzymes involved in HS biosynthesis. This approach should help us to understand how the appearance of inductive sequence motifs in HS is regulated. We are also extending this analysis to the mesodermal lineage (with Georges Lacaud and Valerie Kouskoff) in which other HS-dependent factors (*e.g.* VEGF) play a critical role. Mice that harbour mutations in HS biosynthetic enzymes present developmental defects ranging from a failure to gastrulate in HS null animal to limited and tissue specific effects such as renal agenesis in mice deficient in HS-2-O-sulphotransferase. We have established ES cells from these mutant embryos and are in the process of studying their *in vitro* differentiation properties with a view to identifying which signalling pathways are disrupted.

RNA interference of HS biosynthetic enzymes

RNA interference is the silencing of gene expression by double stranded RNA, which elicits degradation of target mRNA in a homology-dependent manner. Due to the persistence of HS biosynthetic enzymes, any silencing effect needs to be long term (10-20 days). To achieve this we have utilised a plasmid-based expression of short hairpin RNA (shRNA) constructs. These were designed against the HS-polymerase gene Ext1 and used to silence its expression and decrease HS production in murine ES cells, CHO cells and chondrocyte precursors. Silencing was evident up to 21 days after transfection, although only 50-60% of the cells were affected compared to levels of 85-90% seen in the first 10 days. We are now expressing tetracycline-inducible shRNA to Ext1, and also to Oct4, a transcription factor associated with ES cell pluripotency.

FGF1-FGFR2-heparin binding studies

HS, or its chemical analogue heparin, are co-receptors for the fibroblast growth factors (FGFs). We have investigated the interaction of heparin saccharides with FGF1 and its cognate receptor FGFR2. In the absence of FGF1, FGFR2 interacts with heparin oligosaccharides in a 1:1 ratio. We have previously shown that although FGF1 binds heparin hexasaccharides (dp6) as a monomer, it is dimerised by longer saccharides (*e.g.* dp12). It is likely that these two modes of interaction differ in their stability. Our findings indicate that dimerisation of FGF1 is essential for the formation of a stable signalling complex with FGFR2. It is possible that a cooperative mode of binding between FGF1 and heparin saccharides favours the formation of FGF1 dimers. This could explain how FGF1 drives FGFR dimerisation in the presence of large amounts of HS at the cell surface.

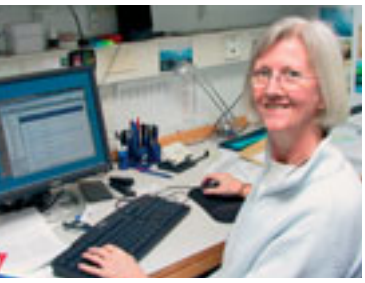


Identification and function of $GlcNH_3^+$ residues in HS

The rare N-unsubstituted glucosamine ($GlcNH_3^+$) residues in HS have important cell-biological and pathophysiological roles. However, we lack methods for isolation and identification of $GlcNH_3^+$. We have used the bacterial heparinases commonly employed for analysis of HS. Digestion of HS (chemically enriched in $GlcNH_3^+$), using a mixture of heparinases I, II and III, yielded four novel $GlcNH_3^+$-containing disaccharides that were subsequently identified as HexA-$GlcNH_3^+$, HexA-$GlcNH_3^+$(6S), HexA(2S)-$GlcNH_3^+$ and HexA(2S)-$GlcNH_3^+$(6S). Testing of individual heparinases revealed that heparinase I did not cleave at $GlcNH_3^+$ residues, whereas heparinase II and III were active. The action of heparinase II on $GlcNH_3^+$ requires additional O-sulphation, whereas heparinase III acts only in the absence of sulphation. This study has revealed striking distinctions in the substrate specificities of bacterial heparinases that can be exploited to analyse and quantify $GlcNH_3^+$ in HS.

Figure 2:
Domain structure of a section of the heparan sulphate chain. K5 lyase attacks the HS chain in non-sulphated regions of the polymer chain; its site specificity has enabled us to propose the new model of HS shown above in which the highly sulphated S-domains are flanked by regions of intermediate sulphation (T zones) forming areas of hypervariable composition with distinct growth factor binding properties. S-domain sequences can be disrupted by Heparinase I.

Publications listed on page 52



HEAD OF RESEARCH SERVICES
Jenny Varley

DEPUTY
Caroline Chadwick

Research Services

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

2004 has been a very exciting year for the Research Services at the Paterson Institute. We have again invested heavily in state-of-the-art equipment which we consider to be essential to maintain the high level of competitive science. We have purchased and installed a Deltavision System for multi-dimensional restorative microscopy, several items for the Histology Unit and a Becton Dickinson FACSAria cell sorter. In addition we have now greatly expanded our ability to carry out protein analysis with the joint purchase of an ABI Q Star XL Quadrupole Time of Flight (Q-ToF) tandem MS and an ABI 4000 Q Trap tandem MS, together with associated chromatography equipment.

Advanced Imaging Facility

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

Owing to the expansion of the Advanced Imaging Facility, the acquisition of new imaging tools and an escalation in the breadth of microscope users' applications, a large proportion of this year has been concerned with commissioning of equipment, the development of new techniques, training and consolidation. The facility is undergoing a period of upheaval as the microscopes have been relocated to temporary accommodation whilst new laboratories are being developed. The purpose of the new laboratories is for enhanced temperature control and environmental stability around each of the microscopes. The equipment will be located in the new laboratories by June 2005.

A multi-dimensional (*x, y, z, t*) fluorescent time-lapse system has been acquired for primarily mammalian investigations. The microscope permits high-resolution UV imaging along with a variety of lasers for photo-activation investigations. The microscope has the ability of multiple lateral and axial positions; thus the process of data acquisition is more meaningful and statistically relevant. The optics are well characterised which enables image reconstruction via deconvolution; consequently noise can be removed from the images which are generally of low signal and defects in the microscope light path can be corrected. This equipment has been in the laboratory for four months and is already over subscribed with four research groups within the Paterson utilising the equipment for multi-position volume imaging over time of both mammalian and yeast imaging.

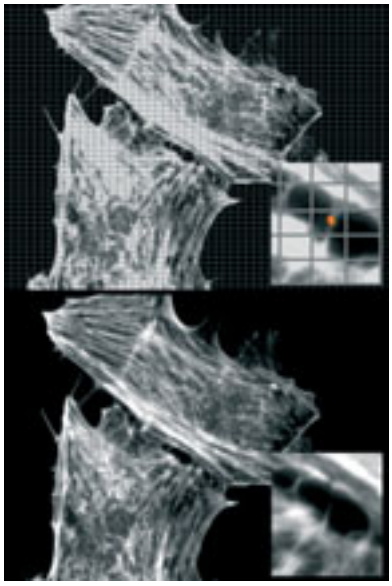
The procurement of an Argon Ion 200 mW laser and the design of a microscope coupling allows for the rapid illumination of samples with a high output laser light for photo-bleaching and ablation studies. The system runs under Metamorph which integrates laser exposure with multidimensional time lapse. Development of the equipment and technique is continuing, a laser spot size of 300 nm is presented to a sample currently and will prove to be invaluable in dynamic studies.

Three papers of note have utilised the facility and extensively employed techniques available within the visualisation laboratory over the last twelve months.

Grallert *et al.* (Genes Dev 2004; 18: 1007) showed that maturation of the *S. pombe* spindle-pole body occurs over consecutive cell cycles and reveals a role for NIMA-related kinase, Fin1, in modulating septum initiation network (SIN) activity. This study was dependent upon the time-lapse system built within the facility.

In a second study, Maekawa *et al.* (Genes Dev 2004; 18: 1709) showed that Cdk1-Clb4 controls the interaction of astral microtubule plus ends with subdomains of the daughter cell cortex. This study utilised spinning disk microscopy to generate 4D images at a high frequency.

The third report by Hart *et al.* (Br J Cancer 2005; 92: 503) examines the invasive characteristics of human prostatic epithelial cells utilising laser scanning



Bovine pulmonary artery endothelial cells (BPAEC) stained with BODIPY FL phallacidin for labelling F-actin visualised on the laser ablation/photobleaching system. A 300nm focus laser spot has been manipulated to bleach the fluorescent Actin near the cell/cell contact point. The upper image shows a low power laser spot and its position over the object to be irradiated by the laser. After an exposure time of 600msec the area of interest has been photobleached (lower image). These images demonstrate the utility of the laser ablation/photobleaching system and the precise definition of the laser spot.

confocal time lapse microscopy. A series of co-culture models of invasion were defined which simulated the blood/bone marrow stroma boundary and allowed the elucidation of the signalling and mechanics of trans-endothelial migration within the complex bone marrow environment. This study highlighted the requirement to examine the potential roles of signalling molecules and/or inhibitors, in co-culture models which mimic the complex environment of the bone marrow.

Over the coming year the development of new techniques with respect to presentation to the microscope and environmental control will be a necessity. The examination of some of the emerging novel fluorescent proteins will be vital for the field of co-localisation volume imaging.

Biological Resources

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

It is now three years since the total refurbishment in 2001 of the Biological Resources Unit and we can hardly remember what the old unit looked like. Today we are all used to working in a modern rodent facility, which runs smoothly and efficiently, providing research support to many different groups

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.

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 inbred 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 genetically 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 and in conjunction with the Molecular Biology Core Facility, 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.

CR-UK GeneChip Microarray Service

Head: Stuart Pepper

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

At the end of 2003 the GeneChip service had gained an extra member of staff so that we could provide a labelling service for our users. This extra service has proved very popular and we are now labelling the samples for all new projects. Given that at any one time we have 20-25 ongoing projects, this provides plenty of work in the lab. This year we passed two milestones – our first 1000 arrays hybridised (now over 1400) and over 100 projects approved by our review panel. We have now reached 110 approved projects distributed between 67 different CR-UK research groups.

Caroline Chadwick

In 2003 we were given funds to upgrade our scanner to a new high resolution system with an autoloader, and add a second fluidics station for performing staining and washing of arrays. With this improved set up, plus an extra member of staff, we expected to be able to process around 800 samples a year, even allowing for time to perform labelling reactions. In fact, by the end of October this year over 800 arrays had already been processed, and we are confident that over the coming year we will be able to keep up with demand for the service.

The most commonly used expression arrays are the Human Genome U133 plus 2.0 array for human work (with 54,000 probe sets) and the Mouse Expression 430 2.0 array for murine samples (with 45,000 probe sets). Use of these new larger arrays has lead to an increase in the size of data files; we now routinely supply results to our users on DVD rather than CD, as even small projects were spanning multiple discs. Alongside the human and mouse chips, Affymetrix have been expanding their range of genomic arrays and now offer 20 different species, including both *S. pombe* and *S. cerevisiae.*

There has been some development in labelling protocols this year, with Affymetrix releasing two new validated protocols. For the standard labelling protocol we now ask users to supply 5µg, rather than 25µg as for the previous protocol. Where projects utilise laser capture techniques, or use small biopsies, it is not always possible to obtain microgram quantities of RNA, for these projects we have a 2 cycle labelling protocol which works with less than 0.5µg total RNA.

During the year we have run a small project using SNP arrays for mapping chromosomal changes in tumours. The 10K SNP array has 11,500 probe sets each interrogating an individual SNP; for each SNP there are actually 10 probe pairs (perfect match and mismatch control) to confirm the SNP base on each strand. These arrays were initially designed for linkage studies, but are rapidly becoming accepted as an excellent means for high resolution mapping of loss of heterozygosity (LOH) in tumours. In collaboration with Maggie Knowles (CR-UK Clinical Centre, St James's University Hospital, Leeds) we have compared the results obtained using the SNP arrays to previous results from CGH experiments and found excellent concordance. With further analysis we have also used the SNP array data in a quantitative manner to show regions of amplification in cancer cell lines, again with excellent correlation to CGH data.

Central Services

Head: Caroline 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 preparation and sterile services, central stores and porters. The services provided can often go un-noticed by staff as the facility runs smoothly and efficiently, which is a sign that the group works well. During the year there have been a few changes with new members of staff arriving and some leaving. The new staff have fitted in well and have shown the potential to reach the eventual goal of staff being able to interchange between the roles provided by the group without problem or disruption to the end users.

The service has been going through some modernisation recently, with email being used to order goods from central stores which will lead to a new more efficient ordering system in the New Year linked to the development of the new intranet. More modernisation will include the new bar code system to be installed in lab services and central stores which aims to help with the increased growth in use of the stores which now includes well over one hundred different items from twenty different suppliers. There is also a continued expansion of the institute freezers for the supply of enzymes, reagents and media which in late 2004 will number five.

The media and plate pouring service is proving more popular than ever with three members of staff having to work hard to keep up with increasing demand, and the service continues to grow each month. All the lab aides are assigned labs in which

they tend to spend a few hours a week in helping with basic technical duties together with the laboratory staff. Alongside 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.

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 and delivering all goods delivered to the institute. They also deliver goods ordered from the central stores to the labs in the afternoons.

Flow Cytometry Unit

Head: Mike Hughes

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

The Flow Cytometry unit has four Becton Dickinson instruments. The FACSVantage SE Turbo sorter, the recently acquired FACSAria and the FACSCalibur analyser are located in the Flow Cytometry laboratory and the FACScan analyser is located in the Kay Kendal Laboratory. The unit has two full time staff who run the cell sorters. All users of the analysers undergo a training course followed by supervision before they can run their own samples. At present there are about one hundred users of the service from all groups within the institute.

The FACSVantage cell sorter has three lasers and can sort cells at speeds up to 15,000 per second whilst collecting data from two scatter parameters and up to five fluorescent parameters. The new FACSAria sorter is a bench top machine with three air cooled lasers. Its new fully digital electronics enables sorting at rates up to about 25,000 cells per second. It is also equipped with a new optics system comprising of an octagon and two trigons in order to minimise loss of light and therefore improve sensitivity. At present there are two scatter and nine fluorescence detectors on the Aria but there are also four spare detectors that can be used as new fluorochromes become available. The new FACSAria also has the ability to sort four populations of cells at the same time and multiple parameters can be used as thresholds making it easier to sort rare populations of cells.

The single cell sorting option is available on both the FACSVantage and the FACSAria, and sample temperature control and sterile sorts are also possible on both sorters. Analysis of the stem cell "side population" is possible on both sorters but the FACSVantage give a significantly better profile because of the 357 nm laser excitation.

This year, one of our major projects has been the sorting of mesodermal subpopulations of embryonic stem (ES) cells for the Stem Cell Biology and Stem Cell Haematopoiesis groups using green fluorescent protein (GFP) and Flk1 expression. The haematopoietic and endothelial lineages derive from mesoderm and develop through the maturation of a common progenitor, the haemangioblast, which expresses the tyrosine receptor Flk1. To investigate the developmental processes regulating mesoderm induction and specification to the haemangioblast, the *in vitro* differentiation of ES cells is used as a system model. An ES cell line that expresses GFP exclusively in the mesoderm germ layer, under the control of *Brachyury* regulatory sequences has been engineered to help track mesodermal subpopulations and study their specification toward blood lineages.

Following the *in vitro* differentiation of these ES cells, developing mesodermal progenitors can be isolated based on GFP expression. Co-expression of GFP with Flk1 reveals the emergence of three distinct cell populations, GFP^{-} $Flk1^{-}$, GFP^{+} Flk^{-} and GFP^{+} $Flk1^{+}$ cells, which represent a developmental progression ranging from pre-mesoderm to pre-haemangioblast to the haemangioblast. We have sorted these mesoderm subpopulations allowing the stem cell groups to study their molecular and biological characteristics.

The chamber in the FACSAria showing the flow cell illuminated by lasers.

Histology

Head: Garry Ashton

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

Over the last twelve months the unit has been fortunate enough to be able to replace three major pieces of equipment. A cryostat, which allows for the use of disposable blades, whilst also greatly reducing the problems associated with static, has seen heavy demand. A new Shandon Excelsior tissue processor, that relies on specific gravity to assess the quality of reagents, and a multi-programmable automated stainer have both brought major improvements to the service. Linked to the new equipment, the unit participates in an external quality assessment program to ensure the highest standards in both tissue processing and staining are achieved.

The Arcturus Laser Capture Microdissection equipment in the Histology Unit.

Generally all areas of the service have seen an increase in workload, particularly immunocytochemistry (ICC) and *in situ* hybridisation (ISH). 5T4 and cytokeratin expression in tumour cell lines was identified using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Other examples of the ICC performed include (a) the optimisation and validation of anti-XIAP antibodies for use as a pharmacodynamic endpoint assay, to determine the biological efficacy of antisense treatment in target tissue, (b) validation at protein level of genes that have been identified on a cDNA array based approach as up-regulated in pre-malignant and normal cervix, ultimately leading to the identification of patients who may not respond well to treatment.

Additionally, double immunostaining was used to demonstrate that apoptotic events in ATF2 knock out mice were derived from the cells of the endothelial compartment.

ISH has been used to show the expression pattern of genes expressed specifically in endothelial cells after induction by stress / inflammatory signals, and also to show that factors involved in the oxidative stress response, including members of the GPX and GST families, show a highly enriched expression pattern specific to vascular endothelial. An ISH protocol is also currently being developed to confirm the differential expression of the chemokine Eotaxin 2 in colorectal hepatic metastasis.

In conjunction with the Molecular Biology Core Facility, we have now validated protocols that will allow expression analysis on the Affymetrix platform of samples obtained by laser capture microdissection (LCM). Initially this was carried out on Haematoxylin & Eosin stained sections, with target cells identified by morphology. We are now evaluating techniques for identifying cells by immunophenotype prior to microdissection and analysis using Affymetrix GeneChips.

Finally, over the coming months the unit will purchase a tissue microarray instrument, which allows for the generation of multiple specimen slides that contain potentially hundreds of individual tissues. This technology enables researchers to perform high throughput microscopic studies using standard histological techniques. As well as the obvious advantages of speed and throughput, tissue microarrays conserve valuable material whilst also making standardisation of the methodology much simpler.

Molecular Biology Core Facility

Head: Stuart Pepper

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

The last year has seen numerous changes in the core facility, from minor upgrades to major acquisitions. Undoubtedly the most exciting new initiative has been the purchase, in collaboration with the University of Manchester (Professor Tony Whetton), of two state-of-the-art mass spectrometers for protein based applications: an ABI Q Star XL Quadrupole Time of Flight (Q-ToF) tandem MS and an ABI 4000 Q Trap tandem MS.

The Q Star offers excellent mass accuracy and resolution, and is well suited to 'routine' identification of sequencing of peptides, whether derived from gel or non-gel based separation approaches. The Q Trap is a unique hybrid instrument in that it is a triple quadrupole instrument with ion trapping capabilities. This makes the instrument amenable to studies on post-translational modifications such as phosphorylation and has allowed development of novel strategies for identification of phosphorylation sites on known, enriched proteins. To provide a suitable range of delivery options to match the capabilities of the two mass spec systems we have three LC Packings Ultimate Nano LC systems, two of which are able to perform two-dimensional chromatography. The LC systems are fitted with Famos autosamplers to allow fully automated sample injection with maximum precision and zero sample loss. These machines have been used in a number of pilot studies and are already demonstrating very impressive performance for routine protein identification, either from Silver stained gel spots or from complex LC fractionation, as well tremendous sensitivity for detecting post translational modifications such as phosphorylation.

Recently a non-gel based relative quantification method has become available. The method allows parallel analysis of up to four samples, and is capable of generating relative quantitation results on hundreds of proteins. This Isotope Tag Relative Absolute Quantification (iTRAQ) technique has

The Molecular Biology team receiving the Christies 2004 "Research Team of the Year" staff award from Sir Alex Ferguson. From left to right, Stuart Pepper, Yvonne Hey, Gillian Newton, Sir Alex, Yvonne Connolly and Emma Saunders.

huge benefits and has been trialled at the Paterson Institute in collaboration with ABI. We are now undertaking a large scale comparison looking at the correlation of protein and transcript levels in normal versus tumour cell lines.

Whilst these developments have been happening, other areas of the core facility have had some improvements too. We now have a new digital imaging system, primarily for chemiluminescent imaging, plus image analysis software available across the PICR network. A new ABI7900 has been installed for real time PCR analysis; this system has a 384-well card platform that allows high throughput PCR without the need for complex robotics to facilitate 384-well pipetting.

Alongside these developments the core services of plasmid preparation and DNA sequencing have also been maintained, with both services having a significant increase in demand. The combination of an automated fluidics system for DNA purification, coupled with a 16 capillary sequencer, has allowed us to manage a throughput of up to 150 samples a day for these services. As 2004 comes to a close we are looking at new reagents for DNA preparation that will allow faster processing times and even higher daily throughputs.

Kostoris Medical Library

Head: Steve Glover

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

In 2004 the medical library has continued to move towards a predominantly online service and now provides the staff of the Paterson Institute with access to over 2.2 million full text articles available from the desktop. In collaboration with the University of Manchester John Rylands Library, this figure is closer to 3.5 million articles. Access to this service is centred on the Library's Intranet pages and 2004 has seen a high volume of page requests by Paterson staff. Some of this year's web-log figures are as follows:

Electronic journals	7461
Electronic databases	2802
Impact factor queries	975

In addition to the high-volume of internal web page requests, the medical library delivered electronic tables of contents from more than 120 journal titles over the course of the year. This equated to approximately 14500 alerts delivered on or ahead of publication to individual mailboxes.

As a result of these services e-journal usage has climbed throughout 2004. Most publishers now supply usage data to a common standard known as COUNTER. This allows the library to assess how well our paid resources are being utilised by PICR staff. Some of the top utilised titles (number of full text downloads) from our various licences are:

Nature	5488
New England Journal of Medicine	4580
Cancer Research	4206
Journal of Clinical Oncology	3936
Proc Natl Acad Sci USA	3538

The Library provides a series of pro-active Current Awareness Services (CAS) with monthly searches of databases such as Medline, Embase, BIOSIS, and ISI's Web of Science. In 2004 the library will have conducted over 370 database searches for institute staff, mainly on Web of Science and PubMed and delivered via email.

In September 2004 Anne Webb and Riz Zafar were invited to present to the European Association for Health Information and Libraries (EAHIL) biennial conference in Santander, Spain. The presentation discussed how the medical library has integrated local, regional, and national electronic resources into a hybrid library setting.

In 2005 the library hopes to further develop the information intelligence side of the operation to help support the needs of the research groups and support services.

Staff make use of the library facilities

Publications

BIOINFORMATICS GROUP *(page 4)*
Crispin J Miller

Refereed Research Papers

Erler, J.T., Cawthorne, C.J., Williams, K.J., Koritzinsky, M., Wouters, B.G., Wilson, C., Miller, C., Demonacos, C., Stratford, I.J. and Dive, C. (2004) Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. *Mol Cell Biol*, **24**, 2875-2904.

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CARCINOGENESIS GROUP *(page 6)*
Geoff Margison

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CELL DIVISION GROUP *(page 8)*
Iain Hagan

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CELL REGULATION GROUP *(page 10)*
Nic Jones

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CELL SIGNALLING GROUP *(page 12)*
Angeliki Malliri

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CELLULAR AND MOLECULAR PHARMACOLOGY GROUP *(page 14)*
Caroline Dive

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Caroline Dive & Malcolm Ranson

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FUNCTIONAL GENOMICS AND CELL CYCLE REGULATION GROUP *(page 18)*

Karim Labib

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GENE THERAPY GROUP *(page 20)*

Lez Fairbairn

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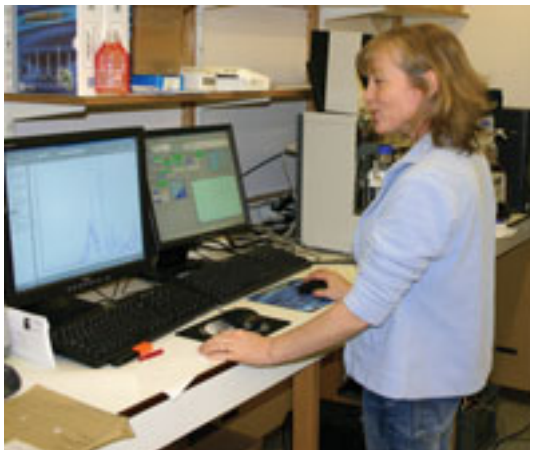

IMMUNOLOGY GROUP *(page 22)*
Peter L Stern

Refereed Research Papers

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MITOTIC SPINDLE FUNCTION AND CELL CYCLE GROUP *(page 24)*

Elmar Schiebel

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RADIOCHEMICAL TARGETING AND IMAGING GROUP *(page 26)*

Jamal Zweit

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STEM CELL BIOLOGY GROUP *(page 28)*

Georges Lacaud

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STEM CELL AND HAEMATOPOIESIS GROUP *(page 30)*

Valerie Kouskoff

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STRUCTURAL CELL BIOLOGY GROUP *(page 32)*

Terence D Allen

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Other Publications

Drummond, S. and Allen, T.D. (2004) Structure, function and assembly of the nuclear pore complex. In: *The Nuclear Envelope*, Editors: Evans, D.E., Hutchison, C.J. and Bryant, J.A. SEB Symposium Series **Vol 16** pp 89-106 Garland Science/ Bios Scientific, Oxford UK.

Allen T.D., Hutchison, E., Murray, S. and Rutherford, S.A. (2004) Surface visualisation and immunological characterisation of the nuclear cytoplasmic interface *in situ*. *Microscopy and Microanalysis* **10,** (Supp 2) 1218-1221.

Kiseleva, E. (2004) Nuclear pore complexes in a *Saccharomyces cerevisiae* nucleus [Cell of the Month] *Nature Rev Mol Cell Biol,* **5**, 427.

MEDICAL ONCOLOGY: GENE-IMMUNOTHERAPY GROUP *(page 34)*

Robert Hawkins

Refereed Research Papers

Armstrong, A.C., Dermime, S., Mulryan, K., Stern, P.L., Bhattacharyya, T. and Hawkins, R.E. (2004) Adoptive transfer of anti-idiotypic T cells cure mice of disseminated B cell lymphoma. *J Immunother*, **27**, 227-231.

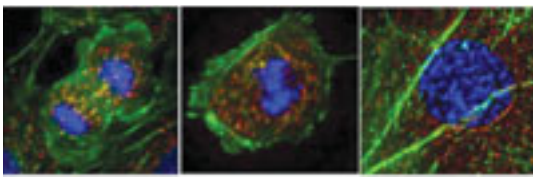
Li, Y., Tew, S.R., Russell, A.M., Gonzalez, K.R., Hardingham, T.E. and Hawkins, R.E. (2004) Transduction of passaged human articular chondrocytes with adenoviral, retroviral, and lentiviral vectors and the effects of enhanced expression of SOX9. *Tissue Eng*, **10**, 575-584.

Meziane el, K., Bhattacharyya, T., Armstrong, A.C., Qian, C., Hawkins, R.E., Stern, P.L. and Dermime, S. (2004) Use of adenoviruses encoding CD40L or IL-2 against B cell lymphoma. *Int J Cancer*, **111**, 910-920.

Other Publications

Dermime, S., Gilham, D.E., Shaw, D.M., Davidson, E.J., Meziane el, K., Armstrong, A., Hawkins, R.E. and Stern, P.L. (2004) Vaccine and antibody-directed T cell tumour immunotherapy. *Biochim Biophys Acta Rev Cancer*, **1704**, 11-35.

Pedley, R.B., Sharma, S.K., Hawkins, R.E. and Chester, K.A. (2004) Antibody-directed enzyme-prodrug therapy. *Methods Mol Med*, **90**, 491-514.



MEDICAL ONCOLOGY: GLYCOANGIOGENESIS GROUP

(page 36)

Gordon Jayson

Refereed Research Papers

Clamp, A., Adams, M., Atkinson, R., Boven, E., Calvert, A.H., Cervantes, A., Ganesan, T., Lotz, J., Vasey, P., Cheverton, P. and Jayson, G.C. (2004) A phase IIA study of the topoisomerase I inhibitor, exatecan mesylate (DX-8951f), administered at two different dose schedules in patients with platinum- and taxane-resistant/refractory ovarian cancer. *Gynecol Oncol*, **95**, 114-119.

Davies, E.J., Blackhall, F.H., Shanks, J.H., David, G., McGown, A.T., Swindell, R., Slade, R.J., Martin-Hirsch, P., Gallagher, J.T. and Jayson, G.C. (2004) Distribution and clinical significance of heparan sulfate proteoglycans in ovarian cancer. *Clin Cancer Res*, **10**, 5178-5186.

He, X., Brenchley, P.E., Jayson, G.C., Hampson, L., Davies, J. and Hampson, I.N. (2004) Hypoxia increases heparanase-dependent tumor cell invasion, which can be inhibited by antiheparanase antibodies. *Cancer Res*, **64**, 3928-3933.

Hipkins, J., Whitworth, M., Tarrier, N. and Jayson, G. (2004) Social support, anxiety and depression after chemotherapy for ovarian cancer: a prospective study. *Br J Health Psychol*, **9**, 569-581.

Jayson, G.C., Parker, G.J., Mullamitha, S., Valle, J.W., Saunders, M., Broughton, L., Lawrance, J., Carrington, B., Roberts, C., Issa, B., Buckley, D.L., Cheung, S., Davies, K., Watson, Y., Zinkewich-Peotti, K., Rolfe, L. and Jackson, A. (2004) Blockade of platelet-derived growth factor receptor-beta by CDP860, a humanized, PEGylated di-Fab', leads to fluid accumulation and is associated with increased tumor vascularized volume. *J Clin Oncol*, epub Oct 4, printed 2005, **23**, 973-981.

Vasey, P.A., Jayson, G.C., Gordon, A., Gabra, H., Coleman, R., Atkinson, R., Parkin, D., Paul, J., Hay, A. and Kaye, S.B. (2004) Phase III randomized trial of docetaxel-carboplatin versus paclitaxel-carboplatin as first-line chemotherapy for ovarian carcinoma. *J Natl Cancer Inst*, **96**, 1682-1691.

Whitworth, M.K., Whitfield, A., Holm, S., Shaffer, J., Makin, W. and Jayson, G.C. (2004) Doctor, does this mean I'm going to starve to death? *J Clin Oncol*, **22**, 199-201.

Other Publications

Duff, S.E. and Jayson, G.C. (2004) Vascular endothelial growth factor D: biology, functions and role in cancer. In *VEGF and Cancer*. Editor: Harmey, J.H. Publisher: Kluwer Academic/Plenum Publishers/Landes Bio-science.

Hasan, J., Shnyder, S.D., Bibby, M., Double, J.A., Bicknel, R. and Jayson, G.C. (2004) Quantitative angiogenesis assays in vivo - a review. *Angiogenesis*, **7**, 1-16.

Ton, N.C. and Jayson, G.C. (2004) Resistance to anti-VEGF agents. *Curr Pharm Des*, **10**, 51-64.

MEDICAL ONCOLOGY: PROTEOGLYCAN GROUP

(page 38)

John T Gallagher

Refereed Research Papers

Davies, E.J., Blackhall, F.H., Shanks, J.H., David, G., McGown, A.T., Swindell, R., Slade, R.J., Martin-Hirsch, P., Gallagher, J.T. and Jayson, G.C. (2004) Distribution and clinical significance of heparan sulfate proteoglycans in ovarian cancer. *Clin Cancer Res*, **10**, 5178-5186.

Lyon, M., Deakin, J.A., Lietha, D., Gherardi, E. and Gallagher, J.T. (2004) The interactions of hepatocyte growth factor/scatter factor and its NK1 and NK2 variants with glycosaminoglycans using a modified gel mobility shift assay. Elucidation of the minimal size of binding and activatory oligosaccharides. *J Biol Chem*, **279**, 43560-43567.

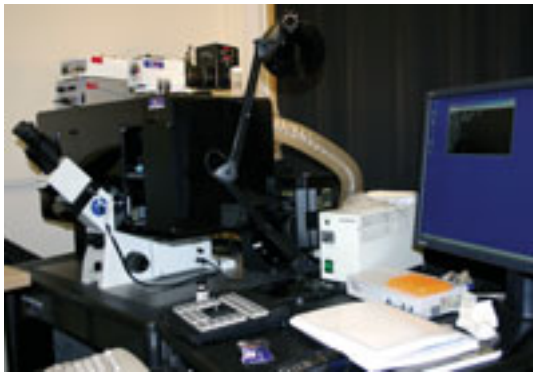
Murphy, K.J., Merry, C.L., Lyon, M., Thompson, J.E., Roberts, I.S. and Gallagher, J.T. (2004) A new model for the domain structure of heparan sulfate based on the novel specificity of K5 lyase. *J Biol Chem*, **279**, 27239-27245.

O'Keeffe, D., Olson, S.T., Gasiunas, N., Gallagher, J., Baglin, T.P. and Huntington, J.A. (2004) The heparin binding properties of heparin cofactor II suggest an antithrombin-like activation mechanism. *J Biol Chem*, **279**, 50267-50273.

Pankonin, M.S., Gallagher, J.T. and Loeb, J.A. (2004) Specific structural features of heparan sulfate proteoglycans potentiate neuregulin-1 signaling. *J Biol Chem*, epub Nov 04, printed 2005, **280**, 383-388.

ten Dam, G.B., van de Westerlo, E.M., Smetsers, T.F., Willemse, M., van Muijen, G.N., Merry, C.L., Gallagher, J.T., Kim, Y.S. and van Kuppevelt, T.H. (2004) Detection of 2-O-sulfated iduronate and N-acetylglucosamine units in heparan sulfate by an antibody selected against acharan sulfate (IdoA2S-GlcNAc)n. *J Biol Chem*, **279**, 38346-38352.

Viviano, B.L., Paine-Saunders, S., Gasiunas, N., Gallagher, J. and Saunders, S. (2004) Domain-specific modification of heparan sulfate by Qsulf1 modulates the binding of the bone morphogenetic protein antagonist Noggin. *J Biol Chem*, **279**, 5604-5611.



Roberts, S.A., Hendry, J.H., Swindell, R., Wilkinson, J.M. and Hunter, R.D. (2004) Compensation for changes in dose-rate in radical low-dose-rate brachytherapy: a radiobiological analysis of a randomised clinical trial. *Radiother Oncol*, **70**, 63-74.

Scott, D. (2004) Chromosomal radiosensitivity and low penetrance predisposition to cancer. *Cytogenet Genome Res*, **104**, 365-370.

Zoubiane, G.S., Valentijn, A., Lowe, E.T., Akhtar, N., Bagley, S., Gilmore, A.P. and Streuli, C.H. (2004) A role for the cytoskeleton in prolactin-dependent mammary epithelial cell differentiation. *J Cell Sci*, **117**, 271-280.

ADDITIONAL PUBLICATIONS

Refereed Research Papers

Barlow, J.W., Mous, M., Wiley, J.C., Varley, J.M., Lozano, G., Strong, L.C. and Malkin, D. (2004) Germ line BAX alterations are infrequent in Li-Fraumeni syndrome. *Cancer Epidemiol Biomarkers Prev*, **13**, 1403-1406.

Booth, C., Booth, D., Williamson, S., Demchyshyn, L.L. and Potten, C.S. (2004) Teduglutide ([Gly2]GLP-2) protects small intestinal stem cells from radiation damage. *Cell Prolif*, **37**, 385-400.

Kim, J.Y., Kim, C.H., Stratford, I.J., Patterson, A.V. and Hendry, J.H. (2004) The bioreductive agent RH1 and gamma-irradiation both cause G2/M cell cycle phase arrest and polyploidy in a p53-mutated human breast cancer cell line. *Int J Radiat Oncol Biol Phys*, **58**, 376-385.

Kim, J.Y., Patterson, A.V., Stratford, I.J. and Hendry, J.H. (2004) The importance of DT-diaphorase and hypoxia in the cytotoxicity of RH1 in human breast and non-small cell lung cancer cell lines. *Anticancer Drugs*, **15**, 71-77.

Seminar Speakers 2004

Our external seminar series was curtailed in late 2004 by the onset of building works associated with refurbishment of areas in the North end of the building adjacent to our lecture theatre. Unfortunately this disruption will continue into 2005, but the refurbishment will include a new ventilation system in the lecture theatre, which will be welcomed by all. In addition to our regular Paterson seminars, there are also seminars organised by the gene and immunotherapy groups and the Christie Oncology groups, as well as postgraduate seminars. Last but not least we held our annual Institute Colloquium in September, this year in Chester. We were fortunate to have tempted Gerard Evan away from the Californian sun to be our guest speaker, and he not only gave a spectacular plenary lecture, but also provided excellent feedback throughout the meeting. We always greatly appreciate the time that our external guests give us in their extremely busy schedules and every Colloquium benefits immensely from their presence.

January 2004

19 Jan	Sergio Moreno (Cancer Research Institute, Salamanca, Spain)
22 Jan	Alan Clarke (University of Cardiff)
29 Jan	Bruno Amati (Department of Experimental Oncology, Milan, Italy)

February 2004

12 Feb	Austin Smith (Institute for Stem Cell Research, Edinburgh)
18 Feb	Thomas Goehler (University of Hamburg, Germany)
19 Feb	Tony Kouzarides (Wellcome Trust/CR-UK Institute, Cambridge)

March 2004

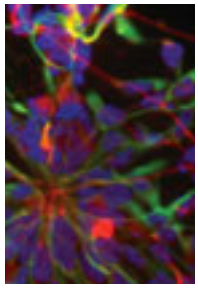
4 Mar	Brad Ozanne (Beatson Institute)
11 Mar	Margaret Frame (Beatson Institute)
18 Mar	Clemens Schmitt (Humboldt University, Berlin, Germany)
25 Mar	Francis Barr (Max Planck Institute, Martinsried, Germany)

April 2004

1 Apr	Manfred Schmidt (University of Freiburg, Germany)
15 Apr	Herbie Newell (Northern Institute of Cancer Research, Newcastle)
22 Apr	Simon Boulton (Clare Hall Laboratories, CR-UK London Research Institute)
22 Apr	Brian Huntly (Harvard University, USA)
29 Apr	Keith Caldecott (Genome Centre, University of Sussex)

May 2004

6 May	Francisco Vega (Cancer Research Institute, Salamanca, Spain)
6 May	Jan-Michael Peters (IMP, Vienna, Austria)
13 May	Michael Bornens (Institut Curie, Paris, France)
27 May	Dennis Headon (University of Manchester)

June 2004

10 Jun David Ish-Horowitz (CR-UK London Research Institute)
16 Jun Adrian Thrasher (Institute for Child Health, London)
21 Jun Nagesh Kalakonda (Memorial Sloan Kettering, New York, USA)
28 Jun Andrew McAinsh (MIT Department Of Biology, Cambridge, USA)

July 2004

12 Jul Jan Paluh (Boston College, USA)
15 Jul Ludger Hengst (Max Planck Institute, Martinsried, Germany)

September 2004

16 Sep Steve Jackson (Wellcome Trust/CR-UK Institute, Cambridge)
30 Sep Norman Maitland (University of York)

October 2004

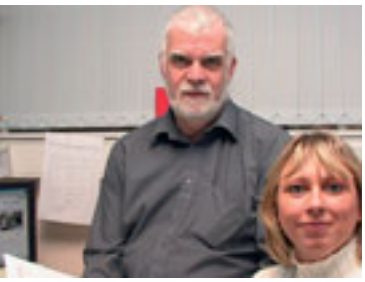
19 Oct Caroline Alexander (McCardle Cancer Centre, Wisconsin, USA)
28 Oct Constanze Bonifer (University of Leeds)

November 2004

16 Nov Nimesh Joseph (Bangalore)

December 2004

2 Dec Clive Stanway (Cancer Research Technology)
21 Dec Rene Bernards (Netherlands Cancer Institute, Amsterdam)



POSTGRADUATE TUTOR
Graham J Cowling

EDUCATION COORDINATOR
Julie Edwards

EDUCATION COMMITTEE 2004
Terry Allen (Chairman, until September)
Iain Hagan (Chairman, from September)
Graham Cowling
Caroline Dive
Dave Gilham
Valerie Kouskoff
Catherine Merry
Crispin Miller
Jenny Varley

Postgraduate Education

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

The Paterson Institute and Christie Hospital is the major centre for research and teaching in the Division of Cancer Studies, School of Medicine, University of Manchester. We continue to provide an excellent cancer research training programme for postgraduate students and clinical research fellows studying for MPhil, MD and PhD degrees by research. This active research environment serves all our students equally. They draw on the wide expertise of our scientists and clinicians and gain benefit from our modern laboratories and first-rate service units. Our training programme aims to improve effectiveness in 'cutting edge' research, provide the latest professional and management skills and enhance chosen career development.

The Paterson PhD Programme

We welcomed five new research students and four clinical research fellows to the Institute to join over 50 postgraduates who are at various stages of their degrees within the Division of Cancer Studies. Their contributions to the scientific work of the Institute are described elsewhere in this report. This year the Institute has four CR-UK funded Paterson students, from the UK, Ireland, Germany and Japan. They were selected from over 250 applicants to begin our PhD training programme. The CR-UK Department of Medical Oncology also welcomed postgraduates from the around the world. Our new student from Hong Kong won a prestigious Manchester University Dorothy Hodgkin's International Studentship to allow her to study in Manchester. The PhD student who pioneered our studentships which offer three 10-week rotation periods in different labs mastered a remarkable range of skills in his first year. The remaining students based in single labs for the entirety of their studies have achieved a strong basis for their final thesis research and several have already published parts of their work. Our programme is still evolving and, in 2005, we are offering an even more flexible approach to PhD training with many more CR-UK studentships on offer. Successful UK, European and International candidates will be offered single four-year projects in one lab, but we will be also able to support a first year rotation option in our research groups if appropriate. The former scheme aims to appeal to students who have completed a masters degree or equivalent or who may have spent a year working in a research laboratory and have a focused view of the research area they wish to pursue.

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. To further enhance our training programme, in 2004 a monthly series of cancer biology master-classes has been introduced; this allows group heads in the Paterson and Christie Hospital to explain the "what", the "how" and the "why" of their area of research. These monthly evening sessions are proving a success with all of our postgraduate students and many of the postdoctoral fellows taking part in scientific discussions, often outside of their area. Students also participate in a structured faculty graduate training programme of lectures and short courses. 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). 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 Universities. Individual students, with advice from their supervisor and advisor, take combinations of these courses that enhance their field of study over the 3-4 years of study.

In 2004, the Division of Cancer Studies introduced a more formalised system for assessing the quality of all MPhil, MD and PhD projects. Based on research council principles, the Education Committee, with help from internal assessors, ensure that the work will form the basis of a stimulating and intellectually challenging postgraduate programme. This process takes place well before the candidate is chosen. The Education Committee, a body made up of senior scientists, postdoctoral fellows and student representatives, continue to assess student progress throughout their 3-4 years of study and ensure, along with their supervisors and advisors, that the student achieves their degree goal. Assessment is through written reports at 3 months (literature review), 1 year and 2 years and short talks to the Institute at similar times (6, 12, 24 months and as part of the final *viva voce*). Students self-assess their own performances with input from their supervisors and advisors at regular intervals throughout their programme. All students have the opportunity to give written feedback on any aspect of their degree programme using 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.

Students are offered advice and training in poster and oral communication. All postgraduates are expected to present their research at group, Education Committee, national or international scientific meetings including the annual Paterson Colloquium. Course units taken, seminars attended feedback from the Education Committee on various student assessment talks and meetings with supervisors and advisors are recorded in a Personal Development Record called the Postgraduate Handbook issued by the University to each student. This is an audited account of progress and achievement throughout their years of study. Workshops are held at the University in the first week of study and at the end of each year covering different skills including communication and exploring future career pathways.

Chained to the bench for 4 years?

In the few hours when our students are not working, our student representatives Kelly Chiang, Laura Hollins, Becky Baldwin 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 the annual student social to meet other new postgraduates and, in 2004, this took the form of a "Games" night at a local bar (see picture).

Paterson students enjoying pizza before "the games" began.

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. They comprise Admin Services (including Human Resources & Reception), Director's Office, Estates & Facilities, Finance and Purchasing, Health & Safety and IT. It has been a very busy year for the team; firstly all the administrative services were reviewed to ensure that they were working in the most efficient and effective way and secondly (and more importantly) the team undertook a lot of the preparatory work to support the Director for the CR-UK Institute Review in July.
In addition to this, the department commissioned a new logo and 'advertising brand' for the Institute, redesigned the Institute's Reception area and oversaw the development of the Paterson's new website.

Admin Services (including Human Resources & Reception)

Manager: Julie Hallett
Ben Bridgeman, Trevor Haughton, Shirley Leonard, Anna Pearson

It has been another demanding and fulfilling year for the Admin team. The department has continued to develop its services to the groups and has, once again, undertaken some very unusual, challenging and rewarding tasks.

Anna, the Institute's HR Advisor, has continued to develop her role and knowledge, under the Associate Director's guidance, and has helped and advised a number of existing staff and new recruits on a diverse number of subjects including pensions, paternity leave and work permits. She should be congratulated for successfully completing another two electives towards her CIPD (Employment Law and Employee Reward). Shirley, the Institute's Recruitment Officer, played a key role in the specification and development process for the new recruitment software which was installed in September.

It was also "all change" on Reception in the summer with the unveiling of the stunning new reception area. To accompany this change a security company has been employed to staff reception and Trevor, the new security/receptionist, has proved to be an invaluable member of the team.

Director's Office

Director's PA: Elaine Mercer

2004 was a busy year for the Director's Office; comprehensive support was provided for the Institute Review and for the Director. Assistance was also given to various special projects and the editing and the production of the Paterson Newsletter was undertaken by this office.

Estates & Facilities

Manager: Steve Alcock
John Lord, Dennis O'Shea

2004 was another busy year for the Estates & Facilities team; the department gained two new members of staff: John Lord, who was appointed as the Institute's Maintenance Technician, and Dennis O'Shea who is on secondment from the Christie Hospital to act as the liaison officer for the duration of the Translational Research Facility (TRF) project. Major areas of the Institute have undergone an upgrade of the ventilation system, and the benefit will certainly be felt by Institute staff in summer 2005. The TRF contract is now under way, which has resulted in parts of the Institute undergoing some upheaval whilst the enabling works are completed and staff are relocated to their temporary accommodation.

ADMINISTRATIVE SERVICES

Finance & Purchasing

Manager: Margaret Lowe
Catherine Bentley, Liz Fletcher, Kate McCoy, Denise Owen, Debbie Suthern

In the light of new groups joining the Institute and the corresponding increase in demands on the department, the opportunity was taken in 2004 to review the finance and purchasing services to ensure that they were tailored to the future needs of the Institute, whilst still complying with the legal requirements of the Christie Hospital NHS Trust. Consequently, the team is confident that it can provide the high levels of service required for the continuing growth of the Institute. Each member of staff is familiar with all aspects of the services provided by the department to ensure adequate cover at all times.

Software for a new ordering system has now been identified and is currently being adapted to the Institute's specific requirements. It is hoped that this will be in place for the new financial year in April 2005.

Health & Safety

Manager: Colin Gleeson

A series of planned safety inspections of the Institute has been developed and is being implemented. Following inspection, each department and /or facility will receive a report detailing any appropriate remedial actions to improve and safeguard the health, safety and welfare of their staff. The inspection programme includes participation by staff, including union members, to encourage active participation in health and safety and compliance with legislative requirements.

A number of guidance documents and forms have been produced relating to work with biological agents, genetically modified organisms, compressed gases, liquid nitrogen, fire, lone working and area and equipment decontamination. It is envisaged these documents, and others still in development, will be rolled out to staff via an updated Institute intranet. They will form a valuable source of information concerning the key work-related hazards in the Institute.

The Translational Research Facility project has now begun in earnest. The Institute is working hard to develop a constructive working relationship with the contractors and their workforce, in order to minimise the disruption and impact of the building work on Institute activities and maintain a safe working environment for Institute staff and construction workers alike.

The Environmental Agency visited and inspected the Institute to review its arrangements for the use, storage and disposal of radio-chemicals. The report received was favourable with only one minor recommendation which has subsequently been implemented.

The *in situ* organisational arrangements of the Institute Joint Safety Committee and the Christie Hospital Trust Health and Safety, Genetic Modification and Radiation Protection committees continue to play an important role in overseeing and monitoring health and safety performance within the Institute.

IT

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

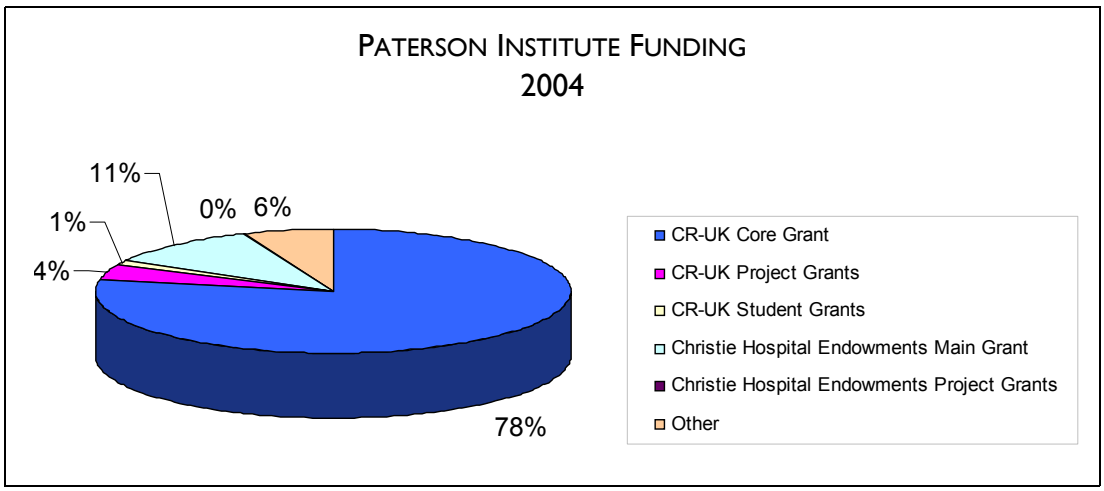
It has been another busy year for IT developments. The team is building upon a robust infrastructure to provide a faster, more secure working environment. New ways of providing enhanced productivity and access to systems have been identified and will be put into practice in early 2005. One such project will deal with the provision of full independence from the desktop by enabling 'Wire' free access within the Institute. A number of IT challenges are ahead including the provision of Disaster Recovery Services, the expansion of network services and the development of systems to encompass the new Translation Research Facility building.

Margaret Lowe

The Admin team

Acknowledgement for Funding of the Paterson Institute

The major source of funding (78%) 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 the 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 just over 11% of the total income.

The final 6% of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- National Translational Cancer Research Network (NTRAC)
- European Commission
- Centocor
- Medical Research Council
- Association of International Cancer Research
- Astra Zeneca
- Wellcome Trust
- Active Biotech
- Action Research
- Friends of Rosie

We are immensely grateful to all our sponsors.

Donations to the Institute in 2004

Legacies

- In memory of Gladys Maud Emerson
- In memory of Florence Butler
- In memory of Mrs CM Ord
- In memory of Elsie Pierpoint

Donations

- Dr TW Brindle & Mrs D Brindle
- Oldham SNU Church
- Mrs E Longden
- Mrs P Johnson
- Mr Neil Raghib
- Miss Carol Davidson
- Mr R Longden
- Mr & Mrs G Mellor in memory of Lucinda Shallcross and Lucinda Henry
- Mr N Drysdale, Streetbridge Inn
- Mrs M Collier in memory of Bill Collier
- Rotary Club of Colne Noyna
- Mrs P Watkinson in memory of Irene Brown
- The Ladies Committee of Prosperity Lodge No 5206, Accrington
- Women's Trust Fund
- Holt's Brewery
- East Lance Christie Crusaders in memory of Simon Brown
- SL Yearsley

Representatives from Holt's brewery and Sir Bobby Charlton present a cheque to the Paterson Institute to enable us to purchase the new Shandon Excelsior tissue processor for the Histology Unit *(Photograph courtesy of the Christie Hospital)*

Career Opportunities

The Paterson Institute is located alongside the Christie Hospital, and has a strong programme of basic and translational research. In addition, there are very close links with the University of Manchester. 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 56). 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.

Fletcher Moss Botanical Gardens, Didsbury
(Photograph courtesy of Steve Royle)

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.

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 by the West Coast main line rail service.