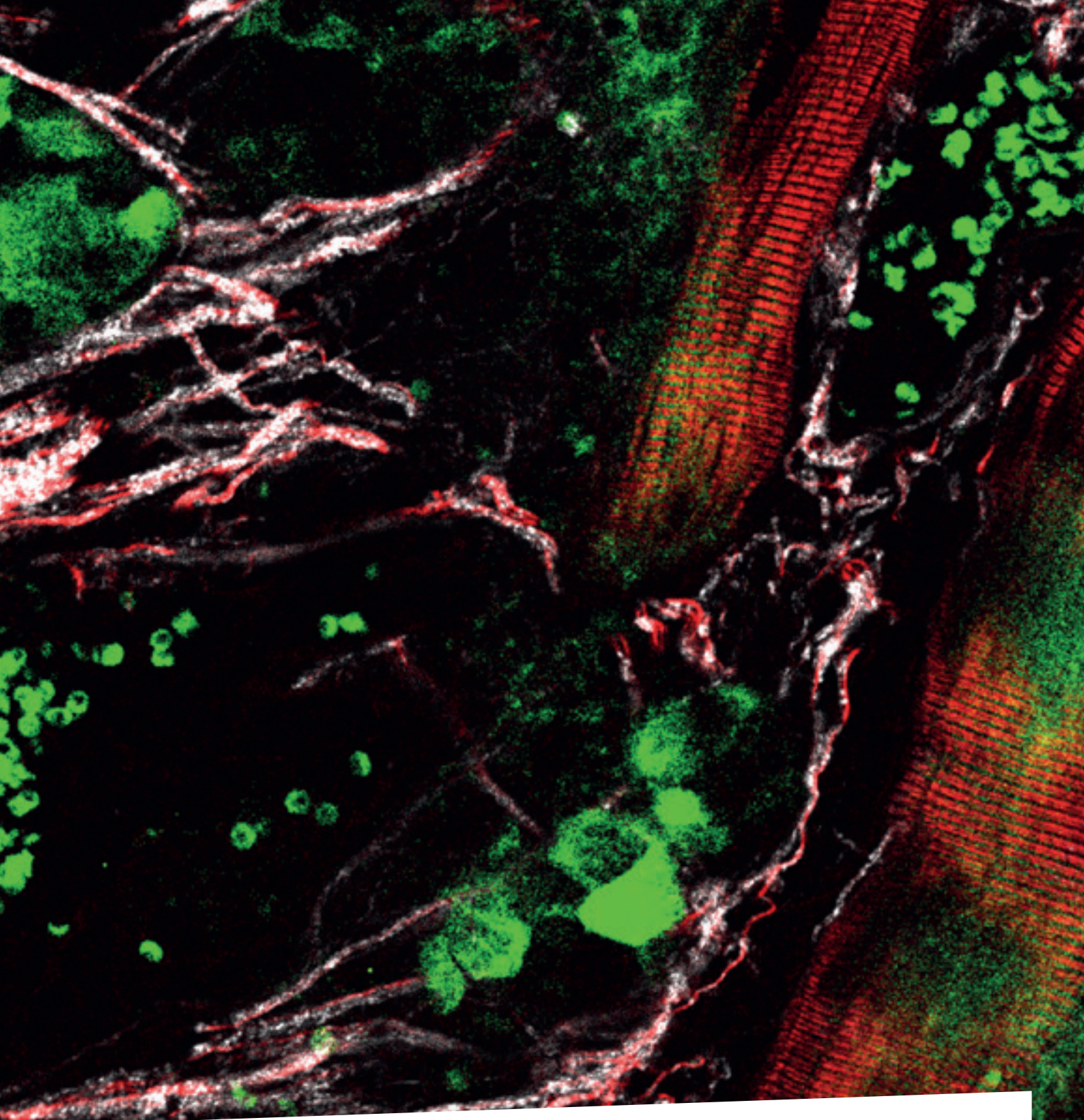

SCIENTIFIC REPORT 2013

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Cover image
Invading cancer cells (green) in muscle layers (red) are surrounded by collagen (red and white).

Image provided by Haoran Tang of the Molecular Oncology group.

SCIENTIFIC REPORT 2013

MANCHESTER INSTITUTE

MANCHESTER 1824
The University of Manchester

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The Cancer Research UK Manchester Institute
is located in The Paterson Building

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DIRECTOR'S INTRODUCTION

Professor Richard Marais
Director of The Cancer Research UK Manchester Institute

Professor Caroline Dive
Deputy Director of The Cancer Research UK Manchester Institute

It has been a highly eventful year for the Institute with a new identity, a major centre award, Group Leader recruitment and the continuation of our drive to extend the capabilities of our core research services. Our two largest research teams underwent very successful Quinquennial Reviews and towards the end of the year, Tim Somervaille was promoted to Senior Group Leader.

Our rebranding to the Cancer Research UK Manchester Institute (CRUK MI), that took place in October, acknowledges the core funding that we receive from the charity and recognises the generosity of our supporters. We renamed the site where we are located as *The Paterson Building* to ensure that we continue to celebrate the legacy of Edith and Ralston Paterson and the impact that they had on cancer research and treatment in Manchester. During the year, the finalisation of the management structure of the Institute was completed with the appointment of Caroline Dive as Deputy Director and Stuart Pepper and Caroline Wilkinson taking on the roles of Chief Laboratory Officer and Chief Operating Officer respectively.

Major research highlights from 2013 included the identification of driver mutations for non-small cell lung cancer; a study which uncovered a novel mechanism by which melanoma develops resistance to BRAF targeted drugs; the identification of critical oncogenic factors in acute myeloid leukaemia; the development of a unique approach to study small cell lung cancer using patient-derived circulating tumour cell explant mouse models; and the elucidation of key mechanistic insights into the control of mitosis.

The Drug Discovery Unit, led by Donald Ogilvie, and the Clinical Experimental Pharmacology Group, led by Caroline Dive, performed extremely well at their respective Quinquennial reviews. These groups have a particularly strong translational focus that lies at the heart of our strategy at the CRUK MI. The expertise of the CEP group in circulating tumour cells will play a key role in the ground-breaking TRACERx (Tracking Cancer Evolution through Treatment) lung cancer study announced by CRUK in 2013. This £14 million study spanning nine years will be performed by a network of teams around the UK with the aim of tracking how lung tumours develop and evolve as patients receive treatment.

Following a successful tenure review, Tim Somervaille has been promoted to Senior Group Leader and will now continue to build on the platform of basic and translational research into acute myeloid leukaemia that he has established over the last six years. Tim's success to date was also recognised through the award of International Researcher of the Year by The Christie NHS Foundation Trust.

Recruitment was one of the major goals for the year and resulted in the appointment of three new Junior Group Leaders who will join the Institute in 2014. Claus Jorgensen, starts in early 2014 and will study tumour-stromal signalling in pancreatic cancer; he will be joined later on in the year by Michela Garofalo who will establish a group to examine the role of micro-RNAs in lung cancer, and Esther Baena whose team will investigate the molecular mechanisms driving tumorigenesis in prostate cancer. The addition of prostate cancer to our areas of interest is in light of a successful major funding application in collaboration with Queen's University, Belfast, to establish a *Prostate Cancer UK Movember Centre of Excellence*. The programme provides £5m over five years (split equally across the two sites) with the aim of improving prostate cancer survival rates via personalised delivery of DNA-damage based therapy and is a good strategic fit with the personalised medicine agenda that runs through all of our priority areas.

In July, Peter Stern retired after twenty-four years as a Senior Group Leader in the Institute. During this time he made valuable contributions to the field of cancer immunology, in particular with his work in harnessing the immune system to attack the 5T4 protein which is highly expressed in many carcinomas. He will remain actively involved in research for some time through an honorary position with the University's Institute of Cancer Sciences.

Karim Labib joined the MRC Protein Phosphorylation and Ubiquitylation Unit in Dundee, as well as becoming Professor of Genome Integrity at the College of Life Sciences at the University of Dundee. Nullin Divecha will take up a position at the University of Southampton in 2014 while Ivan Ahel moved to the William Dunn School of Pathology at the University of Oxford. Two of our Post-doctoral Research Fellows, Giacomo de Piccoli and Dragana Ahel, were both awarded Career Development Fellowships from Cancer Research UK to start their own research groups studying the regulation of genome stability at The University of Warwick and The University of Oxford respectively.

In the summer, we welcomed Wei Xing, who joined us to lead the new Scientific Computing Facility, which will provide a High Performance Computing and data management service allowing us to fully exploit the complex datasets that we obtain from the technological platforms that are now available in our core research facilities. These facilities will serve scientists in both the new MCRC research building as well as throughout the Paterson Building and were given a significant boost towards the end of 2012 with an £8.7M investment from the UK Research Partnership Investment Fund (UKRPIF). To help with their development and plan for expansion, I commissioned a review of these facilities, which was conducted by an external panel chaired by Professor Owen Sansom from the CRUK Beatson Institute. This was a highly valuable exercise and we shall continue to implement recommendations from the review throughout the coming year. In addition to several major items acquired during 2013 with the UKRPIF investment, we also purchased a super resolution gSTED microscope with funds from the MCRC, which has significantly enhanced our imaging capabilities.

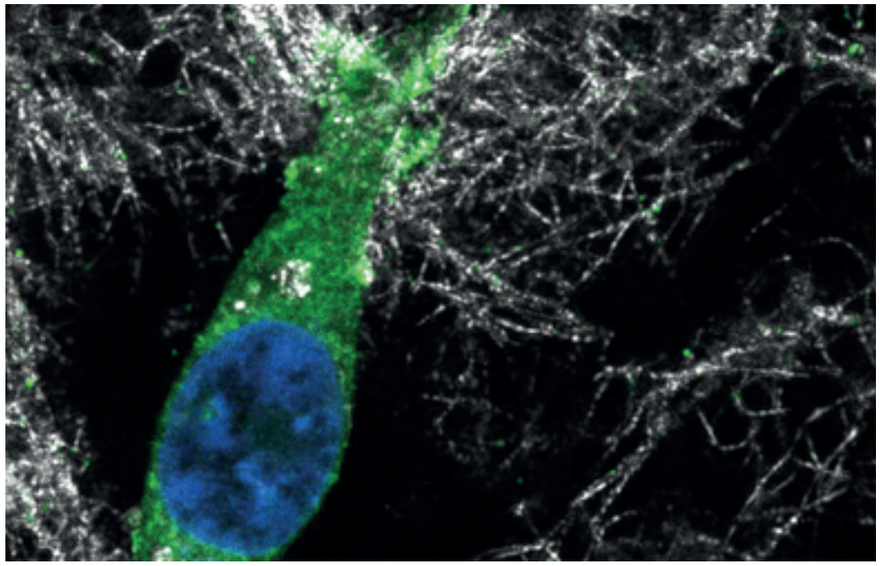
Towards the end of the year, the renewal of funding for the Manchester Cancer Research Centre (MCRC) was announced by CRUK. This partnership between the University of Manchester, Cancer Research UK and The Christie NHS Foundation Trust provides infrastructure and training support that facilitates interactions between scientists and clinicians. The next five years of funding will play a crucial role in driving the personalised cancer medicine agenda in Manchester and the North West. The MCRC building that is currently being constructed opposite the Paterson Building is due for completion in Autumn 2014 and is set to provide space for both the expansion of CRUK MI as well as recruitment of further University of Manchester cancer research groups. An exciting development is that Manchester has been shortlisted for further discussions with CRUK regarding Major Centre status which would greatly accelerate achievement of our research aims.

It is a pleasure to note a number of prizes and awards to members of the Institute. Eva Barkauskaite, was the recipient of the Institute's Dexter Award for Young Scientists. This honour was in recognition of her highly successful structural and mechanistic work on poly(ADP-ribose) glycohydrolase, which has culminated in an impressive set of publications in just over three years during her PhD studies in the DNA Damage Response group. In addition, Eva was awarded a prize for the best talk at 7th International PhD Student Cancer Conference hosted by students from the CRUK London Research Institute as well as receiving the Ruth Bowden Scholarship from the British Federation of Women Graduates.

Professor Richard Marais was awarded the second Griem Lectureship in Molecular and Cellular Oncology from University of Chicago Comprehensive Cancer Center (UCCCC) and will travel there in 2014 to receive the award and deliver a research lecture; Ged Brady, Dominic Rothwell and Debbie Burt of the Clinical and Experimental Pharmacology group, received first prize for their poster presentation at the 9th International Symposium on Minimal Residual Cancer, held in Paris. Ivan Ahel's success during his time at the Institute was acknowledged with the award from CRUK of their 2013 Future Leaders' Prize which was presented to him at the NCRI conference in Liverpool. Drug Discovery won the S-Lab Effective Laboratory Award while Daniel Mould won the Society of Chemical Industry North West Industrial placement student of the year for his undergraduate project with this group.

A migrating breast cancer cell in a 3D collagen gel. Grey represents collagen; green represents active integrin and the cell nucleus is shown in blue.

Image provided by Haoran Tang (Molecular Oncology)



Elli Marinopoulou from the Stem Cell Biology group won the Young Investigator Award at the EMBO Workshop, "*RUNX Transcription Factors in Disease and Development*". The first North West Bio-Pharma Postdoctoral Symposium was held in March at AstraZeneca's Alderley Park and was well attended by numerous postdoctoral scientists from AstraZeneca and the Universities of Manchester, Liverpool, Leeds and Sheffield. James Lynch of the Leukaemia Biology Group won the prize for best talk while Urszula Polanska (Stromal-Tumour Interactions group) won the prize for best poster. The best oral abstract presentation at the Melanoma Research Congress was awarded to Malin Pedersen of the Molecular Oncology group. Hadir Marei from the Cell Signalling group was awarded a prize for the best presentation at University of Manchester, Faculty of Medical and Human Sciences, Postgraduate Showcase while Shameem Fawdar from the Signalling Networks in Cancer group won a poster prize at CRUK Postdoctoral Researcher Career Development Day. Other notable achievements included the election of Ian Waddell to the BACR and Richard Marais as President of the EACR; Angeliki Malliri was awarded a research grant by the MRC to study the regulation of the Rac activator Tiam1 by ubiquitylation.

Our highly successful programme of public engagement continued throughout the year. We welcomed Eve Hart into the role of Research Engagement Manager in June, replacing James Dunphy who took up a new post as CRUK Senior Research Engagement Manager for the north of the UK. Between them, James and Eve co-ordinated over 1000 visitors to CRUK MI during 2013 during numerous lab tours and 12 other events including the annual open day which welcomed 150 visitors and tours/talks for major donors, CRUK shop managers and race for life volunteers. For the first time, the Institute participated in the Manchester Science Festival and we developed a Social Media presence with the development of a Facebook site and the opening of a twitter account.

The coming year promises a number of exciting developments, including further Group Leader recruitment in Melanoma Immunology and Molecular Pathology, as we continue to strengthen the research portfolio of the Institute and develop the necessary research facilities to drive forward our translational research agenda.

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2013 which report significant advances in specific areas. The selected papers demonstrate the breadth and the quality of the research being undertaken by the groups at the Cancer Research UK Manchester Institute.

Grallert A, Patel A, Tallada VA, Chan KY, Bagley S, Krapp A, Simanis V, Hagan IM.
Centrosomal MPF triggers the mitotic and morphogenetic switches of fission yeast.
Nature Cell Biology, 2013; 15:88-95.

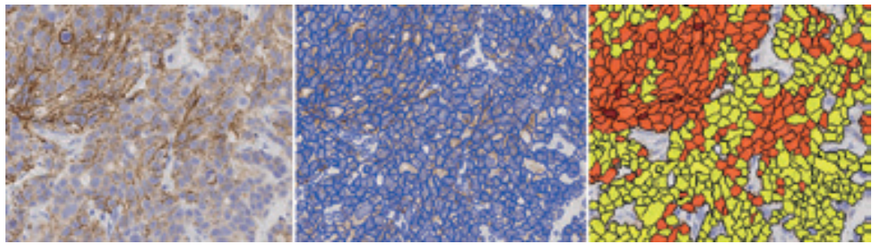
Mitotic commitment is driven by the activation of the Cdk1-Cyclin B protein kinase complex. The Cell Division Group wanted to ask whether the initial appearance of Cdk1-Cyclin B on human centrosomes indicates that the decision to divide is driven by events at spindle poles. Using fission yeast as a model system, they generated a fusion between the single chain llama antibody GBP (GFP Binding Protein) that has a very high affinity for Green Fluorescent Protein, and a mutant version of Cdk1 that was unique amongst all kinases in the cell because it could be inhibited by a specific ATP analogue. Because GFP is widely used to study protein distribution in fission yeast, they were able to generate an array of strains in which the chimeric kinase was targeted to a variety of subcellular structures. Targeting was initiated in the presence of ATP analogue before removal of the analogue released a pulse of kinase activity at each location. Of the many locations tested, the only one at which Cdk1-Cyclin B activation promoted mitosis was the spindle pole, suggesting that this organelle does indeed act as a site that integrates signal from diverse transduction pathways to generate the coherent decision to initiate division.

Girotti MR, Pedersen M, Sanchez-Laorden B, Viros A, Turajlic S, Niculescu-Duvaz D, Zambon A, Sinclair J, Hayes A, Gore M, Lorigan P, Springer C, Larkin J, Jorgensen C, Marais R.
Inhibiting EGF Receptor or SRC Family Kinase Signaling Overcomes BRAF Inhibitor Resistance in Melanoma.
Cancer Discovery, 2013; 3: 158-167.

BRAF inhibitors, such as vemurafenib, target mutations in the BRAF protein, found in around half of all melanomas. Treatment with vemurafenib is initially effective, however patients often develop resistance to this drug after a short time. Here, the Molecular Oncology Group show that using drugs which target a different molecular pathway can halt the growth of BRAF inhibitor-resistant cancer cells. They developed vemurafenib-resistant melanoma cell lines and measured the phosphorylation state of receptor tyrosine kinases present. It was observed that, while EGFR phosphorylation was increased, the negative regulator of EGFR was inactivated, indicating a role for EGFR in vemurafenib resistance. Increased SRC activation was also detected in the resistant samples and it was further discovered that drug resistant cell lines are more invasive than sensitive cell lines, but that invasion could be blocked by inhibiting the EGFR pathway or SRC, suggesting that EGFR/SRC activation not only increases resistance to BRAF inhibitors but also increases ability to invade and metastasise. The authors posit that combining both BRAF inhibitors and EGFR inhibitors or treating resistant cells with SRC inhibitors alone could provide an effective new treatment for certain BRAF mutated melanoma patients.

Huang X, Spencer GJ, Lynch JT, Ciceri F, Somerville TD, Somervaille TC.
Enhancers of Polycomb EPC1 and EPC2 sustain the oncogenic potential of MLL leukemia stem cells.
Leukemia, 2013 Oct 29. doi: 10.1038/leu.2013.316.

While genetic mutations initiate acute myeloid leukaemia (AML), epigenetic dysfunction plays a critical but incompletely understood role in



Tumour tissue visualised at x400 magnification; Definiens Tissue Studio was used to classify tumour (orange) and stroma (blue) in tumour tissues stained for CD44. Image 1: subset of the total tissue; Image 2: colour mask applied during training of the two data subsets; Image 3: separation of tissue and stroma with the mask removed.

Image supplied by Francesca Trapani, Clinical and Experimental Pharmacology.

disease pathology. The Leukaemia Biology Group hypothesised that a lentiviral knockdown screen of genes coding for proteins which regulate the structure or function of chromatin would reveal novel and hitherto unappreciated critical regulators of leukaemogenic potential. This approach identified the EP400 complex components EPC1 and EPC2 as critical oncogenic co-factors in AML. *EPC1* and *EPC2* were required for the clonogenic potential of human AML cells of multiple molecular subtypes. *Epc1* or *Epc2* knock down (KD) induced apoptosis of murine MLL-AF9 AML cells and abolished leukaemia stem cell potential. By contrast, normal haematopoietic stem and progenitor cells (HSPC) were spared. In keeping with these distinct functional consequences, *Epc1* or *Epc2* KD induced divergent transcriptional consequences in murine MLL-AF9 granulocyte-macrophage progenitor-like (GMP) cells versus normal GMP, with a signature of increased MYC activity in leukaemic but not normal cells. This was caused by accumulation of MYC protein. Pharmacological inhibition of MYC:MAX dimerisation, or concomitant *MYC* KD, reduced apoptosis following *EPC1* KD, linking the accumulation of MYC to cell death. Therefore, EPC1 and EPC2 are components of a complex which directly or indirectly serves to prevent MYC accumulation and AML cell apoptosis, thus sustaining oncogenic potential.

Foltman M, Evrin C, De Piccoli G, Jones RC, Edmondson RD, Katou Y, Nakato R, Shirahige K, Labib K.
Eukaryotic replisome components cooperate to process histones during chromosome replication.
Cell Reports, 2013; 3(3):892-904.

All eukaryotic cells create a near-perfect copy of their chromosomes in order to survive cell division. However, chromosome replication severely disrupts the chromatin, the integrity of which is preserved by the transfer of parental histones and the deposition of new histones. In this study the Cell Cycle Group screened for replisome components that bind to histone complexes released from chromatin in cell extracts of yeast. Gene expression patterns demonstrated that the Mcm2 helicase subunit and FACT complex (facilitates chromatin transcription) bind together to parental histone complexes that have been released from chromatin. FACT is not recruited to Mcm2-7 at replication origins before initiation but is present at DNA replication forks, indicating that FACT and Mcm2 help to retain parental histones transiently at the fork before deposition onto nascent DNA just behind the replisome. This study illustrates that the eukaryotic replication and transcription machineries use similar histone-binding modules to process parental histones in order to preserve chromatin integrity during chromosome replication. The activation of oncogenes is believed to cause defects in replication, which drive tumour development, so a deeper understanding of the mechanics of chromosome replication may help optimise novel strategies that better exploit replication defects in tumour cells.

Sharifi R, Morra R, Appel CD, Tallis M, Chioza B, Jankevicius G, Simpson MA, Matic I, Ozkan E, Golia B, Schellenberg MJ, Weston R, Williams JG, Rossi MN, Galehdari H, Krahn J, Wan A, Trembath RC, Crosby AH, Ahel D, Hay R, Ladurner AG, Timinszky G, Williams RS, Ahel I.
Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease.
The EMBO Journal, 2013; 32: 1225–1237.

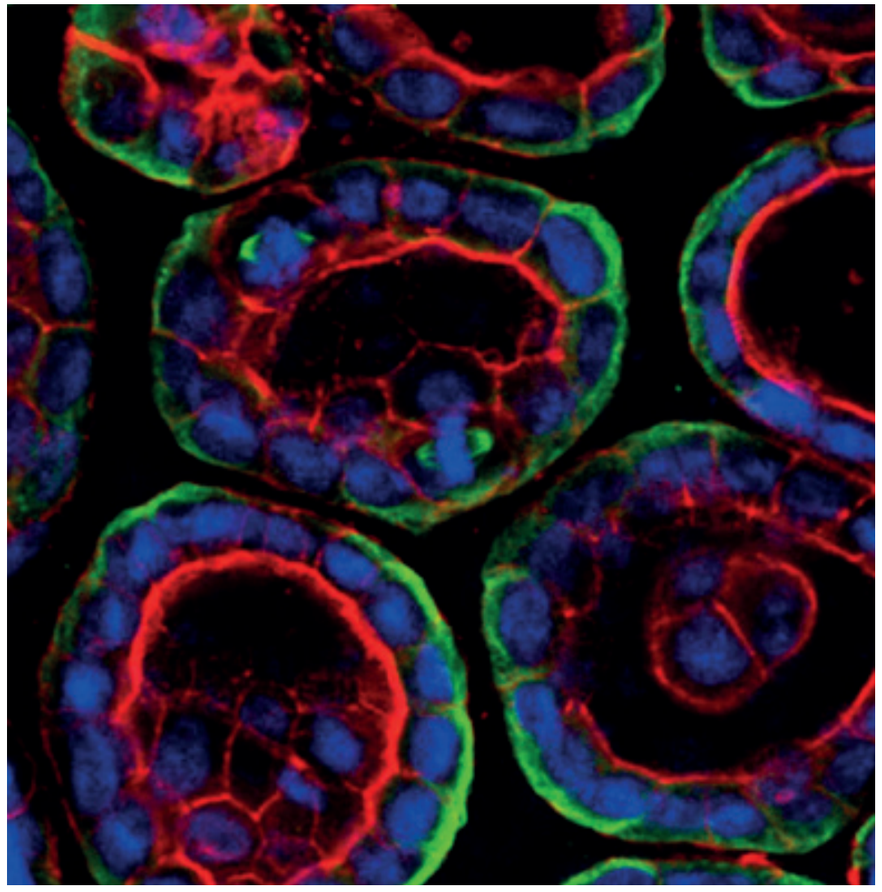
Adenosine diphosphate (ADP)-ribosylation is an evolutionarily conserved, reversible post-translational protein modification, catalysed by poly(ADP-ribose) (PAR) polymerases (PARPs). The hydrolysis of PAR chains is catalysed by PAR glycohydrolase (PARG). ADP-ribosylation regulates a wide range of cellular processes, including DNA repair, so the timely modulation of this dynamic post-translational modification is critical. In this study, defects in the c6orf130 gene were identified in patients with severe neurodegeneration. X-ray structures and biochemical studies showed that the product of this gene is a PARP-interacting macrodomain protein, capable of removing the mono(ADP-ribose) from PARP-modified proteins. The data suggests that the c6orf130 protein causes a defect in these patients' cells that ultimately leads to cell death and neurodegeneration. The authors propose that the c6orf130 protein either directly reverses protein mono(ADP-ribosyl)ation or completes the reversal of protein poly(ADP-ribosyl)ation following the PARG reaction, and so renamed this protein Terminal ADP-Ribose protein Glycohydrolase (TARG1). Overall, the study reveals that the function of TARG1 is important for normal cellular proliferation and cellular response to DNA damage.

Fawdar S, Trotter EW, Li Y, Stephenson NL, Hanke F, Marusiak AA, Edwards ZC, Ientile S, Waszkowycz B, Miller CJ, Brognard J.
Targeted genetic dependency screen facilitates identification of actionable mutations in FGFR4, MAP3K9, and PAK5 in lung cancer.
Proc Natl Acad Sci U S A, 2013; 110(30):12426-31.

Lung cancer is the most common cause of cancer-related death in the UK. Although the implementation of kinase-specific targeted therapy has improved the overall survival rate of lung cancer patients in recent years, we are still faced with the enormous challenge of deciphering the mutated drivers of lung cancer in about 50% of cases. In an attempt to elucidate the low frequency novel drivers of lung cancer, the Signalling Networks in Cancer group, supported by the Bioinformatics and Drug Discovery Groups, undertook a functional genomics approach to screen a panel of six previously sequenced adenocarcinoma cell lines (datasets accessible on COSMIC and CCLE databases). A targeted siRNA genetic dependency screen was designed to deplete all somatically mutated genes in the cancer cell lines. Three targets conferring a survival advantage to the cancer cells were identified: FGFR4, MAP3K9 and PAK5. The mutations in each of the kinases were further functionally and structurally validated to be gain-of-function mutations by their enhanced activity towards

Mitotic cells in cysts – stained with actin (red), Hoescht (blue) and Tubulin (green).

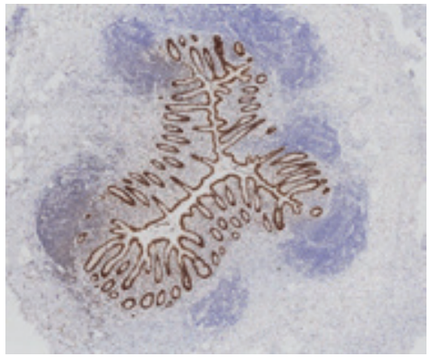
Image provided by Andrew Porter, Cell Signalling.



the pro-proliferative signalling pathway MEK/ERK. This approach provides a stratification strategy to select patients that may benefit from a MEK inhibitor or alternatively encourage the development of inhibitors specific to FGFR4, MAP3K9 and PAK5 to be used in the clinic.

Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, Desjardins L, Turajlic S, Piperno-Neumann S, de la Grange P, Roman-Roman S, Stern MH, Marais R.
SF3B1 mutations are associated with alternative splicing in uveal melanoma.
Cancer Discovery, 2013; 3(10):1122-9.

Uveal melanoma, the most common eye malignancy, causes severe visual morbidity and is fatal in approximately 50% of patients. Primary uveal melanoma can be cured by surgery or radiotherapy, but the metastatic disease is refractory to treatment. Furthermore, the role of ultra-violet radiation in uveal melanoma aetiology is unclear. Researchers from the Molecular Oncology Group conducted single-nucleotide polymorphism arrays and whole-genome sequencing of 12 primary uveal melanomas and matched normal DNA to understand the somatic genomics of uveal melanoma comprehensively. Whole genome sequencing revealed a remarkably low mutation burden in the tumour genomes and the researchers did not observe an ultraviolet radiation DNA damage signature. They identified *SF3B1* mutations in three samples and a further 15 mutations in an extension cohort of 105 samples. Interestingly, these mutations were associated with good prognosis. *SF3B1* encodes a component of the spliceosome, and the authors conducted RNA sequencing of the tumour samples to investigate differences in splicing between the *SF3B1* mutant and wild-type tumours. Sequencing revealed that *SF3B1* mutations were associated with differential alternative splicing of protein coding genes, including *ABCC5* and *UQCC*, and of the long non-coding RNA *CRNDE*, highlighting a novel potential mechanism of tumourigenesis in melanoma.



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

Image provided by Darren Roberts (Immunology)

Walczynski J, Lyons S, Jones N, Breitwieser W
Sensitisation of c-MYC-induced B-lymphoma cells to apoptosis by ATF2.
Oncogene, 2013; Feb 18. doi: 10.1038/onc.2013.28.

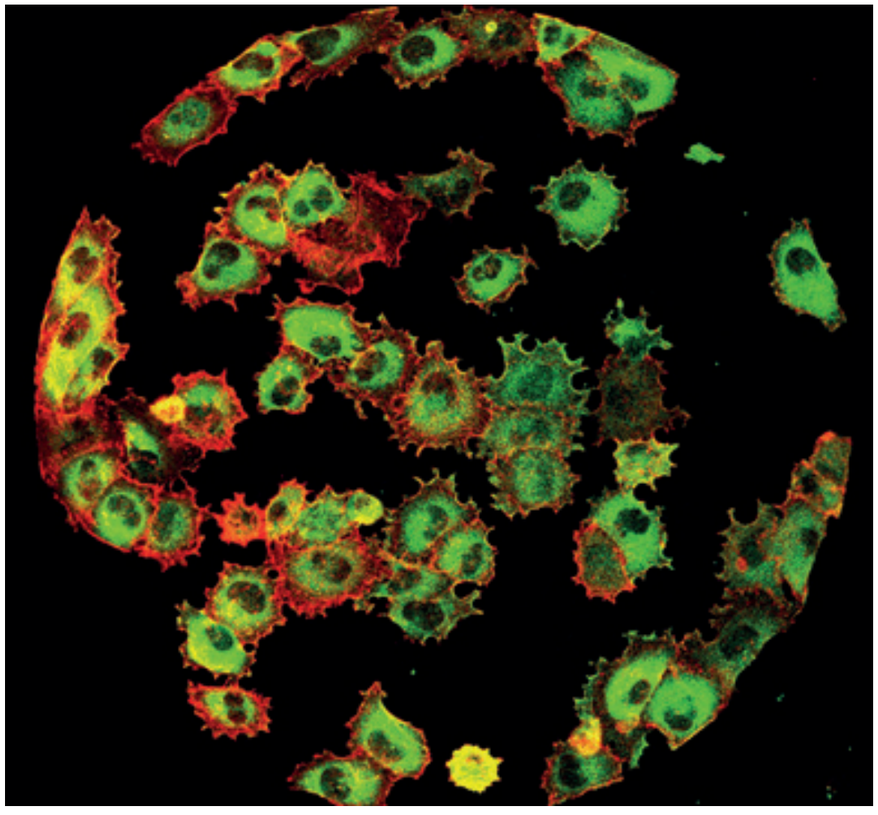
A common feature in B lymphoma, as found in many other tumour types, is the aberrant activity of the c-MYC gene. It has been known for some time that hyperactive c-MYC not only regulates tumour cell growth, but also induces apoptosis. A project led by Jacek Walczynski of the Cell Regulation Group found that lymphomas that have highly active c-MYC also often show strongly active JNK kinase, as well as activated ATF2, a stress-induced transcription factor and phosphorylation target of JNK. Furthermore, in a mouse model of c-MYC driven B lymphoma, the B cell specific inactivation of ATF2 led to significantly reduced apoptotic cell death triggered by c-MYC and to significantly more aggressive behaviour of lymphomas. In addition, JNK and ATF2 were shown to direct the induction of apoptosis in response to chemotherapeutic drugs but only once B lymphocytes have progressed towards lymphoma stages by the actions of c-MYC. Thus, ATF2 is an effector of c-MYC induced apoptosis through activation by JNK.

Simpson KL, Cawthorne C, Zhou C, Hodgkinson CL, Walker MJ, Trapani F, Kadirvel M, Brown G, Dawson MJ, MacFarlane M, Williams KJ, Whetton AD and Dive C.
A caspase-3 `death-switch' in colorectal cancer cells for induced and synchronous tumor apoptosis in vitro and in vivo facilitates the development of minimally invasive cell death biomarkers.
Cell Death & Disease, 2013; 4: e613.

The ability to determine the success of anti-cancer therapies is critical; however, for many cancers this is only possible by invasive surgery, biopsies or expensive imaging techniques. Consequently, the ability to detect drug action in the bloodstream of the patient has become an attractive option. Many cancer therapies work by inducing cell death in the tumour, which led the Clinical and Experimental Pharmacology Group to establish a model system, entitled the "death-switch", to control the timing and extent of cell death induced in a tumour. Colorectal cancer cells were manipulated to express and activate a key component of the apoptosis pathway, which induced widespread and synchronous apoptosis. Using proteomic methodologies, they identified and validated four proteins released into the bloodstream from cells undergoing apoptosis. The death switch was found to induce regression of tumours with the release of the previously established biomarker, cytokeratin 18. This model was also used to evaluate a novel imaging probe which could be applied in positron emission tomography (PET) to image tumour cell death. These findings mark an important development in the assessment of anti-cancer therapies that can be used in clinical trials.

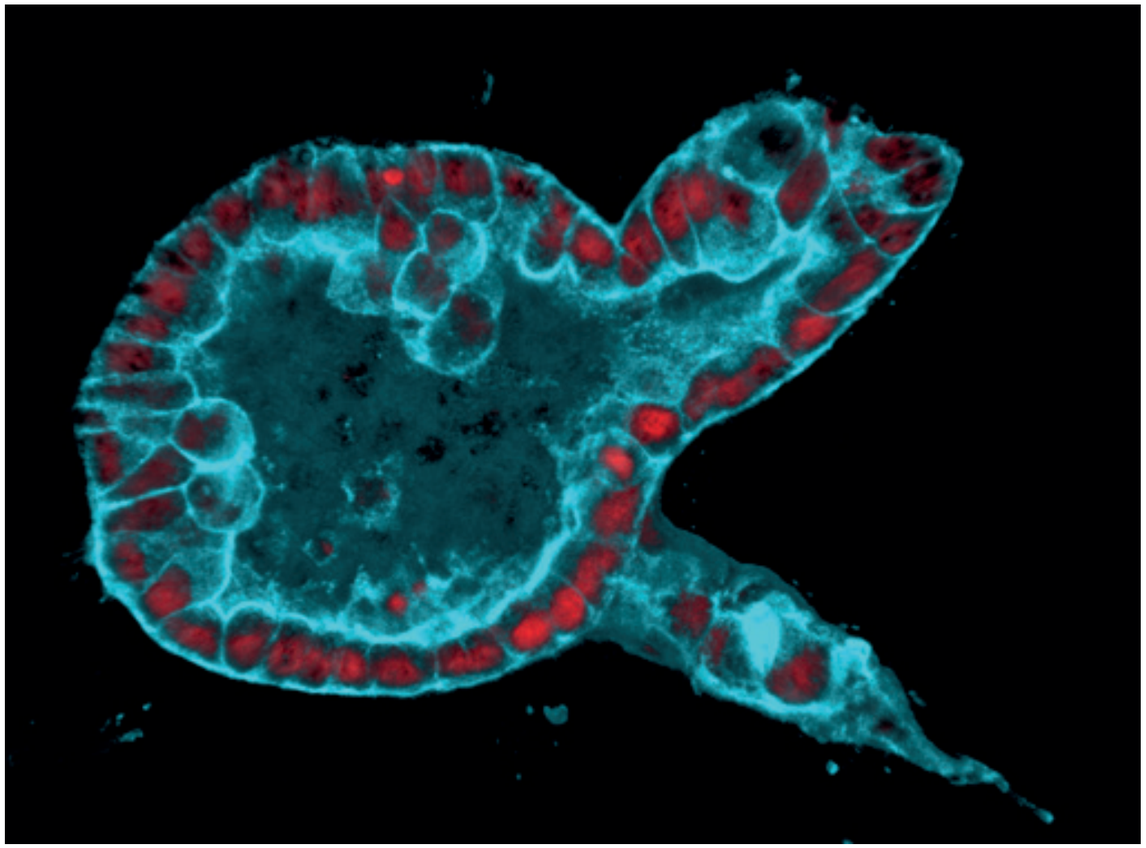
Polanski R, Hodgkinson C, Fusi A, Nonaka D, Priest L, Kelly P, Trapani F, Bishop P, White A, Critchlow SE, Smith PD, Blackhall FH, Dive C, Morrow CJ.
Activity of the Monocarboxylate Transporter 1 inhibitor AZD3965 in Small Cell Lung Cancer.
Clinical Cancer Research, 2013 Nov 25. [Epub ahead of print]

Small cell lung cancer (SCLC) patients have poor prognosis and there has been little improvement in treatment for 30 years. SCLC tumours rapidly proliferate and are hypoxic, suggesting a reliance on glycolysis which could be targeted by inhibiting lactate transport with monocarboxylate transporter (MCT) inhibitors. Whilst AZD3965 (MCT1 inhibitor) is in Phase I clinical trials, activity in SCLC has not been assessed and predictive biomarkers are unproven. Preclinical research undertaken by the Clinical and Experimental Pharmacology Group suggests that hypoxic SCLC cells which do not express MCT4 (an alternate lactate transporter), are sensitive to AZD3965. Analysis of SCLC tissue microarray of 78 SCLC biopsies demonstrated that 21% express MCT1 but not MCT4, predicting response to AZD3965. MCT1 expression also correlated with reduced survival time. Taken together, these data support clinical evaluation of AZD3965 in a SCLC enriched cohort, coupled with assessment of hypoxia, MCT1 and MCT4 expression as a predictive signature for AZD3965 response.



Breast cancer cells (green) on an extra-cellular matrix (ECM) array, which is used to study cell adhesion and spreading on various types of ECM. This picture shows a single spot of the array. Actin cytoskeleton is in red.

Image provided by Haoran Tang (Molecular Oncology).



MDCK cells, grown in a collagen matrix, where they form hollow spheres, called cysts. When the growth factor HGF is added to the cysts, they start to sprout, with actin-rich projections emerging from the surface of some of the cells. After one or two days, some of these cells undergo EMT, and re-orientate their axis of cell division, forming long chains of cells which can start to hollow out to form hollow tubules. This recapitulates stages in normal kidney development, and is a useful model for studying EMT and oriented cell division, and the way in which these might contribute to tumour progression. Actin is cyan, and nuclei are red.

Image supplied by Andrew Porter, Cell Signalling.

THE CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH GROUPS

APPLIED COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

www.cruk.manchester.ac.uk/bioinformatics

Group Leader
Crispin Miller

Postdoctoral Fellows
Jing Bi
Hui Sun Leong
Janet Taylor[2, 3]

Scientific Officer
Keren Dawson

Graduate Students
Elli Marinopolou[4]
Danish Memon
Sharmin Naaz[5]
Maria José Villalobos Quesada[6]

Computational Biologist
Yaoyong Li

Bioinformatics Analyst
Phil Chapman[7]
Catriona Tate[1, 8]

Software Architect
Tim Yates

Bioinformatics Programmer
Chris Wirth

Systems Administrator
Zhi Cheng Wang[9]

[1]joined in 2013
[2]left in 2013
[3]joint with Translational Radiobiology
[4]joint with Stem Cell Biology
[5]joint with Stem Cell Haematopoiesis
[6]joint with Cell Division
[7]embedded within Drug Discovery Unit
[8]embedded within Clinical and Experimental Pharmacology
[9]joint with IT department

The Applied Computational Biology and Bioinformatics Group is interested in the computational analysis of genome-wide datasets arising from deep sequencing and quantitative proteomics experiments. It collaborates widely within the Institute and has its own research programme focused on developing a better understanding of the transcriptional systems that control gene expression within cells. It is particularly interested in the role played by long non-coding RNAs (lncRNAs) in regulating these processes and how these are disrupted in tumours.

This year, the Institute has invested further in deep sequencing, with the purchase of a new Illumina HiSeq 2500 and a MiSeq. These platforms offer exciting opportunities, but come with an increased computational burden, not only in the sheer volume of information (each run of the 2500 can require terabytes of disk space to analyse) but also in the computational power required to process them.

Scientific Computing

In order to address the computational burden associated with our new sequencing platforms, we have established a new Scientific Computing Team, led by Wei Xing (page 53), to provide the next generation of High Performance Computing (HPC) necessary to deal with future deep sequencing, proteomics and imaging data. Wei has already helped manage the installation of additional processing and storage capacity, and over the next year will be coordinating the development of a new Cancer Genomics Data Centre to further extend our computing capabilities.

Computational Biology Support

As computational biology becomes central to increasing numbers of groups within the Institute, our collaborations have grown significantly. The close of 2013 saw a restructuring of the ACBB group through the formation of a distinct Computational Biology Support Team, to complement a new and separate RNA Biology Research Group focused on regulatory RNAs (see below).

A major aspect of the support team's work has been to develop pipelines that streamline the pre-processing and alignment of deep sequencing data. These are parallelised across our HPC system, and handle many of the routine tasks associated with Illumina data, freeing valuable time to conduct downstream analysis and data interpretation. A significant part of this work has been to evaluate different aligners and to develop an understanding of the influence different parameter settings have on the performance of these tools.

Pipelines for DNA-, RNA- and ChIP-seq have all been built, and will continue to be refined in order to keep track with a fast moving field. The team has also developed proteomics tools to complement those provided for deep sequencing. These bring peptide-level tandem mass spectrometry datasets into a format that supports integrated analysis with RNA-sequencing data, in part by mapping them into a common genome-level coordinate system. This further extends approaches we developed to support the integration of genomics data (e.g. Bitton et al., Genetics, 2011; Bitton et al., PLoS ONE, 2010; and Bitton et al., BMC Bioinformatics, 2008).

Since most of our downstream data analysis is performed using R and Bioconductor, these tools bring the data into the R programming environment, where they can be analysed using the comprehensive statistical toolkits that accompany this programming language, complementing other work in the ACBB group that has focused on bringing genome annotation data into the same environment (see http://annmap.cruk.manchester.ac.uk/, for example).

Collaborative Analysis

Additional collaborative support is provided by computational analysts that work closely with other research groups. Where demand is sufficient, these can be provided by full time embedded posts; both the CEP and the DDU Groups embed analysts in this way. In addition, we have established an exciting collaboration with the CEP group (page 24) to develop bioinformatics approaches for the analysis of whole genome and transcriptome data derived from single cell genomics experiments.

While much of our work is focused on genome-wide datasets, we are also interested in statistical methods, and were able to contribute to work with the Signalling Networks in Cancer Group (page 40) by providing the mathematical modelling necessary to analyse data from genetic dependency screens (Fawdar et al., Proc Natl Acad Sci U S A, 2013).

We have been collaborating with the Translational Radiobiology Group (part of the University of Manchester Institute of Cancer Sciences) on the development and application of RNA signatures of tumour hypoxia. (Eustace et al., Clin Cancer Res., 2013; Ramachandran , et al., Eur J Cancer, 2013 ; and Hall et al., P Int J Radiat Oncol Biol Phys., 2013). Many of these studies required the analysis of microarray data generated from Formalin fixed Paraffin Embedded Tissue (FFPET) samples, which presents a challenge because the RNA is degraded and chemically modified, requiring new bioinformatics strategies.

RNA Biology Research Group

Although less than 2% of the human genome encodes protein sequences, the majority is transcribed, raising the possibility that many of these non-coding RNAs may play an important but currently unknown role in the cell. We are integrating bench-, clinical- and computational science to identify novel lncRNAs that play an important role in cancer.

Some of our work uses the model system fission yeast *(Schizosaccharomyces pombe)* to investigate the basic functions of non-coding RNAs (Figure 1), since many of the core pathways known to be involved in their action are conserved with human cells. Our interest in non-coding RNAs and transcription has led to an emphasis on genome annotation, and the development of computational tools to support de novo re-annotation of samples from RNA-sequencing and protein mass spectrometry data. Our discovery of novel protein coding genes has added almost 1% to the protein coding complement of fission yeast. Publications are now emerging that describe these loci in more detail (see, for example, Dhani et al., Mol Biol Cell., 2013).

We are currently applying similar approaches to the ones we pioneered in fission yeast to the analysis of human cell lines and clinical datasets, allowing us to identify novel long intergenic non-coding-coding RNAs (lincRNAs) of potential relevance in cancer. We are in the process of characterising these further at the bench.

Publications listed on page 56

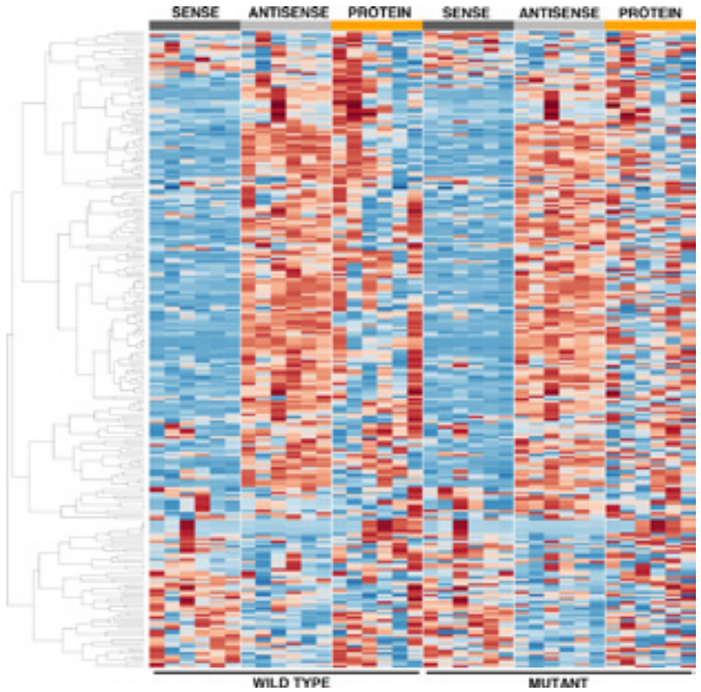


Figure 1
Integrating strand specific RNA sequencing data with global quantitative protein mass spectrometry to identify gene expression changes in fission yeast cells. Rows in the heat map correspond to individual coding regions for which quantitative proteomics data were also available. Columns correspond to individual samples in a time course. A wild type and mutant strain were compared. Sense: coding sequence expression levels. Antisense: antisense expression levels corresponding to these coding regions. Protein: protein abundance. Blue: low abundance, red high abundance.

CELL CYCLE

Group Leader
Karim Labib[1]

Postdoctoral Fellows
Giacomo de Piccoli[1]
Cecile Evrin[1]
Luis Garcia-Rodriguez[1]
Tim Maculins[1]

Scientific Officers
Frederick van Deursen[1]
Pedro Junior Nkosi[1]

Graduate Student
Marija Maric[1]

[1] left in 2013

Chromosome replication is still understood poorly in eukaryotic cells, but is intimately linked to our understanding of the development and treatment of human cancer. Defects in replication arise early in cancer development, so that agents perturbing chromosome replication are often able to kill cancer cells preferentially. A multi-protein machine known as the replisome is assembled at DNA replication forks, and the mechanisms and regulation of the replisome form the focus of our research. During the last year, we identified conserved histone-binding activities within the replisome, which might contribute to the retention of parental histones at sites of replication, in order to maintain epigenetic histone marks that control gene expression. We also discovered how the replicative DNA helicase is connected to the leading strand DNA polymerase, making a physical link that is likely to play an important role in regulating the progression and function of the eukaryotic replisome.

Copying chromatin during DNA replication

Eukaryotic chromosomes contain a single molecule of DNA that is packaged into nucleosomes by association with histones. The density of nucleosomes along each chromosome is an important determinant of gene expression, as are post-translational modifications of the extended tails at the amino termini of the histone proteins. In addition to making a near-perfect copy of the DNA double helix during chromosome replication, cells need to ensure that the complex patterns of nucleosome density, and post-translational modifications to histone tails, are conserved when the chromosomes are duplicated, in order to preserve patterns of gene expression. Unwinding of the DNA duplex displaces histones, and a long-standing model supposes that parental histones must then be retained locally at DNA replication forks, so that they can be re-deposited immediately onto the nascent DNA, thus preserving the parental chromatin features throughout each chromosome (Figure 1). Until now, nothing was known about how this feat is actually achieved during chromosome replication.

We are searching systematically for histone-binding activities in the eukaryotic replisome, and this year we described the first stage of this work (Foltman et al., Cell Rep, 2013). Using a simple assay in which histones are released into a yeast cell extract by digestion of the chromosomal DNA, Magda Foltman discovered that the Mcm2 subunit of the replicative DNA helicase was able to pick up the displaced histones. Interestingly, Mcm2 binds to histones together with another replisome component called FACT, which is known to help process parental histones during transcription. Magda found that the extended amino-terminal tail of Mcm2 contains a histone-binding motif that is conserved from yeast to humans. In budding yeast, this motif is important to preserve the nature of sub-telomeric chromatin, although it is not required for DNA synthesis *per se*.

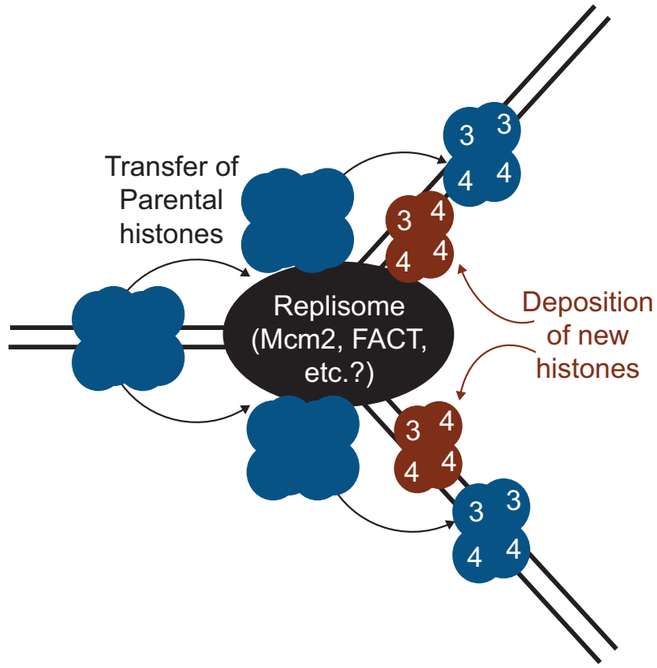


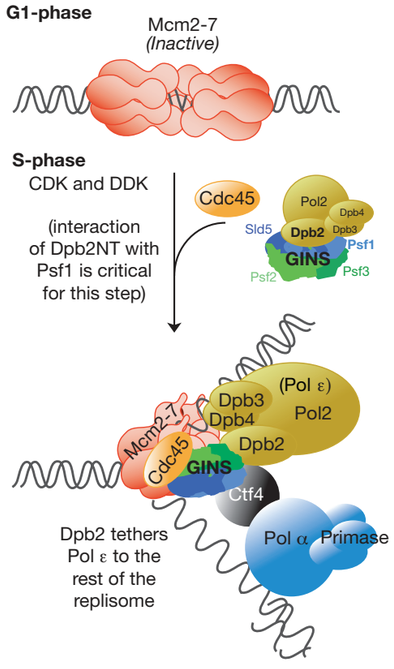
Figure 1
The eukaryotic replisome contains multiple histone-binding activities, which are likely to cooperate with each other, in re-depositing parental histones onto nascent DNA during chromosome replication.

Our goal for the future will be to identify all the remaining histone binding activities within the eukaryotic replisome and then map and mutate the relevant domains, so that by combining these mutations we will be able for the first time to assess the contribution of the replisome to the preservation of parental chromatin during chromosome replication.

Dpb2 connects the leading strand DNA polymerase to the Cdc45-MCM-GINS DNA helicase within the eukaryotic replisome

In all species, a DNA helicase is required to unwind the parental DNA duplex at DNA replication forks, so that DNA polymerases can then synthesise the leading and lagging strands. Seminal work with *E. coli* showed that the replicative helicase and DNA polymerases are connected to each other by other factors, to form a large assembly known as the replisome. Assembly of the replisome allows the rate of DNA unwinding to be co-ordinated with the rate of DNA synthesis, thus minimising the exposure of single-strand DNA that might otherwise be a dangerous target for nucleases or recombination enzymes in the cell. More surprisingly, it turns out that replisome assembly is a critical determinant of the rate of fork progression. The DNA polymerases can only act on a single-strand template generated by the helicase, so that it would be natural to think that the helicase might determine the rate of fork progression. But the polymerase is inherently faster than the helicase, and replisome assembly allows the polymerase to set the rate of fork progression, making the helicase unwind DNA much more quickly than it would normally have been able to.

Figure 2
Dpb2 incorporates DNA polymerase epsilon into the eukaryotic replisome, by direct association with the GINS component of the Cdc45-MCM-GINS DNA helicase.



Until now, it has not been possible to address these issues in eukaryotes, as the connections between helicase and polymerases have not been understood. We reported this year that the Dpb2 subunit of the leading strand DNA polymerase epsilon plays a key role in this regard (Sengupta et al., Curr Biol, 2013), as it connects the polymerase to the Cdc45-MCM-GINS DNA helicase, by binding directly to the GINS component of the latter (Figure 2). Previous work from the lab of Hiro Araki showed that GINS and DNA polymerase epsilon are recruited together to replication origins as a complex, during the initiation of replication. We found that the amino terminal domain of Dpb2 (Dpb2NT) is required for this recruitment. Interestingly, if cells express Dpb2NT as the only form of Dpb2, this is sufficient to allow recruitment of GINS and assembly of active Cdc45-MCM-GINS, but leads to the production of a replisome that lacks DNA polymerase epsilon. Cells are viable under such conditions (presumably because another DNA polymerase can make the leading strand in the absence of Pol epsilon), but growth is very poor, indicating that the connection between DNA polymerase epsilon and the helicase is important for normal replisome function. Future studies will explore why this is the case.

Publications listed on page 56

CELL DIVISION

www.cruk.manchester.ac.uk/celldivision

Group Leader
Iain Hagan

Associate Scientist
Agnes Grallert

Postdoctoral Fellows
Ye Dee Tay
Kuan Yoow Chan

Scientific Officer
Ben Hodgson

Graduate Student
Maria-José Villalobos Quesada[1]

[1]joint with RNA Biology Group

The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways or a simultaneous defect in both. Understanding how the diverse external and internal cues are integrated to co-ordinate cell division and death therefore sits at the heart of our need to understand the basic biology of cancer. Because the regulatory networks that control cell division are highly conserved, understanding how the relatively simple unicellular yeasts take the decision to divide greatly accelerates the analysis of the more complex issue of cell division controls 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 is regulated by the activity of the Cdk1-Cyclin B protein kinase complex. Prior to mitosis the complex is inhibited through the phosphorylation by Wee1 kinase of a residue within the ATP binding pocket of the Cdk1 catalytic subunit. The timing with which the Cdc25 removes this phosphate dictates the timing of Cdk1-Cyclin B activation and so the timing of mitotic commitment. The balance between Wee1 and Cdc25 activities therefore determines when a cell will divide. Once a critical threshold level of Cdk1-Cyclin B activation is exceeded a positive feedback loop simultaneously boosts Cdc25 activity and suppresses Wee1 activity to convert the hitherto gradual accumulation of Cdk1-Cyclin B activity into an all or nothing bi-stable switch that drives the cell into mitosis. The protein kinase Polo plays a key positive role in this feedback loop.

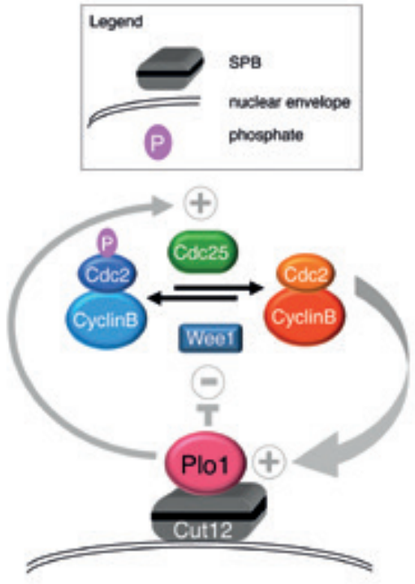
Mitotic commitment and the centrosome/spindle pole body

Centrosomes nucleate the two microtubule arrays that inter-digitate to form the mitotic spindle. The initial appearance of active Cdk1-Cyclin B on human centrosomes and modelling of Cdk1-Cyclin B activation in frog egg extracts suggests that the trigger for mitotic commitment stems from this organelle. Our studies of a component of the fission yeast centrosome equivalent, the spindle pole body (SPB), strongly endorse this view. Gain of function mutations in the SPB component Cut12 compensate for an otherwise lethal loss of Cdc25 function arising from the conditional *cdc25-22* mutation. This influence appears to stem from Cut12's impact upon the activity of the fission yeast polo kinase Plo1. The *cut12* mutations that suppress *cdc25-22* boost Plo1 kinase activity, while none can be detected when Cut12 function is lost. Enhancement of Plo1 activity by mutation of Cut12 would be expected to increase feedback loop inhibition of Wee1, thereby reducing the need for Cdc25 activity.

Triggering mitotic commitment from the fission yeast SPB

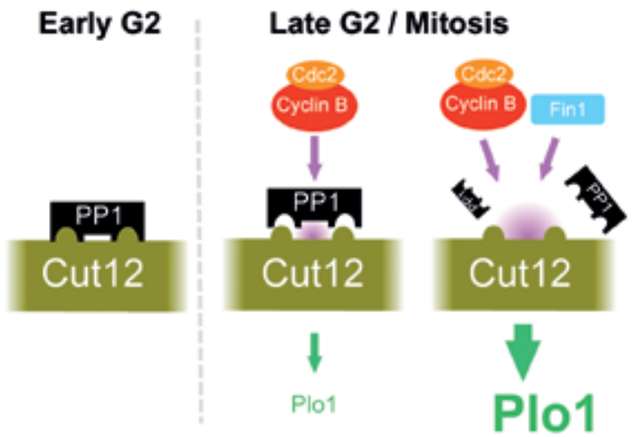
We have taken a direct approach to follow up this correlative evidence, suggesting that centrosomes/SPBs play a key role in mitotic commitment, by asking the simple question "What happens to the mitotic commitment decision when a pulse of Cdk1-Cyclin B or Plo1 activity is released at a particular cell location?" We fused each kinase to a single chain llama antibody called GFP binding protein (GBP). GBP has a very high affinity for the Green Fluorescent Protein (GFP) that is widely used in cell biology because its intrinsic fluorescence reveals the location of any fusion protein. A large number of fission yeast strains have been modified to generate fusions between individual genes of interest and sequences encoding GFP. We therefore used GBP to direct Plo1 and Cdk1 kinases to a wide range of structures defined by

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



this library of GFP fusions. The catalytic pocket of each kinase was modified to incorporate mutations that conferred sensitivity to inhibition by ATP analogues. We could then restrain the activities of the targeted kinases with inhibitory ATP analogues until removal of these analogues by a simple exchange of culture medium provided a burst of kinase activity at the subcellular location of interest. We used this approach to release each kinase activity at diverse subcellular locations including nuclear pores, centromeres and the cell cortex. The only location at which the release of either kinase activity triggered mitosis was the SPB. Moreover, of the three SPB components tested, the drive into mitosis was greatest when the chimeric kinases had been recruited by Cut12-GFP.

Figure 2
Model illustrating the inverse correlation between the degree of PP1 recruitment to Cut12 and the activity of the feedback loop kinase Plo1 during late G2/mitosis.



Recruitment of Protein Phosphatase 1 to the SPB controls mitotic commitment

Protein Phosphatase 1 (PP1) is recruited by defined docking motifs to both direct targets and scaffolds from which it can dephosphorylate neighbouring proteins. The *cut12* mutations that suppress *cdc25-22* reside within a perfect match for a PP1 docking site. We employed a range of approaches to show that PP1 recruitment to this site in Cut12 sets the requirement for Cdc25 in fission yeast. Strikingly, cells could tolerate the otherwise lethal ablation of the *cdc25*$^{+}$ gene when the PP1 docking site had been removed from Cut12. We are yet to identify the target for the Cut12-PP1 complex however we did find an inverse correlation between the degree of PP1 recruitment to Cut12 and the activity of the feedback loop kinase Plo1. Furthermore, PP1 recruitment to Cut12 constitutes yet another level of feedback control because PP1 docking was inhibited by phosphorylation of the docking site by Cdk1-Cyclin B and the NIMA kinase Fin1. Thus, elevation of Cdk1-Cyclin B kinase activity triggers a loop that reduces PP1 recruitment to Cut12 to boost Plo1 activity to further enhance Cdk1-Cyclin B activity towards the PP1 docking site. This loop will greatly accelerate the mitotic commitment switch.

A novel approach for the study of Wee1 function *in vivo*

The sensitisation of Plo1 to inhibition by ATP analogues that we used to study Plo1 function in the SPB control of mitotic commitment relied upon a novel methionine to phenylalanine mutation within its ATP binding pocket. The same position is occupied by methionine in the catalytic pocket of Wee1. Because Wee1 is only marginally sensitised to analogue inhibition by the canonical sensitising mutations, we introduced the M > F mutation that had worked so effectively with Plo1 into Wee1. The mutation gave a profound enhancement of Wee1 sensitivity to inhibition by ATP analogues. Proof of principle experiments then established that this allele provides a powerful tool for the dissection of the conserved pathways that control mitotic commitment and execution.

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 iterative cycle of comparative studies ensures that great strides are being made in understanding the molecular basis of cell division.

Publications listed on page 56

CELL REGULATION

www.cruk.manchester.ac.uk/cellregulation

Group Leader

Nic Jones

Associate Scientists

Wolfgang Breitwieser

Scientific Officer

Steve Lyons

Graduate Students

Emily Holmes

Alexander Thapa

Stress Signalling Pathways describe the molecular activities emanating from (generally adverse) external stimuli that are received by cells via surface or internal receptors and transmitted via relays of protein kinases. Depending on the type of stimulus and cellular context, activation of these pathways may lead to induction of diverse cellular programmes, ranging from growth to growth arrest, differentiation, or cell death.

One such signalling pathway is described by the stress activated protein kinase (SAPK) pathway. Formally, this has been categorised as a specific branch of the wider acting MAPK (mitogen activated protein kinase) pathways and involves a series of protein kinases which act as signalling molecules that transmit information via phosphorylation of their protein substrates. Thus, SAPKs (including members of the JNK and p38 subfamilies) are activated by upstream acting layers of kinases comprising SAPKKs (e.g. MAP2K3, 4, 6, and 7) and SAPKKK (e.g. MAP3K1, 2, etc). At the base of this hierarchy lie cellular effector proteins that include other kinases, apoptosis regulators, and transcription factors. Together, the actions of the effector molecules, in response to activation by the SAPKs determine the cellular response to a specific stimulus.

Stress Kinase pathways in cancer

Recent advances in cancer genome analyses have highlighted the frequency at which stress signalling pathways are deregulated, or mutated, in various tumour types. For example the signalling kinase MEKK1 (MAP3K1) is mutated in 9% of breast invasive and endometrial carcinoma and 6% in prostate adenocarcinoma (source: cbioportal.org), while its substrate, MAP2K4, is mutated in 6-7% of breast and colon cancers. The nature of these mutations suggests that the pathway is aberrantly activated in some types of cancer, but inactivated to a far greater extent in many other types. Thus, stress signalling pathways are thought to be pro-oncogenic or tumour suppressive depending on the cancer context.

ATF2: an effector transcription factor of stress kinase signalling

One focus of research in the Cell Regulation group is the AP-1 transcription factor ATF2, which is an effector substrate of the SAPKs, JNK and p38. Our research work has demonstrated that ATF2 has essential functions during development of the embryonic liver, heart and brain, and that this is dependent on its activation by the SAPK signalling cascade.

High levels of phospho-ATF2 have been detected in both human melanoma and prostate carcinoma samples, and a role for ATF2 in driving progression of these tumours has been suggested in the literature. For example, recent findings indicate that the SS18-SSX2 fusion protein, found in human synovial sarcomas, derives its oncogenicity from the ability to interact with ATF2. Conversely, low levels of ATF2 expression in human breast tumours have also been reported.

Functions of JNK and ATF2 in B lymphoma

Experimental analyses using mouse tumour models carried out by our group as well as by others, have uncovered diverse roles for ATF2 in tumourigenesis. Accordingly, ATF2 was shown to contribute to melanoma development through its role in a melanocyte-specific gene activation programme. In contrast, ATF2 deficient mice are sensitised to carcinogen-induced skin tumourigenesis, underlining the tumour context dependent activities of the stress signalling pathway. In a mouse model of MYC oncogene induced B-cell lymphoma development, we showed that ATF2 deficiency results in accelerated disease onset. Further in vitro analysis revealed that ATF2 responds to oncogene induced cellular stress by inducing programmed cell death. In addition, JNK and ATF2 were shown to direct the induction of apoptosis in response to chemotherapeutic drugs but only once B lymphocytes have progressed towards lymphoma stages by the actions of cMYC. Thus, ATF2 is an effector of cMYC induced apoptosis through activation by JNK.

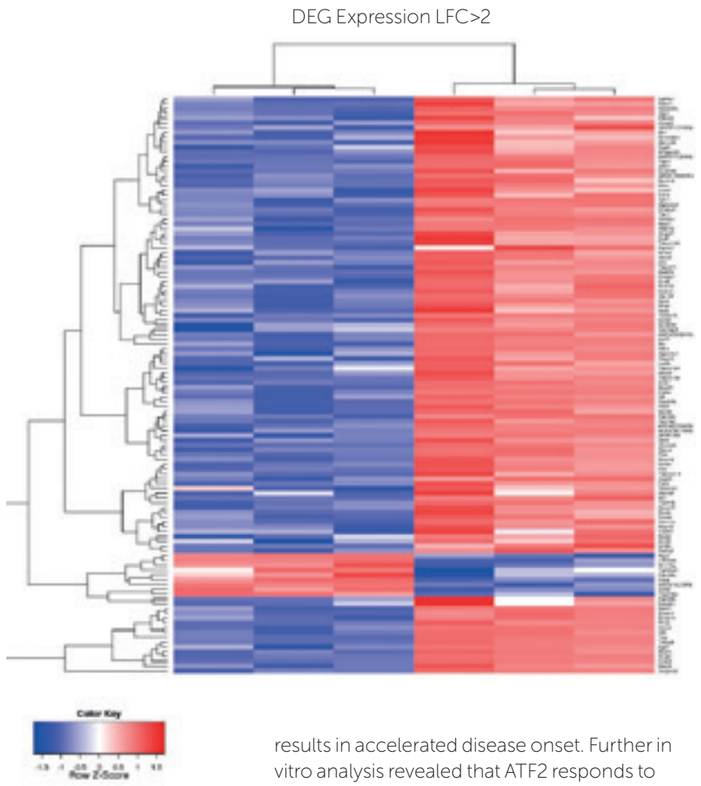


Figure 1
Gene Level Analysis: A heat map depicting differentially expressed genes in hepatoblasts in response to ATF2 activity. Expression array experiments were carried out in triplicate (columns) of control or ATF2 expressing cells. Over 250 up-regulated (red bars) and over 30 down-regulated (blue bars) genes were identified.

Figure 2
The *S. pombe* Sty1 MAPK signalling pathway. The Sty1 kinase is activated by a kinase cascade. The activated MAPK Sty1 then phosphorylates a range of target proteins to mediate the stress response.

Tumour suppressive transcriptional programmes by ATF2

An independent project has focused on the role of SAPK pathway components in hepatocellular carcinoma. Here, we demonstrated that JNK dependent activation of ATF2 is critical in blocking the oncogenic transformation of hepatocyte precursors (hepatoblasts), as well as in suppressing their tumourigenicity after orthotopic transplantation into recipient livers. In addition, we defined a JNK and ATF2 dependent transcriptional programme that acts in a tumour suppressive manner. Further analysis revealed that this programme is frequently found inactivated or genetically altered in a variety of human tumour types, including breast, lung, pancreatic and hepatocellular carcinoma. This analysis therefore confirmed that the experimental tumour models reflect human cancer scenarios. Further analysis of SAPK dependent effectors, as well as other components of stress signalling pathways, will therefore be the focus of future research.

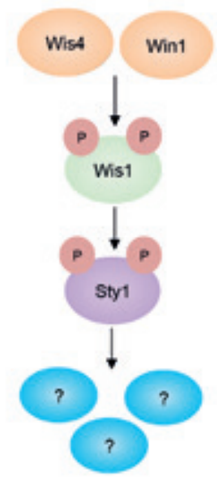
Identification of novel targets of the *S. pombe* MAP kinase Sty1

The SAPK pathways are highly conserved between yeast and mammalian cells, and in *Schizosaccharomyces pombe* the MAPK Sty1 is a key regulator of the stress response. Sty1 is activated following the exposure of cells to a wide variety of environmental stress conditions. Similarly to the mammalian SAPKs, Sty1 is activated through dual phosphorylation by an upstream MAPKK. Due to the high level of conservation between the mammalian and yeast signalling pathways, *S.pombe* provides an ideal model to investigate the function and regulation of the SAPK signalling pathways.

Upon activation Sty1 mediates the appropriate stress response through the phosphorylation of downstream target proteins, the best characterised of which is the transcription factor Atf1. Whilst only a small number of Sty1 targets are currently known, the large number of cellular processes regulated by the Sty1 pathway suggests that there are likely to be a number of, as yet uncharacterised, Sty1 target proteins. To fully understand the stress response, it is vital that we identify targets of SAPKs.

In order to identify novel targets of Sty1 phosphorylation we performed a SILAC screen in collaboration with the laboratory of Boris Maček at the University of Tübingen. Intriguingly, we found that following exposure to oxidative stress, proteins upstream of Sty1 in the MAPK pathway are phosphorylated in a Sty1-dependent manner. Furthermore, one such protein is a potential direct target of Sty1 phosphorylation. Further investigation revealed that Sty1-dependent phosphorylation of upstream components appears to promote Sty1 phosphorylation, thus forming a positive feedback loop to increase the level of Sty1 activity following oxidative stress exposure.

Publications listed on page 57



CELL SIGNALLING

www.cruk.manchester.ac.uk/cellsignalling

Group Leader

Angeliki Malliri

Postdoctoral Fellows

Zoi Diamantopoulou[1]
Andrew Porter
Lynsey Vaughan
Helen Whalley

Scientific Officer

Gavin White

Graduate Students

Hadir Marei
Erinn-Lee Ogg
Anna Woroniuk

[1]joined in 2013

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

RAC1 is active in melanoma

Aberrant expression of RAC or RAC activators in human cancer, and the effect of conditional ablation of *rac1* or Rac activators in mouse tissues on tumour formation and progression, have implicated RAC1 in tumourigenesis. While RAC mutations were not readily detected in human cancer using conventional sequencing, subsequent exome sequencing has revealed oncogenic RAC alleles. In particular, ~9% of cases of melanoma developing in sun-exposed sites possess RAC1 mutated at a common amino acid, Proline 29. Conversion of this residue to Serine alters the conformation of the switch I loop of RAC1 and activates the protein. RAC1 mutation can coincide with gain-of-function of BRAF or NRAS mutation in melanoma, suggesting that RAC1 co-operates with MAPK signalling to induce melanoma.

To investigate possible co-operation between RAC and RAS in melanoma formation, we combined expression of an active RAC1 allele ($RAC1^{G12V}$) with oncogenic RAS ($HRAS^{G12V}$) in zebrafish melanocytes. The combination was significantly more potent at inducing tumour nodules than either mutant gene alone. Surprisingly, expression of $RAC1^{G12V}$ alone did not perturb zebrafish melanocyte development, morphology, proliferation or migration, but in combination with $HRAS^{G12V}$ did induce precocious proliferation and migration of transformed melanocytes, promoting tumour formation. Immunohistochemical staining of human melanoma samples demonstrated widespread overexpression and hyperactivation of RAC1. We also revealed overexpression of the RAC activator TIAM1 (T-lymphoma invasion and metastasis protein) in nodular forms of melanoma (Dalton et al., J Invest Dermatol. 2013). Thus, we conclude that RAC1 deregulation is a common event in the genesis of melanoma, which co-operates with aberrant RAS signalling to drive malignant progression.

Post-translational modifications of Rac1 during cell migration

Recently, regulation by post-translational modification has emerged as a significant means of regulating RAC activity. To gain further insight into the regulation of RAC during cell migration, we performed a screen for proteins that interact with RAC following treatment of cells with a motility-inducing factor, Hepatocyte Growth Factor (HGF). This revealed the small ubiquitin-like modifier (SUMO) E3-ligase, PIAS3, as a novel RAC interacting protein. Subsequently, we demonstrated that RAC1 can be conjugated to SUMO-1 by PIAS3 in response to HGF. PIAS3 interacts better with GTP-bound RAC and the GTP-bound form of RAC is a better substrate for SUMOylation. Furthermore, we demonstrated that PIAS3-mediated SUMOylation of RAC1 controls RAC1-GTP levels and the ability of Rac1 to stimulate lamellipodia, cell migration and invasion (Castillo-Lluva et al., Nat Cell Biol. 2010).

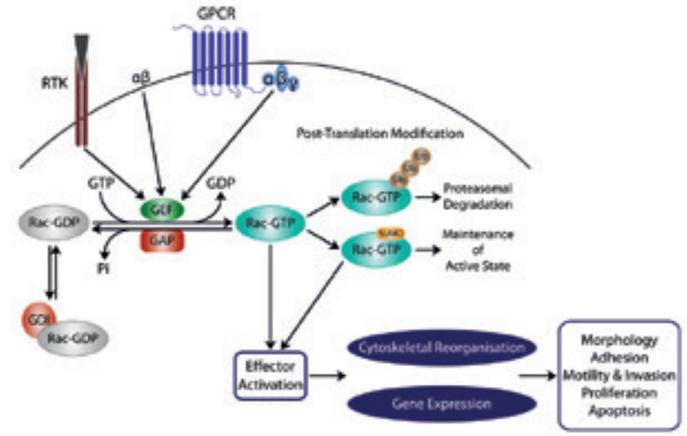


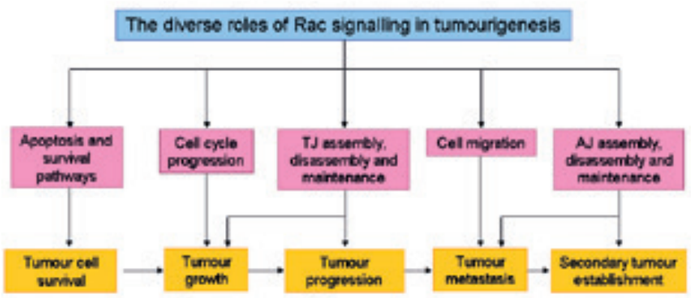
Figure 1
RAC GTPase cycles between inactive GDP-bound and active GTP-bound states. RAC activation is facilitated by the action of GEFs, which promotes GDP dissociation from RAC and allows GTP to bind instead. In turn, GEFs are stimulated by receptor tyrosine kinases (RTK), integrins (αβ), and G-protein coupled receptors (GPCR). Through the association with GAPs, the intrinsic GTPase activity of RAC is accelerated, thereby inactivating RAC. Through association with RhoGDIs (GDI) RAC can be sequestered in its inactive state. Activated RAC can also be removed through ubiquitylation-induced degradation (mediated by HACE1 following a migration stimulus) or it can be maintained following its modification by SUMO (mediated by PIAS3).

RAC activity is also regulated through ubiquitylation and subsequent degradation. Recently, we identified the tumour suppressor HACE1 to be the E3 ubiquitin ligase responsible for RAC degradation following activation by a migration stimulus. We showed that HACE1 and RAC1 interaction is enhanced by HGF signalling and that HACE1 catalyses the poly-ubiquitylation of RAC1 at lysine 147 following its activation by HGF, resulting in its proteasomal degradation. HACE1-depletion is accompanied by increased total RAC1 levels and accumulation of RAC1 in membrane ruffles. Moreover, HACE1-depletion enhances cell migration independently of growth factor stimulation, (Castillo-Lluva et al., Oncogene 2013). Jointly, the above two studies suggest that SUMOylation and ubiquitylation of RAC1 act co-ordinately to fine-tune RAC1 activity in migrating cells, promoting RAC activity at sites where the cell membrane is advancing, while antagonising RAC at sites where membrane protrusion needs to cease.

TIAM1-RAC signalling regulates bipolar spindle assembly, chromosome congression and mitotic progression dependent on phosphorylation of TIAM1 by Cyclin B/CDK1

Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by chemical carcinogens and the few resulting tumours grow very slowly (Malliri et al., Nature 2002). To better understand the role of TIAM1 in promoting tumour growth we have examined its role in the cell cycle. We revealed that TIAM1 and RAC localise to centrosomes during prophase and prometaphase, and TIAM1, acting through RAC, ordinarily retards centrosome separation. TIAM1-depleted cells transit more slowly through mitosis and display increased chromosome congression errors. Significantly, suppression of the microtubule motor Kinesin-5/Eg5 in TIAM1-depleted cells rectifies not only their increased centrosome separation but also their chromosome congression errors and mitotic delay (Woodcock et al., Curr Biol. 2010). Subsequent to this study, we have found that TIAM1 is phosphorylated by Cyclin B/CDK1 in mitosis. This phosphorylation, while not required for TIAM1 localisation to centrosomes, is essential for its role in regulating centrosome separation. Currently, we are investigating the mechanism by which phosphorylation of TIAM1 influences its role at centrosomes.

Figure 2
RAC contributes to several cancer hallmarks that promote tumour formation and progression.



TIAM1 antagonises malignant progression

Despite their slower growth, tumours arising in Tiam1-deficient mice progressed more frequently to malignancy (Malliri et al., Nature 2002). One mechanism by which TIAM1 and RAC suppress malignant progression is through promoting cell-cell adhesion. We further investigated the function of TIAM1 and RAC at cell-cell adhesions. We identified β2-syntrophin, a component of the dystroglycan adhesion complex, as a TIAM1 binding partner. Our study (Mack et al., Nat Cell Biol. 2012) unearthed a novel role for this complex in regulating tight junctions and the generation of apicobasal polarity through the formation of a RAC activity gradient in the membrane region encompassing these junctions.

Malignant progression can entail the loss of cell-cell adhesion. The oncoprotein Src, a non-receptor tyrosine kinase, targets adherens junctions (AJ) for disassembly. Previously, we showed that Src phosphorylates TIAM1, inducing its cleavage by Calpain and its depletion from AJs. Abrogating TIAM1 phosphorylation by Src suppressed AJ disassembly (Woodcock et al., Mol Cell 2009). We have now found that TIAM1, like RAC1, is ubiquitylated and degraded upon treatment of cells with HGF. We have mapped ubiquitylation sites and identified the responsible E3 ligase. Moreover, we show that interfering with TIAM1 ubiquitylation retards the scattering and invasion of cells through delaying AJ disassembly.

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CLINICAL AND EXPERIMENTAL PHARMACOLOGY

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Group Leader
Caroline Dive

Deputy Group Leader
Ged Brady

Disease Focus Team Leaders
Fiona Blackhall
Andrew Renehan
Malcolm Ranson

Staff Scientists
Jeff Cummings
Dominic Rothwell
Jonathan Tugwood

Clinical Lecturers
Alastair Greystoke[2]
Cliona Kirwan
Matthew Krebs

Associate Scientists
Christopher Morrow
Kathryn Simpson

Service Manager
David Moore

Postdoctoral Fellows
Alison Backen
Becky Bola
Radoslaw Polanski[2]

The CEP group places emphasis on the discovery, development and validation of circulating biomarkers to facilitate drug development and to aid cancer patient treatment decision making. Our scientific focus is lung cancer and this year we enhanced our portfolio of clinical trials incorporating Circulating Tumour Cells (CTCs) as biomarkers. A highlight is the enumeration and characterisation of CTCs within the pioneering TRACERx consortium that will map intra-tumour heterogeneity and the evolution of Non Small Cell Lung Cancer (NSCLC). This year our annual report outlines the development of unique patient derived CTC based mouse models, to study the biology of SCLC and to test novel agents, as well as the progress made by our circulating nucleic acids biomarkers team.

Small Cell Lung Cancer – the need for improved preclinical models to explore biology and test novel therapies

Small cell lung cancer (SCLC) represents ~15-20% of all lung cancer cases and is characterised by a high proliferative rate resulting in rapid tumour growth, early metastatic dissemination and an aggressive clinical course. Most cases are initially chemo and radiosensitive, but disease relapse invariably occurs with emergence of treatment resistance such that overall survival rarely exceeds two years. The major genetic aberrations in SCLC involve p53 (75-90%) and Rb (78-90%), along with mutually-exclusive amplification of MYC family genes in 18-31% of patients. Whilst many scientific hypotheses have been generated using long established SCLC cell lines, they have not been upheld in the clinic where trials with targeted therapies in SCLC have proved universally disappointing. Readily accessible patient derived preclinical models are required. The frequent, rapid and marked biological transition from chemotherapy sensitive to resistant disease suggests that SCLC has much to reveal regarding novel drivers of acquired chemotherapy resistance. However, a major barrier to more comprehensive understanding of human SCLC biology is the paucity of fresh, sufficient and sequential tumour biopsies for research as surgery is rarely performed. There are few useful preclinical models of SCLC with which to progress translational research and drug development.

CTC derived patient explant mouse models (CDX), a unique approach to study Small Cell Lung Cancer

Having demonstrated the prevalence of SCLC CTCs, we sought to develop patient derived in vivo models of SCLC, reasoning that tumour initiating cells must be present within this invasive tumour cell subpopulation. Blood samples were obtained from patients with chemonaïve, extensive stage SCLC. To establish whether patients' CTCs could form tumours in immune-compromised mice, 10ml of blood from each patient was enriched for CTCs and the enriched fraction injected subcutaneously into the flanks of NOD.scid IL2γ (NSG) mice. The number of epithelial CTCs ($EpCam^+/CK^+$) implanted was estimated in a paired 7.5ml blood sample using CellSearch technology. Palpable tumours were detected within 4 months of implantation with doubling times ranging from 5–21 days for a high proportion of patient samples. CDX derived from chemosensitive patients were slower growing than those derived from chemorefractory patients and the number of CellSearch detected CTCs in patients whose parallel blood samples gave rise to CDX were all >400/7.5ml.

Francesca Trapani[1]
Cong Zhou

Clinical Fellows
Kyaw Aung
Louise Carter
Leila Khoja[2]
Kalena Marti Marti
Robert Metcalf
Danielle Shaw
Laura Cove Smith

Scientific Officers
Jenny Antonello
Mahmood Ayub[1]
Jessica Booth[2]
Stephen Bramley
Debbie Burt
Fouziah Butt
John Castle
Samson Chinien
Jakub Chudziak
Martin Dawson
Olive Denneny
Suzanne Faulkner
Joseph Halstead
Grace Hampson
Cassandra Hodgkinson
Clare Hodgson
Hana Jelassi
Paul Kelly
Matthew Lancashire
Daniel Morris
Karen Morris
Jackie Pierce
Tony Price
Lynsey Priest
Robert Sloane
Nigel Smith
Nahida SultanaMiah[1]

Graduate Students
Danielle Potter
Shaun Villa[2]

Laboratory Manager
Martin Greaves

Scientific Assistant
Aileen Jardine

Admin Assistant
Lisa Waters

[1]joined in 2013
[2]left in 2013

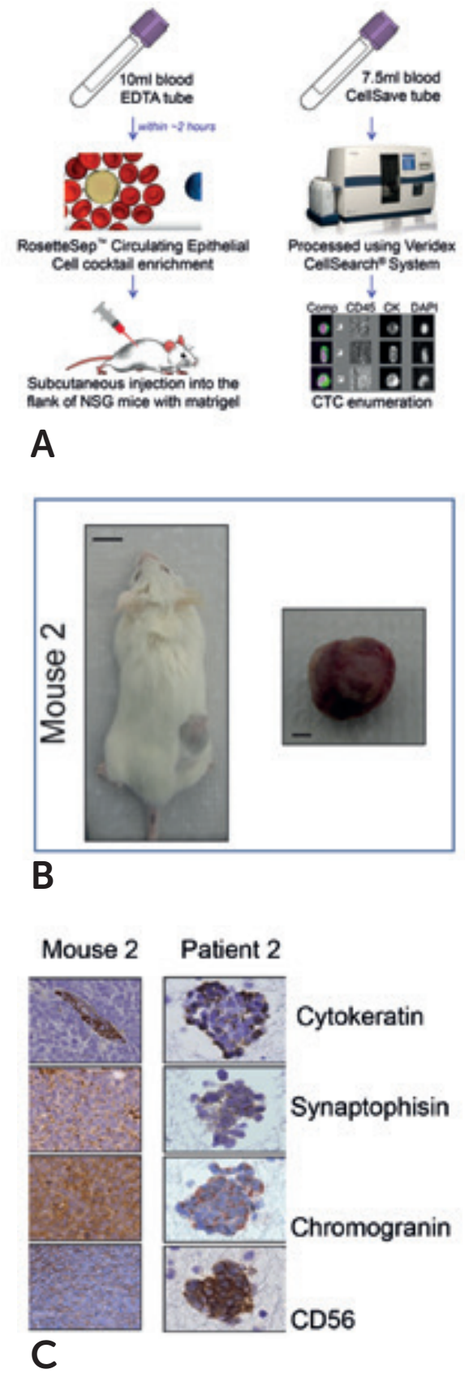


Figure 1
Development of SCLC Patient CTC derived mouse models: A, schematic of protocol for enrichment and enumeration of CTCs from SCLC patients' blood samples; B, example of subcutaneous CDX tumour; C, comparison of the pathology and immunohistochemistry of example CDX tumour with matched patient diagnostic cytology specimen.

The pathology, cytology and immunohistochemistry (IHC) of CTC tumours and micro-metastases mirrored that of the corresponding clinical diagnostic specimens. Mitotic and apoptotic cells were frequently observed in CDX. Detailed histological examination of serial lung sections revealed micrometastases comprised of human SCLC cells in the alveolar wall. CDX tumours have now been repeatedly passaged and on-going studies will show how closely their response to standard of care chemotherapy reflects the chemosensitivity of the patient from which they were derived. Importantly, we have developed sequential CDX models from individual patients' blood samples collected at baseline, prior to patient responses to first cycle of chemotherapy, and again as patients relapse with drug resistant disease. These models are now being interrogated to facilitate an understanding of acquired drug resistance and to identify potential targets that could be evaluated by our Drug Discovery Unit.

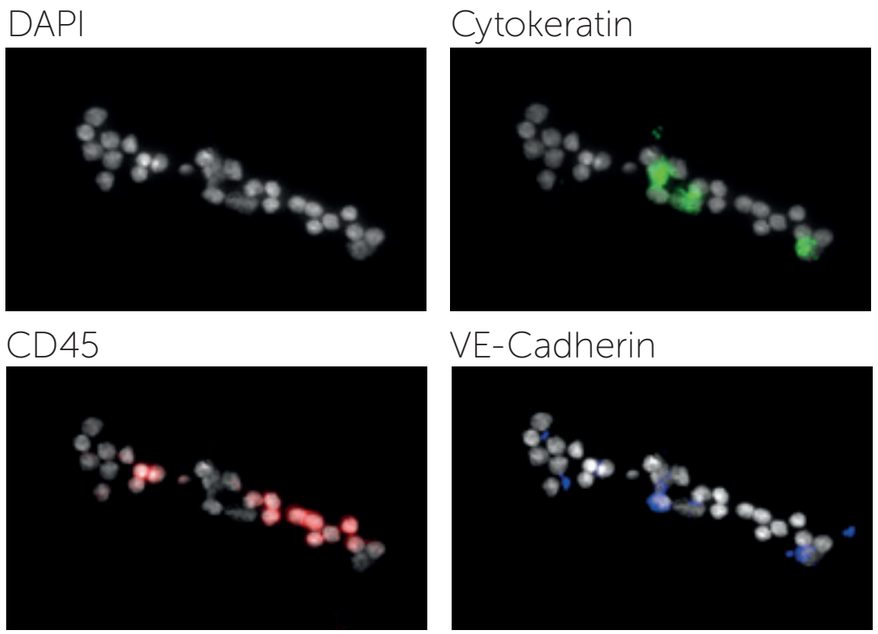
The Nucleic Acids Biomarkers Team (NAB)

Cancers arise and develop through the multistep accumulation of somatic mutations responsible for tumour growth, metastasis and the development of treatment resistance. Circulating biomarkers provide one of the most promising means of serially monitoring a patient's cancer and raises the possibility of more effective personalised therapies. A patient blood sample may contain 0-1000s CTCs in amongst 10^8 normal white blood cells (WBCs) along with nanogram quantities of tumour derived circulating free DNA (cfDNA). The NAB team led by CEP Deputy Group Leader, Ged Brady, was established in 2011 to develop circulating biomarkers in a clinic ready format. In 2013, the NAB team has established the following methodological protocols:

- Novel stable whole blood processing delivering CTCs and cfDNA up to 4 days post collection
- NGS based mutation calling and copy number variation (CNV) of tumour samples

- Routine single CTC isolation, Sanger sequencing and NGS based CNV analysis
- Platforms for multiplex NGS analysis of single CTCs
- Single cell mRNA profiling by array, RNA-Seq and qPCR
- Routine plasma cfDNA qPCR analysis
- Initial NGS of cfDNA including detection of a potential novel resistance biomarker
- Plasma miRNA profiling for clinical and pre-clinical monitoring
- Bioinformatic pipeline for sequencing and CNV analysis in collaboration with Crispin Miller's group

Fundamental to progress with nucleic acids based biomarker development, we improved blood collection and processing by ensuring minimal loss or change in cellular and extracellular nucleic acids following storage of whole blood for four days at room temperature. This simplification will greatly improve sample analysis for multi-site clinical trials and allows the analysis of cfDNA as well as CTC mRNA and DNA from the same blood sample. To establish molecular profiles of individual CTCs we have established a three step protocol (Figure 2), a) CTC enrichment removing >99% of WBCs; b) single cell identification/isolation and c) Genomic DNA (gDNA) and mRNA analysis. For each patient sample the appropriate CTC enrichment protocol was chosen based on the type of cancer and the downstream analysis required. For routine gDNA analysis of single CTCs we established a simple and representative whole genome amplification (WGA) approach. Over the last year we have successfully developed and applied next generation sequencing (NGS) approaches to determine gDNA copy number variation (CNV) and mRNA profiles from individual cells. Bioinformatic analysis of CTC CNV data (in collaboration with Crispin Miller) has established similarities between SCLC CTCs and matching CDX tumours as well as heterogeneity accompanying treatment and relapse.



CTCs from a patient with non small cell lung cancer enriched using ISET microfiltration. Tumour cells are identified through their expression of the epithelial marker cytokeratin (green) and leukocytes through expression of CD45 (red). The co-expression of cytokeratins and VE-cadherin (blue) by tumour cells is under investigation by our laboratory in association with a study of tumour cell vasculogenic mimicry.

Image supplied by Robert Metcalf.

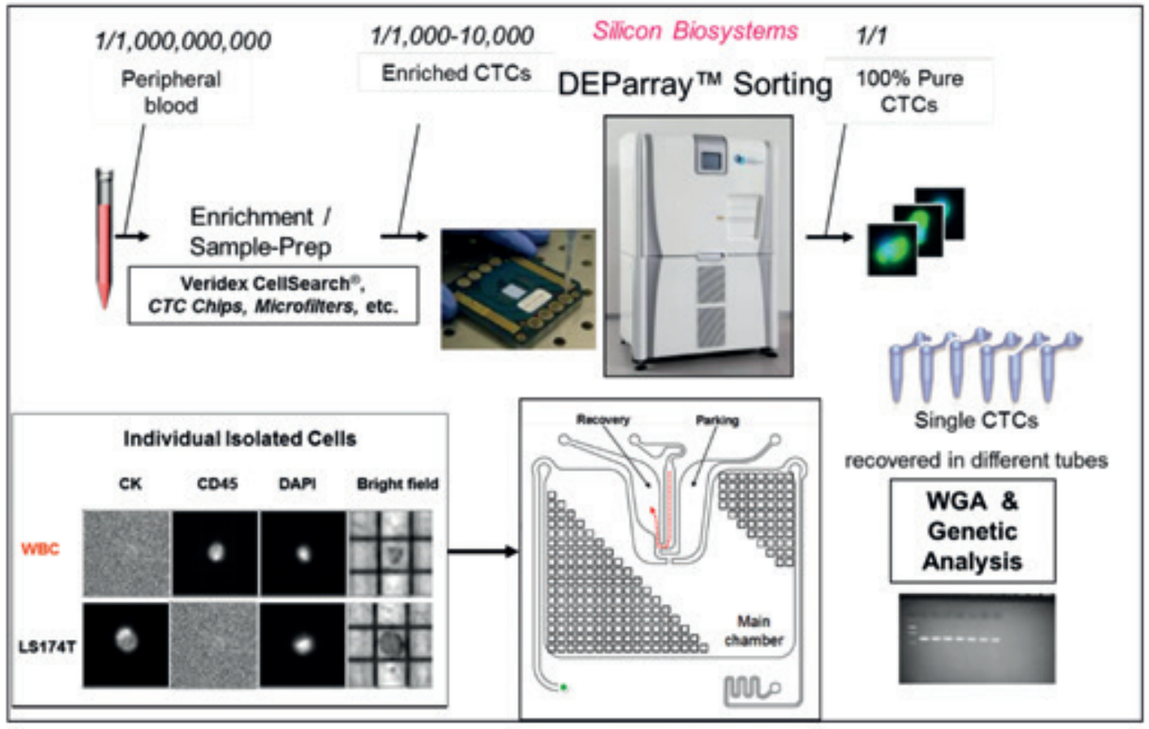


Figure 2
Schematic for the enrichment and isolation of CTCs prior to molecular analysis. WBC are used as negative controls and the protocol was initially validated using LS174T epithelial cells spiked in healthy volunteer blood: (CK= cytokeratins).

In collaboration with Nitzan Rosenfeld (Cancer Research UK Cambridge Institute) we have also established a focussed NGS approach (Tagged amplicon sequencing or TAm-seq) for single CTCs analysis.

For mRNA analysis we can now carry out accurate and reproducible transcriptional profiling at the single cell (CTC) level and have devised approaches that allow profiling of single cells stored at room temperature for four days. We have successfully used the mRNA profiling methods to identify stem cell linked signatures in enriched primary non-small cell lung cancer (NSCLC) cancer initiating cells, and this work was judged as the best poster (from a total of over 100) at the 9th International Symposium on Minimal Residual Cancer (Paris, Sept 2013).

We have developed our cfDNA program by establishing both whole genome and focused cfDNA NGS analysis. Initial longitudinal sequencing of plasma and tumour DNA from patients with advanced colorectal cancer (collaboration with Marilyn M. Li Baylor Genetics Dept) showed mutation profiling of cfDNA can complement tumour profiling by identifying mutations missed by tumour sequencing alone and was also able to detect the emergence of a cfDNA mutation that may be a potential chemotherapy resistance mechanism. We have developed a highly sensitive protocol for plasma based miRNA analysis that can now be utilised for longitudinal monitoring of tumours in mice and patients.

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DNA DAMAGE RESPONSE

Group Leader
Ivan Ahel[1]

Postdoctoral Fellows
Dragana Ahel[1]
Benito Banos[1]
Rosa Morra[1]
Roko Zaja[1]

Senior Scientific Officer
Ria Weston[1]

Graduate Students
Eva Barkauskaite[1]
Michael Tallis[1]

[1] left in 2013

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

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

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

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

Our laboratory is particularly interested in the identification of novel DNA repair pathways and protein functions regulated by poly(ADP-ribosyl) -ation, in order to identify components of these pathways that can be exploited as targets for cancer therapy. For this, we have been screening for proteins that have the ability to respond to DNA damage in a manner that is blocked by treatment with clinically relevant PARP inhibitors, such as olaparib. Our goal is to characterise some of the obtained candidate proteins and elucidate their exact biochemical functions in DNA repair, as well as their mode of regulation in response to DNA damage. Previously, in screening for proteins with the ability to bind poly(ADP-ribose), we discovered a poly(ADP-ribose)-binding zinc finger motif (PBZ). PBZ is a structurally distinctive, atypical type of zinc finger that is associated with several proteins involved in response to DNA damage (Ahel at al., Nature 2008).

One of the human proteins containing a PBZ motif is a protein called Checkpoint protein with FHA and RING domains (CHFR). CHFR is an ubiquitin ligase frequently inactivated in human epithelial tumours, which acts as a key regulator of the poorly understood early mitotic checkpoint that transiently delays chromosome condensation and nuclear envelope breakdown in response to variety of stresses. The elucidation of the function of the PBZ motif gave us a vital clue to discover that the CHFR-dependent checkpoint is regulated by PARPs and that the PBZ motif in CHFR protein is critical for checkpoint activation. Another PBZ-regulated protein we are studying is a protein called Aprataxin-PNK-like factor (APLF). APLF uses tandem PBZ repeats for direct interaction with poly(ADP-ribosyl)ated PARP1, which allows APLF's timely localisation to the sites of DNA damage. We previously discovered that the role of APLF is to act as a histone chaperone to modulate chromatin structure and facilitate DNA repair reactions in response to poly(ADP-ribose) signalling (Mehrotra et al., Mol Cell 2011).

Another class of DNA damage response proteins that is a focus of our research is the macrodomain proteins. The macrodomain is another evolutionary widespread module with the capacity to bind poly(ADP-ribose) and we recently identified several human macrodomain protein factors that are recruited to broken DNA ends in a poly(ADP-ribose)-dependent manner. These include a histone H2A variant called MacroH2A and several other uncharacterised macrodomain proteins, such as TARG1 and MACROD1.

Structural and functional analysis of the poly(ADP-ribose)-degrading enzymes and their validation as a targets for cell-permeable inhibitor design

Available data indicates that inhibiting PARG might offer a promising and beneficial approach in the treatment of cancer and cardiovascular conditions. However, unlike the case of PARP inhibitors, progress in developing permeable, small-molecule PARG inhibitors has been limited, partly due to the lack of functional and structural data for the human PARG protein. Recently, we solved the crystal structures of several PARG enzymes from bacteria and lower eukaryotes, which gave the first insight into the basic principles of PARG structure and its mechanism of catalysis (Slade et al., Nature 2011; Barkauskaite et al., Nat Commun. 2013). These structures revealed that the PARG catalytic centre is a diverged type of macrodomain and demonstrated that they are likely to prove useful in guiding structure-based discovery of new classes of PARG inhibitors. Despite these advances, detailed structural information on the human PARG is still lacking. Our goal is to solve the structures of human of PARG in complex with the substrate analogues and inhibitors which, in combination with solution and cell biology studies, should address the mechanism, structure and regulation of human PARG, as well as providing a foundation for the development of small, cell-permeable PARG inhibitors.

The PARG enzyme is unable to cleave the mono(ADP-ribose) unit directly linked to the protein targets; accordingly, we recently identified TARG1 (C6orf130) protein as an enzyme responsible for this last step of poly(ADP-ribosyl)ation reversal in human cells (Sharifi et al., EMBO J. 2013) (Figure 1). We are studying further this protein to understand its mechanism of action and the exact cellular pathways it regulates.

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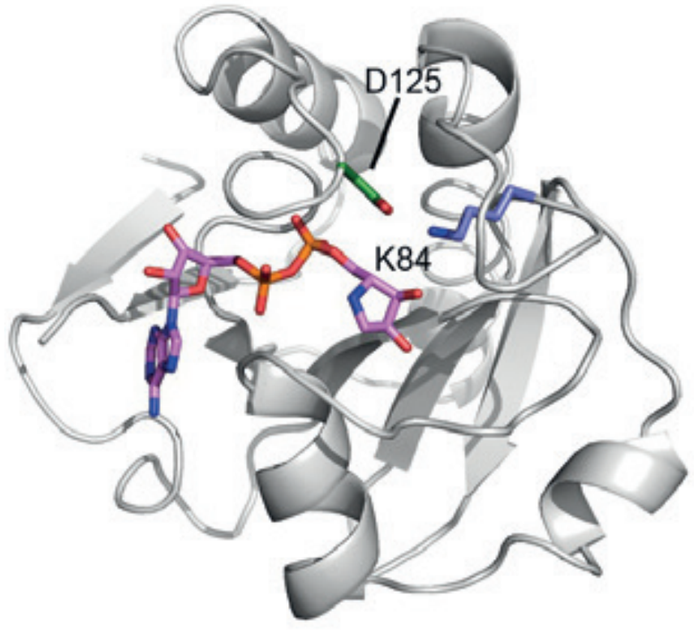
Figure 1
Active site of human TARG1 protein with bound ADP-ribose.



DRUG DISCOVERY

www.cruk.manchester.ac.uk/drugdiscovery

Group Leader

Donald Ogilvie

Head of Chemistry
Allan Jordan

Head of Bioscience
Ian Waddell

Chemists
Ali Raoof
Alison McGonagle
Amanda Lyons[2]
Bohdan Waszkowycz
Colin Hutton
James Hitchin
Kate Smith
Kristen Goldberg
Niall Hamilton
Rebecca Newton
Stuart Jones

Bioscientists
Alex Boakes
Ben Acton[1]
Dominic James
Emma Fairweather
Gemma Hopkins
Graeme Thomson
Helen Small
Mandy Watson
Mark Cockerill
Nicola Hamilton
Nikki March
Phil Chapman
Samantha Fritzl
Sarah Holt

Graduate Student
Daniel Mould[1] (PhD)

Undergraduate Students
Kate Bowler[1]
Eleanor French[2]

[1]joined in 2013

[2]left in 2013

During 2013, we have advanced our drug discovery portfolio significantly and now have two projects in the lead optimisation phase. We also currently have three earlier hit-to-lead projects tackling a variety of cancer targets. These activities are underpinned by multiple collaborations, both within and beyond Manchester. In particular, during 2013, we have signed four collaboration agreements with pharmaceutical and biotech companies to enhance our hit-finding capabilities.

Project Portfolio

Our current portfolio consists of two lead optimisation projects, directed against oncogene and DNA repair targets respectively, and three hit identification projects against two epigenetic and oncogene signalling targets (Figure 1). For the more advanced lead optimisation projects, the pharmacology of our novel compounds is being explored in cells, both internally and with key expert collaborators. In support of all of our projects, we have initiated a range of target biology and technology-related collaborations, both within and beyond Manchester.

Deployment of bioinformatics has had a major impact on our portfolio this year, both upfront in drug target selection, bringing forward new hypotheses for evaluation, and also at later stages. In particular, we have built and deployed an innovative collateral vulnerability bioinformatics pipeline to identify novel candidate drug discovery targets. In this approach, we seek to identify functional homologues of genes that are selectively deleted in cancer cells that are essential for their survival. Selective pharmacological targeting of these homologues is potentially lethal for cancer cells while sparing normal cells. The output from the collateral vulnerability analysis in lung cancer is now being followed up by functional target validation studies in Caroline Dive's Clinical and Experimental Pharmacology group (SCLC) and John Brognard's Signalling Networks in Cancer team (NSCLC). Exploration of additional cancer types, for which the required extensive mutation and expression data are available, is underway and this has led to new collaborations with target validation partners.

Capabilities

Once a target has been selected for drug discovery, the next stage is to try and identify prototype small molecules, or "hits", that interact with the target molecule. Using our fragment-based ligandability assessment platform, we have concluded that some of our novel targets carry a low/medium likelihood of finding new hit matter. In order to maximise our chance of success with these types of target, we have negotiated four screening collaborations with industrial partners giving us access to millions of diverse proprietary compounds. In two cases, with AstraZeneca and GlaxoSmithKline, this has already yielded novel chemical startpoints for our projects. In another of these deals, with the biotech company HitGen, we are accessing >400 million DNA-encoded compounds. Our local Cancer Research Technology representative, Martyn Bottomley, has played a key role in securing these important collaborations.

After hit matter is identified, one of the challenges in a drug discovery project is to assess rapidly and quantitatively the activity and selectivity of novel inhibitor compounds in intact cells. These cell assays are not only important to direct our own drug discovery efforts but also serve to benchmark competitor compounds as they emerge. In the LSD1 project, we have worked closely with James Lynch in Tim Somervaille's Leukaemia Biology group in the development and use of a novel, proximal cell biomarker for LSD1 activity. The DDU provided an irreversible LSD1-selective inhibitor (compound 3) and this was used to treat THP1 AML cells for 24 hours. Transcriptome analysis revealed CD86, a ligand for the co-inhibitory immune response receptors CTLA4 and CD28, as one of the five most upregulated genes. Inhibitor dose-dependent upregulation of the corresponding CD86 protein was then demonstrated using both flow cytometry and ELISA methods. The negative regulation of CD86 expression by LSD1 was confirmed using lentiviral shRNAs (Lynch et al., Anal Biochem. 2013).

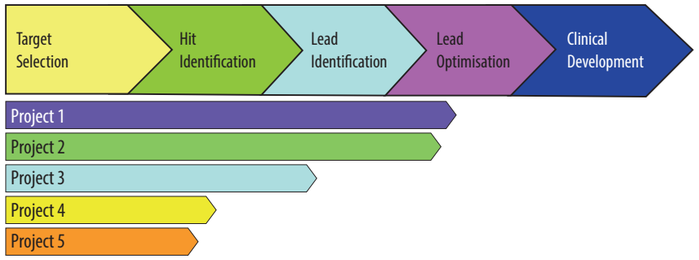


Figure 1
Drug Discovery Unit active portfolio.

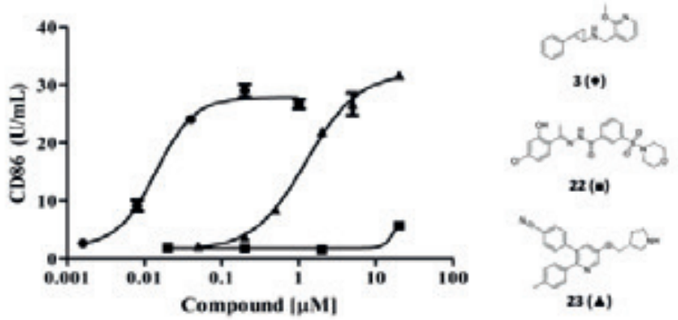


Figure 2
CD86 expression in THP1 cells, as determined by ELISA, following 24hr incubation with various concentrations of LSD1 inhibitors 3, 22 & 23

While this work was progressing, two putative reversible inhibitors of LSD1 were disclosed. These were quickly synthesised in the DDU and rapidly tested in the CD86 cell biomarker assay (see Figure 2 and Hitchin et al., Med Chem Commun. 2013). This experiment confirmed the highly potent, selective activity of the irreversible standard compound 3 (CD86 IC50 14nM; cytotoxicity IC50 >20uM). One of the competitor reversible inhibitors (compound 23) also showed potent, selective activity (CD86 IC50 1.2uM; cytotoxicity IC50 >20uM) in cells, while the other (compound 22) showed little CD86 activity and a negative margin to cytotoxicity (CD86 IC50 >20uM; cytotoxicity IC50 <2uM). These data demonstrate the power of this novel assay to assess and discriminate between these competitor reversible LSD1 inhibitors.

Finally, for our more advanced lead optimisation projects, in collaboration with colleagues in CEP, we have developed pharmacokinetic and biomarker technologies. These are very important capabilities, enabling the selection of the best compound(s) and models for demonstration of preclinical proof of concept and ultimately the identification of cancer patients most likely to benefit from the novel target approach in clinical trials.

Cancer Research UK

We remain actively involved in the broader Cancer Research UK drug discovery programme. During the summer of 2013 we underwent a successful five year (quinquennial) review with an international expert panel. The group was complimented on many aspects of its progress, including our closely integrated multidisciplinary team, clinical alignment of our projects, rapid decision making and project portfolio progression and we have recently been notified that our funding has been extended for a further five years (until March 2019).

People and Publications

During the last year as our project portfolio has moved forward we recruited Ben Acton to facilitate more advanced biological and pharmacokinetic testing of compounds. We have also taken on our first PhD student, Daniel Mould, who previously completed an industrial placement year in our laboratory and was awarded North West student of the year 2013 by the Society of the Chemical Industry for his project report.

Although wider reporting of our most advanced work is usually delayed by the need to file patents first, four papers directly relating to Drug Discovery activities were published in 2013. A further four publications, related to work with collaborators in the Institute and beyond, also appeared in 2013. Moreover, we are now progressing our first patent filings, providing legal protection for our discoveries in the laboratory.

The Future

During 2013, we have advanced our project portfolio into the lead optimisation stage and have enhanced our capabilities both internally and with four industrial collaborations. In 2014, we begin a new five year funding cycle and our ultimate aim for that period is to deliver our first candidate drug. In the short term, we look forward to progressing rapidly our lead optimisation projects while simultaneously bringing forward new target opportunities.

Publications listed on page 59

IMMUNOLOGY

Group Leader
Peter L. Stern[1]

Postdoctoral Fellows
Owen McGinn[1]
Julie Brazzatti[1]
Darren Roberts[1]

Clinical Fellow
Saladin Sawan[1]

Scientific Officer
Cheryl Petit[1]

[1]left in 2013

5T4 oncofoetal glycoprotein expression by many different cancer types with only low level detection in some normal tissues, as well as its mechanistic involvement in cancer spread, has driven the development and clinical testing of several 5T4 directed immunotherapies. Since 1989, our translational research has been powered by pivotal academic/clinical collaborations, most recently with the Groups of Professors Robert Hawkins, Vaskar Saha and Henry Kitchener within the University of Manchester's Institute of Cancer Sciences (ICS), interfaced with commercial partnerships, many of which were initiated through Cancer Research Technology. This final report aims to update progress of this body of work.

5T4 vaccine (TroVax® with Oxford BioMedica)

Vaccine immunotherapy aims to overcome the relative poor immunogenicity of tumour associated antigens (TAA) by presentation in a foreign viral vector with the principle goal of generating effector T cells able to kill 5T4 positive tumours. The highly attenuated and modified vaccinia virus ankara (MVA), expressing either human or mouse 5T4, was used for successful evaluation of immunogenicity and anti-tumour activity in preclinical studies. Following this, a succession of phase I or II clinical trials in colorectal, prostate and renal cell carcinoma (RCC) patients (including with chemotherapy or cytokine treatments) established the optimal dose and route of vaccination as well as safety, tolerability, and vaccine immunogenicity. Two or three TroVax® immunisations were needed to generate 5T4 specific cellular immunity and this was independent of the vector specific response, leading to a protocol of multiple booster vaccinations; there was an association of 5T4 immune responses with better clinical outcome in some cases.

A phase III trial in RCC patients was designed to determine if the addition of TroVax® to available standard of care therapy could improve survival for patients with metastatic RCC. 733 patients were randomised to receive 7/8 injections of TroVax® or placebo, along with either interferon-α, IL-2 or sunitinib as first line treatment (Amato et al. Clin. Cancer Res., 2010). While TroVax® was safe and well tolerated in all these patients, it failed to meet its primary endpoint as there was no significant difference in survival for the TroVax® and placebo treated groups. However, in the subset of patients with a good prognosis and receiving IL-2, there was a significantly improved survival with TroVax® compared to the placebo group. Interestingly, a high antibody response was associated with longer survival within the TroVax® treated group (Harrop et al., Cancer Immunol Immunother. 2011). It is tempting to speculate that such antibodies might be mechanistically involved in reducing cancer spread. This is supported by the observation that monoclonal antibody (mAb) to 5T4 can prevent the spread of 5T4 positive Sup-B15 B-ALL (acute lymphoblastic leukaemia) cells in a xenograft model (Figure 1). It is possible that the observed influence of spread might, in part, derive from inhibition of 5T4 glycoprotein function in regulating chemokine or Wnt signalling pathways (Castro et al., Leukemia 2012; Kagermeier-Schenk et al., Developmental Cell 2011). TroVax® is now being tested in ovarian cancer patients to see if there is benefit from an immunological response with an extended time to progression. A significant number of patients with advanced ovarian cancer develop CA-125 relapse whilst asymptomatic with low volume radiological disease, but there is no survival benefit from early chemotherapy treatment (www.ctc.ucl.ac.uk/TrialDetails.aspx?TrialID=75&TrialName=TRIOC).

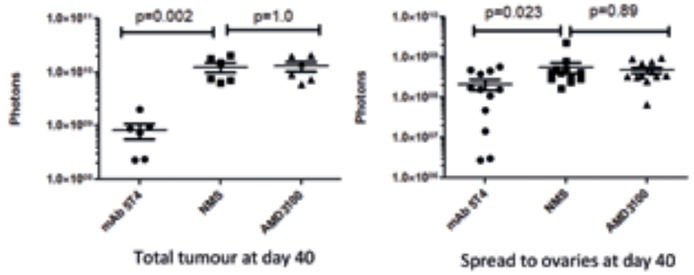


Figure 1
5T4 antibody inhibition of leukaemia spread
100µg mAb 5T4, but not normal mouse serum (NMS), (both given day one and then every other day for 10 days) or AMD3100 (CXCR4 inhibitor- plerixafor at 1.25mg/kg, given daily for 10 days) blocks spread of intravenous Sup5T4 leukaemia (5x10e6). Significant reduction in total tumour load and for spread to the ovaries at day 40 for mAb5T4 compared to either NMS or AMD3100 treated animals (Mann-Whitney).

5T4 antibody targeted superantigen therapy (with Active Biotech)

Bacterial superantigens, such as Staphylococcal Enterotoxin A (SEA), can activate T cells by linking the latter through binding to a particular family of V-beta chain containing T cell receptors (TCRs) to MHC class II molecules on antigen presenting cells. With an antibody-superantigen fusion protein, large amounts of cytotoxic and cytokine producing T cells can be targeted by the antibody specificity for a TAA for in vivo tumour treatment. A succession of 5T4-Fab-superantigen fusions designed to overcome the toxicity associated with MHC class II binding, and avoiding any pre-existing immunity to the bacterial protein, have been developed and tested in preclinical and clinical studies. The latest version (ANYARA or naptumomab estafenatox) incorporates a hybrid SEA/E-120 superantigen sequence with additional point mutations, reducing MHC class II binding and antigenicity (Forsberg et al. J. Immunother., 2010).

A multinational (UK, Russia, Ukraine, Bulgaria, Romania), randomised Phase II/III study of ANYARA, in combination with interferon-alpha versus interferon-alpha alone, in 533 advanced RCC patients has been completed. The treatment was safe but the primary endpoint of survival advantage in the intention to treat population was not reached. This appears to be a consequence of higher levels of pre-formed antibodies against the superantigen component of ANYARA in the patients recruited from East European countries. Thus a subgroup analysis, excluding patients with high levels of pre-formed antibodies, resulted in a trend for survival benefit with ANYARA treatment. Interestingly, high baseline levels of IL-6 were associated with a poorer outcome, and a hypothesis generating analysis of the 25 % of patients with low/normal levels of base line IL-6 and low anti-superantigen antibody levels, a statistically significant treatment advantage for overall survival was seen. In North America and Western Europe, this subgroup accounts for 40-50% of the total number of advanced renal cell cancer patients (J Clin Oncol 31, 2013 (suppl; abstr 3073)). Additional analyses of the ANYARA Phase II/III study data are on-going with future development strategies aiming at a phase II/III study with ANYARA in combination with a tyrosine kinase inhibitor in the favourable RCC subgroup.

5T4 antibody drug conjugates (ADC) (Pfizer)

ADCs chemically combine the specificity of the antibody with a cytotoxic drug. The challenge is to produce an efficacious and safe agent and this demands optimising the properties of a suitable TAA specific antibody in combination with the linkage chemistry and the payload characteristics. A recent development of this approach by Pfizer has used a 5T4 humanised monoclonal antibody (A1) linked by sulphydryl-based conjugation to deliver a tubulin inhibitor, monomethyllauristan F (MMAF) via a maleimidocaproyl linker (Sapra et al. Mol. Cancer Ther., 2013). This conjugate, (A1mcMMAF), showed potent in vivo activity in a variety of tumour models, with induction of long term regression after the last dose. Evidence of the selective accumulation of the 5T4 (but not control) conjugates with release of the payload and consequent mitotic arrest in the tumour tissue was demonstrated.

Depending on the particular tumour, 3-10mg/kg doses given every 4 days were sufficient to produce a complete pathogenic response; this was independent of the degree of heterogeneity in 5T4 expression. This effect was shown to be consistent with the targeting of tumour initiating cells (TICs) within the tumours. The overall therapeutic value is enhanced by the targeting of the most aggressive and tumourigenic populations within tumours (TICs), and its testing in a clinical setting is eagerly awaited.

Building on the above, funding from Leukaemia and Lymphoma Research (Stern, McGinn, Saha) and Wellcome Trust for a Clinical Research Fellow (Louise Wan) on a collaborative project (with Stern, Gilham & Kitchener) are allowing further evaluation of the role of chemokine and Wnt pathways as well as 5T4 targeted immunotherapies in leukaemia and ovarian cancer respectively within ICS.

Publications listed on page 59

INOSITIDE LABORATORY

Group Leader
Nullin Divecha[1]

Associate Scientist
David Jones[1]

Postdoctoral Fellow
Maria Carla Motta[1]

Scientific Officer
Yvette Bultsma[1]

Graduate Student
Rebecca Foulger

[1]left in 2013

Phosphoinositides are a family of lipid second messengers that are regulated in response to environmental changes by a network of kinases and phosphatases. Alterations in phosphoinositide levels can regulate many different cancer-relevant pathways including cell survival, proliferation, migration, cell substratum interactions and transcription.

In cancer cells, PtdIns(4,5)P2 is at the heart of phosphoinositide signalling and can be synthesised by two different kinase pathways. De-regulation of the PIP5K or PIP4K pathway leads to different outcomes in cancer signalling, suggesting that each pathway controls specific downstream targets.

PIP4K and PtdIns5*P*

PtdIns(4,5)P_2 is present in the plasma membrane and in the nucleus and can be synthesised by two different families of kinases utilising two different substrates (Figure 1). There are three isoforms of PIP4Ks of which the α is predominantly cytosolic, β is cytosolic and nuclear, and γ localises to internal membrane compartments. We developed a specific antibody to PIP4Kβ and interrogated tissue micro arrays of 500 advanced human breast tumour samples associated with patient outcome data. These data demonstrated that increased PIP4K2β expression in tumours was highly correlated with ERBB2 expression. Analysis of mRNA profiles derived from 3000 tumours associated with patient outcome supported the above conclusion (Figure 2) and analysis of genome amplifications in breast tumour samples demonstrated that the PIP4K2B gene can be amplified as part of the ERBB2 amplicon. Not surprisingly, increased PIP4K2β expression was associated with poor patient survival, probably as a consequence of its co-amplification with ERBB2 (Figure 2). Interestingly, some tumours also have marked increases in PIP4K2β expression in the absence of ERBB2 amplification, although low patient numbers prevented an analysis of its correlation with patient outcome.

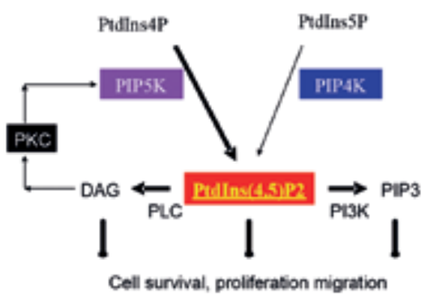


Figure 1
The phosphatidylinositol-5-phosphate kinase (PIP4K) and phosphatidylinositol-4-phosphate kinase PIP5K pathway contribute to phosphatidylinositol(4,5) bisphosphate (PtdIns(4,5)P_2) synthesis, however the major synthetic pathway is likely through PIP5K. PIP4K regulates the levels of phosphatidylinositol-5-phosphate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

Surprisingly, the immunohistochemistry study also showed that low PIP4K2β expression (the enzyme is expressed in normal breast tissue) also correlated with worse patient survival. This is strikingly illustrated in the analysis of the mRNA profiles from 3000 breast tumours (Figure 2). To begin to define if decreased PIP4K2B expression might be causal in patient survival, we analysed its expression and the consequences of manipulating its expression in human breast tumour cell lines. Our studies show that decreased PIP4K2β expression decreases the expression of the tumour suppressor protein E-cadherin. E-cadherin regulates cell-cell adhesion, and loss of E-cadherin expression increases the cells propensity to undergo an epithelial to mesenchymal transition (EMT), an acquired characteristic important in the development of metastatic tumour cells and the dissemination of tumour cells to distant organs.

In other cell types, targeted RNAi studies and RNAi screening strategies demonstrated that decreasing the expression of PIP4Kα specifically reduced tumour cell growth in colorectal, breast, pancreatic and leukaemic human tumour cell lines. Together with Dr. T. Somervaille, we showed that limiting the expression of PIP4K2α reduces cell growth of

Figure 2

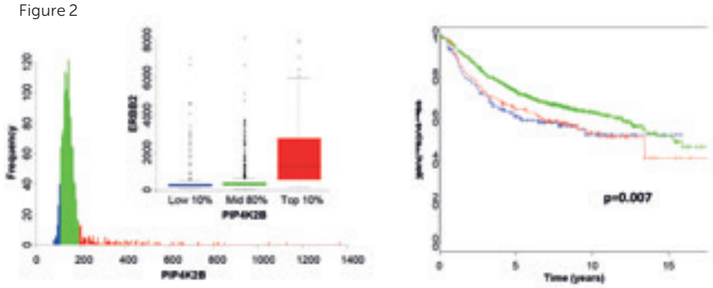


Figure 3

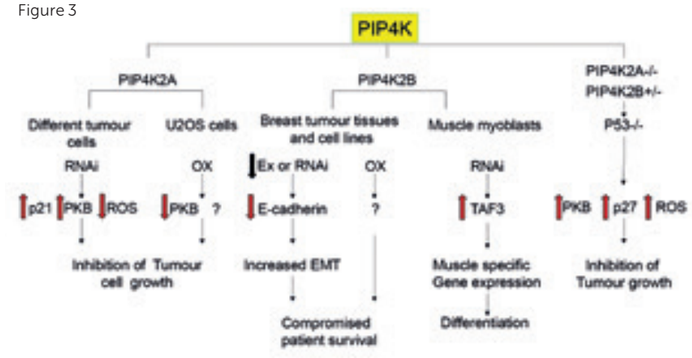


Figure 2
Left panel: Distribution of PIP4K2B gene expression was assessed across 2999 primary breast cancer tumours integrated from 17 studies. The data are presented as a frequency distribution of PIP4K2B expression. The samples were amalgamated into three groups based on PIP4K2B expression for further analysis: Top 10% (red) middle 80% (green) and the lowest 10% (blue). High expression of PIP4K2B (Red) was clearly associated with high ERBB2 expression (inset). Right panel: Cumulative recurrence-free outcome was plotted for low, middle and high PIP4K2B expression with colours defined above.

Figure 3
An illustration of how isoforms of PIP4K control cell fate in different systems. OX= overexpression and Ex= expression. Characterised changes in downstream pathways after manipulation of PIP4K are illustrated with red arrows. For p21, p27, e-cadherin and ROS arrows reflect changes in their levels while for PKB and TAF3 they represent change in activity.

leukaemic human cell lines, murine cell lines and of primary human tumour-derived AML cells. Strikingly, decreased PIP42α expression did not inhibit normal human haematopoietic stem cell growth or differentiation, suggesting that inhibitors of PIP4K2α might be used as therapeutics in AML. Other studies in muscle cells showed that reducing the expression of PIP4K2β, which is normally highly expressed in muscle, stimulated the ability of stem like myoblast cells to differentiate into myotubes. These studies suggest that PIP4K2β expression limits differentiation, perhaps preventing stem cell depletion during successive rounds of muscle injury, a process that is disrupted in diseases such as muscle dystrophies. The effect of manipulating the expression levels of PIP4K isoforms on cell fate in different model systems is outline in Figure 3.

How PIP4Ks control cell fate is far from clear. We have previously hypothesised that PtdIns5*P* is a key signalling intermediate and its sub-cellular compartmental specific regulation by PIP4K will regulate specific downstream pathways. For example, oxidative signalling plays a key role in aging and cancer; in response to oxidative stress, the induction of adaptive responses ultimately controls cell fate. We discovered that oxidative stress leads to a rapid and reversible increase in the levels of both cytoplasmic and nuclear PtdIns5*P* and that PtdIns5*P* signalling impacts on cell survival. The PKB, FOXO and NRF2 pathways are evolutionarily highly conserved regulators that control responses to oxidative insults. Our previous studies showed that nuclear PtdIns5*P* regulates the accumulation of toxic reactive oxygen species (ROS), possibly through regulating the NRF2 transcription pathway. Our recent studies show that oxidative stress induced PtdIns5*P* increases PKB activation and cell survival. Isoform-specific RNAi studies show that the PIP2K2A isoform regulates stress induced PtdIns5*P* accumulation and PKB activation, while paradoxically loss of PIP4K2α also inhibits cell growth in a PKB independent manner. How exactly PtdIns5*P* regulates PKB is under study, the importance of which is underlined by the intensity with which the pharmaceutical industry has pursued inhibition of the PI3K/PKB pathway as a means to halt tumour growth. Thus the inhibition of PIP4K2α might compromise tumour cell growth, however the paradoxical activation of PKB might eventually stimulate tumour growth if tumour cells can bypass the growth inhibition pathway.

In order to unravel how PtdIns5*P* regulates cellular processes, we have searched for PtdIns5*P* interacting proteins and have identified the PHD (Plant Homeo-Domain) finger as a receptor that is regulated by nuclear phosphoinositides. PHD fingers are cross-braced Zinc fingers, some of which can interact with and decode changes in histone tail modifications to regulate chromatin structure and gene transcription. We found that the PHD finger of TAF3, a component of the basal transcription complex that regulates transcription and cell differentiation, interacts with phosphoinositides. We characterised mutants that no longer interact with PtdIns5*P* and used these to demonstrate that changes in nuclear PtdIns5*P* are required for the transcription of a subset of TAF3-regulated genes through its interaction with the PHD finger. Mutants that do not interact with PtdIns5*P* still interact with components of the basal transcription machinery and can interact with H3K4me3 modified histone tails.

We are using TAF3 as a model to understand how changes in nuclear PtdIns5*P* directly regulate gene transcription, which might underlie our observation of the impact of PIP4K2B expression on muscle cell differentiation. Our studies highlight the importance of the expression of different isoforms of PIP4Ks in controlling cell fate and, together with novel studies (Cantley laboratory) showing synthetic lethality of PIP4K2α and β loss in p53 null tumours (Emerling et al., Cell 2013), suggest that isoform specific inhibitors of PIP4Ks might be useful to target cancer cell growth and other disease states.

Publications listed on page 60

LEUKAEMIA BIOLOGY

www.cruk.manchester.ac.uk/leukaemia

Group Leader

Tim Somervaille

Postdoctoral Fellows
Xu Huang
James Lynch

Clinical Research Fellows
Brigit Greystoke[2]
Dan Wiseman

Scientific Officers
Gary Spencer
Filippo Ciceri[1]

Graduate Students
Julian Blaser[2,3]
Filippo Ciceri[2]
Tim Somerville
Emma Williams[1]

[1] joined in 2013

[2] left in 2013

[3] co-supervised with Nullin Divecha until February 2013

Epigenetic dysfunction plays a critical but incompletely understood role in the pathology of myeloid cancers. In the past year, the Leukaemia Biology Laboratory has reported the results of a genetic knockdown screen designed to reveal novel dependencies of myeloid leukaemia cells on key regulators of the structure and function of chromatin.

We discovered that Enhancer of Polycomb (EPC) 1 and 2, components of the EP400 chromatin regulatory complex, are required to prevent AML cells (but not normal cells) undergoing apoptosis. Identification of genes selectively required for the function of leukaemia cells, but not normal bone marrow cells, is an important strategy to pinpoint new therapeutic targets for drug development.

To identify novel chromatin genes that regulate leukaemogenic potential, we adopted a targeted shRNA knockdown screening approach. The knockdown library consisted of 1040 individual lentiviral vectors targeting 272 genes with Gene Ontology chromatin annotations, and the screen was performed in human THP1 cells as a representative myeloid leukaemia line. Lentiviral supernatants were prepared individually in 96-well plates and the consequences for THP1 cell proliferation or apoptosis following infection by each were assessed using an alamarBlue cell biomass assay. In addition to *MLL* itself and *MEN1*, which is essential for the oncogenic potential of *MLL* fusion oncogenes, this approach identified the homologous Enhancer of Polycomb genes *EPC1* and *EPC2* as required for AML cell proliferation and/or survival. *EPC* is conserved and essential in yeast, fly and mouse and its gene product forms part of the EP400 chromatin regulatory complex, variants of which include the TIP60 histone acetyltransferase complex and a MYC binding complex. To date there is no information as to any role for EPC1/2 in normal or malignant haematopoiesis.

Knockdown of *EPC1* or *EPC2* in mouse MLL-AF9 AML cells, human THP1 cells and primary patient cells, abrogated clonogenic potential in vitro (Figure 1A) through induction of apoptosis, and abolished leukaemia-initiating potential in vivo as demonstrated by syngeneic and xenogeneic transplantation (Figures 1B and 1C). Forced expression of human *EPC1* or *EPC2* rescued the clonogenic potential of knockdown AML cells. Similar results were obtained following EPC knockdown in cell lines representative of other disease subtypes (e.g. Kasumi1, NB4, U937, HL60), suggesting a wide dependency of AML cells on EPC. By contrast, *Epc1* or *Epc2* knockdown did not significantly affect the clonogenic (Figure 1D) or reconstitution potential of normal murine haematopoietic stem and progenitor cells (HSPC). This was even though levels of *Epc1* and *Epc2* expression and efficiencies of knockdown of both transcript and protein were similar to those observed in AML cells. Experiments with normal human CD34$^+$ HSPC gave similar results: the clonogenic and multilineage differentiation potentials of *EPC1* and *EPC2* knockdown CD34$^+$ cells were maintained with respect to myeloid lineage colonies, although a reduction in erythroid burst-forming units was noted. Thus, there is a selective dependency of AML cells, but not normal HSPC, on *EPC1* and *EPC2*.

Using exon arrays, we next investigated the transcriptional consequences of *Epc1* and *Epc2* knockdown in murine normal and leukaemic granulocyte-macrophage progenitor cells. Changes in gene expression following *Epc1* knockdown were highly correlated with those following *Epc2* knockdown, suggesting their gene products have similar but non-redundant functions. The most remarkable observation was that prior to apoptosis of AML cells, there was relative up-regulation of transcriptional programs associated with the oncogenic potential of MLL leukaemia stem cells, including

Figure 1
In vitro and *in vivo* effects of EPC knockdown.
(A) Representative images show *EPC1* and *EPC2* knockdown abolishes the clonogenic potential of primary human AML cells from a patient with a t(9;11) translocation. Primary AML cells from a patient with a t(9;11) were infected with lentiviruses targeting *EPC* for knockdown, or a non-targeting control (NTC), with GFP as the selectable marker. (B) Graph shows percentage human AML cell engraftment in mouse bone marrow (BM) 150 days following transplant of 1.25 x10^5 FACS-purified GFP$^+$ *EPC* knockdown or control cells into sub-lethally irradiated neonatal immune deficient mice (n=4 per cohort). (C) Representative FACS plot shows engraftment in BM of primary human AML cells in a control mouse from (B). (D) Representative images show *Epc1* or *Epc2* knockdown spares the clonogenic potential of murine KIT$^+$ HSPCs.

Figure 2
Accumulation of MYC after EPC knockdown.
Murine MLL-AF9 leukaemic splenocytes (A) or KIT$^+$ HSPCs (B) were infected with lentiviruses targeting *Epc1* or *Epc2* for knockdown, or a non-targeting control (NTC), with GFP as the selectable marker. Cells were FACS-purified 48 hours following lentiviral infection. Representative western blots show expression of the indicated proteins in FACS-purified cells.

Figure 1

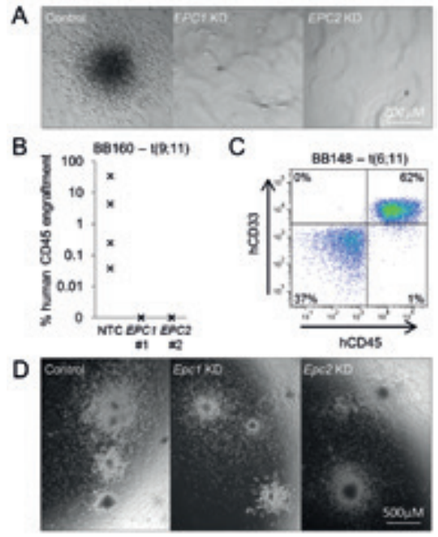
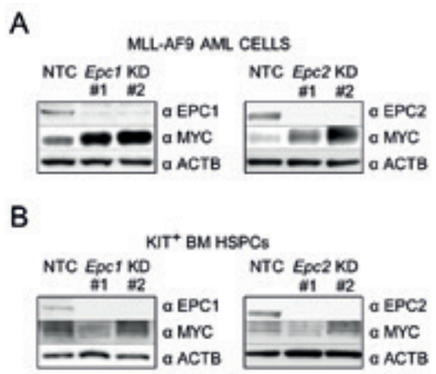


Figure 2



transcriptional changes previously associated with a MYC oncogenic signature. These changes were not seen following *Epc1* or *Epc2* knockdown in normal granulocyte-macrophage progenitor cells; indeed the changes in gene expression observed were quite distinct from those observed in AML cells, as expected given the different functional outcomes. Our data raised a question as to whether *Epc* knockdown activated MYC in leukaemia cells.

Western blotting demonstrated that in AML cells there was a marked accumulation of MYC protein, but not transcripts, following *Epc1* or *Epc2* knockdown (Figure 2A) which was not observed in normal HSPCs (Figure 2B). Cycloheximide chase assays demonstrated an increased half-life of MYC in *Epc1* knockdown AML cells by comparison with control cells. Similar accumulations of MYC protein (and induction of apoptosis) were observed following knockdown of genes coding for other members of the EP400 complex (which physically interacts with MYC) in murine and human AML cells. Together, these data suggest a role for EP400 complex members in regulating MYC turnover in AML cells, and in preventing leukaemia cell apoptosis.

We further demonstrated that the acute accumulation of MYC following *EPC1* knockdown contributed to apoptosis. This was suggested by four lines of evidence. First, both the accumulation of MYC and onset of apoptosis after *EPC1* knockdown in THP1 cells were found to be MAP kinase dependent, because both were significantly reduced by treatment of cells with MEK inhibitors U0126 or PD184352. Second, treatment of cells with 10058-F4, a small molecule inhibitor of MYC:MAX dimerisation (which did not prevent accumulation of MYC), also significantly reduced *EPC1* knockdown-induced apoptosis. Third, concomitant knockdown of *MYC* and *EPC1* delayed apoptosis by comparison with *EPC1* knockdown alone. Finally, acute induction of MYC levels in THP1 cells using a doxycycline-inducible system led to a significant and persistent increase in apoptosis and a modest but significant reduction in proliferation. These data were published as an article in *Leukemia* in October, with Xu Huang as the first author.

Inhibition of LSD1 as a potential therapy for AML

The last 12 months have also seen progress towards a first-into-man trial of a first-in-class inhibitor of the histone demethylase, LSD1. Our publication of data demonstrating that pharmacological inhibition of LSD1 promoted differentiation of human AML cells (Harris et al., Cancer Cell 2012) has led to a fruitful collaboration with Oryzon Genomics based in Barcelona, Spain. Their advanced lead molecule, ORY1001, which is a derivative of the monoamine oxidase inhibitor tranylcypromine, is ready for the clinic. Contingent upon ethical approval, we plan to initiate, in early-mid 2014, a clinical trial of this molecule in patients with relapsed AML at The Christie NHS Foundation Trust, Manchester. In parallel, we have continued to work on understanding the mechanisms by which tranylcypromine derivate LSD1 inhibitors, such as ORY1001, promote differentiation of AML cells; we plan to report our findings in this area in 2014. More generally, our future plans will focus on the identification and validation of additional candidate therapeutic targets in AML, as well as the evaluation of candidate compounds using in vitro and in vivo model systems that take advantage of patient AML cells as a prelude to early phase clinical trials.

Publications listed on page 61

MOLECULAR ONCOLOGY

www.cruk.manchester.ac.uk/molecularoncology

Group Leader
Richard Marais

Senior Clinical Scientist
Amaya Viros

Postdoctoral Fellows
Franziska Baenke
Kelly Brooks
Simon Furney
Romina Girotti
Gabriela Gremel[1]
Koorosh Korfi
Matthew Martin[2]
Berta Lopez Sanchez-Laorden
Grazia Saturno
HaoRan Tang

Bioinformatician
Mike Gavrielides

Scientific Officers
Sarah Ejiama[1]
Lesley-Anne Foster[1]
Clare McManus
Eamonn Morrison

Graduate Student
Kate Hogan

MSc Student
Courtney Thwaites[1]

[1] joined in 2013

[2] left in 2013

Our group focuses on cancer cell biology, with particular emphasis on melanoma, a form of skin cancer that affects over 12,000, and kills over 2,000 people in the UK each year. The *NRAS* gene is mutated in 20% of melanomas and *BRAF* is mutated in a further 40% of cases and the proteins these genes express are components of the RAS/RAF/MEK/ERK pathway, a conserved signalling module that regulates cell growth and survival. Importantly, drugs that inhibit this pathway can achieve impressive responses in melanoma patients whose tumours carry mutations in *BRAF*, but most patients develop resistance to these drugs after a relatively short period of disease control. An important concept to emerge from these observations is the need to understand the genetics and biology of individual patients' tumours so that their treatment can be tailored for their particular disease. Thus, a key aim of our group is to develop "personalised medicine" protocols for melanoma patients.

A key approach we take is to develop melanoma models driven by expression of oncogenes in melanocytes. In humans, these specialised pigment cells largely reside in the skin, where one of their major functions is to provide protection from the harmful effects of the ultraviolet radiation (UVR) that is present in sunlight. However, melanocytes also reside in organs such as the brain, eyes, ears and heart where they are presumed or known to perform other specialist functions. We previously demonstrated that oncogenic BRAF drives melanomagenesis, but although it can initiate this process, it does so by cooperating with additional genetic abnormalities. In 2013, we reported developmental melanoma models driven by oncogenic NRAS (Pedersen et al, 2013). We found that somatic mutations in NRAS in embryonic melanocytes appear to be a risk factor for leptomeningeal melanosis, a rare, but inevitably fatal form of childhood melanoma of the CNS. These studies have improved our understanding of the genetics underlying this particular disease and provided a relevant and tractable model system for its further study.

Drugs that inhibit BRAF can drive a curious paradox in cells. When BRAF is mutated they inhibit MEK/ERK signalling, but when NRAS is mutated, they hyper-activate MEK/ERK signalling. This is because in the presence of active RAS, BRAF inhibitors drive BRAF into a complex with a closely related protein called CRAF, activating CRAF and thereby MEK/ERK signalling. In 2013, we demonstrated that a third closely related protein called ARAF is not functionally redundant with CRAF in this process (Rebocho and Marais, 2013). It appears also to be activated by BRAF, but rather than binding to BRAF, it appears to stabilise the BRAF:CRAF complex, at least in some cells. These data add another layer of complexity to the paradox, but the consequences of this are currently unclear.

In 2012, we demonstrated that a consequence of the BRAF inhibitor paradox is induction of secondary non-melanoma skin cancers in ~30% of patients (keratoacanthomas and squamous cell carcinomas). This is because the paradox accelerates growth of pre-existing tumours, bearing mutations in one of the RAS genes, whose growth is then accelerated by the drugs. Single lesions can be easily removed by surgical approaches, but removal of multiple lesions can prove problematic. We demonstrated that topical application of 5-fluorouracil elicits regression in paradox-induced lesions, providing a relatively simple and inexpensive treatment for patients with large fields of tumours for whom surgery is not desirable (Viros et al, 2013).

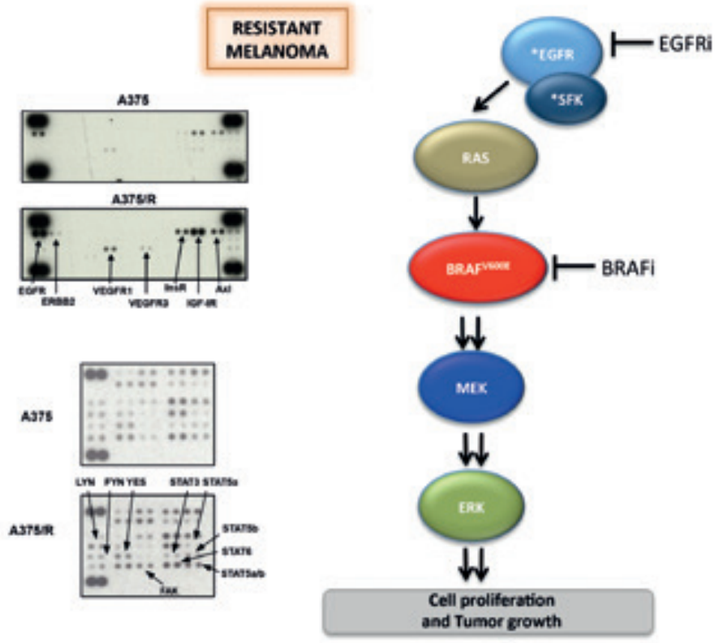


Figure 1
The EGF receptor confers BRAF inhibitor resistance in BRAF-mutant melanoma cells. LHS Upper panels: phospho-protein arrays for A375 and A375/R cell lines. Lower panels: The SFKs confer BRAF inhibitor resistance in BRAF-mutant melanoma cells: phospho-protein arrays. RHS: Drugs that target both the BRAF (BRAFi) and EGFR (EGFRi) pathways may provide a treatment strategy for patients who develop resistance to BRAF inhibitors.

Although BRAF drugs are effective in the majority of melanoma patients with a BRAF mutation, approximately 20% of melanoma patients do not respond to these drugs despite the presence of a BRAF mutation (primary resistance), and the majority of patients who do respond, relapse on treatment due to the development of secondary resistance. In 2013, we reported that hyper-activation of epidermal growth factor receptors (EGFR) can drive resistance in some patients (Girotti et al. 2013). We developed cell lines resistant to BRAF inhibitors and discovered that they had elevated EGFR, and SRC family kinase (SFK) signalling. Importantly, the combination of EGFR and BRAF drugs was able to inhibit the growth of the resistant lines both *in vitro* and *in vivo*. Importantly, we observed increased EGFR and SFK activity in tumours from patients who had developed resistance to BRAF drugs and showed that tumours from resistant patients were susceptible to the combination of BRAF and EGFR drugs. These data established that increased EGFR signalling can mediate resistance to BRAF drugs, adding to the complexity of mechanisms of resistance to these drugs (Figure 1). Our studies suggest that drugs that target both the BRAF and EGFR pathways may delay the onset of resistance in some patients and may even provide second-line treatments for patients who have failed treatment.

Finally, we have continued to use next generation sequencing to characterise the genomes of human melanomas. We performed whole genome, or whole exome sequencing on ten mucosal melanomas. The mutation signature did not implicate UVR exposure in the genesis of this disease and we observed a significantly lower number of single nucleotide variants (SNVs), but a significantly higher number of copy number variations and structural changes than are seen in mucosal melanoma (Furney et al, 2013a). These data show that mucosal and common cutaneous melanomas are different diseases with a distinct genesis. The data suggest that structural variations in the chromosomes play a more significant role in mucosal than in common cutaneous melanomagenesis, but the consequences of these differences to disease progression and treatment are currently unclear.

We have also performed whole genome sequencing on 12 uveal melanoma samples (Furney et al, 2013b). Uveal melanoma is the most common eye malignancy and it has a very poor prognosis, being fatal in about half of patients. Surprisingly, uveal melanoma had a very low tumour burden, with very small number of SNVs (only ~2,000 per genome) and a small number of structural chromosome variations. Again we did not observe a UVR mutation signature, suggesting that UVR does not play a role in uveal melanomagenesis. In addition to recurrent mutations in GNAQ or GNA11 (11/12 samples) and BAP1 (7/12 samples), previously described as the most common oncogenes and tumour suppressors in uveal melanoma, we also identified mutations in *SF3B1* in ~15% of the tumours. The tumours with *SF3B1* mutations had a better prognosis than those with loss of chromosome 3 and these events generally appear to be mutually exclusive. *SF3B1* encodes for a component of the splicosome machinery and accordingly, the mutations in *SF3B1* were associated with differential splicing of specific coding and non-coding RNA. Again, these data suggest that uveal melanoma is a distinct disease that is driven by distinct genetics, highlighting the need to improve our understanding of melanoma biology to allow us to develop new treatment approaches for melanoma patients.

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SIGNALLING NETWORKS IN CANCER

www.cruk.manchester.ac.uk/signallingnetworks

Group Leader
John Brognard

Postdoctoral Fellows
Shameem Fawdar[1]
Anna Marusiak

Clinical Fellow
Andrew Hudson

Scientific Officers
Natalie Stephenson
Eleanor Wendy Trotter

Graduate Students
Zoe Edwards
Ewelina Testoni

[1] left in 2013

Signalling pathways dictate a range of important cellular outcomes, ranging from cell death to replication and cellular migration. Genetic lesions that skew the balance of these pathways towards abnormal growth, proliferation, and cell survival, are the fundamental mechanisms that cause normal cells to become premalignant. Kinases are the key regulators of signalling pathways (similar to transistors in a circuit) and dictate the activation or amplification of a given signal that ultimately leads to cellular fate decisions. When these kinases are hyperactivated, or inactivated by genetic mutations, they become the main drivers of tumourigenesis and thus serve as primary targets for the development of small molecule inhibitors.

Cancer genomic sequencing studies and genome-wide siRNA screens are highlighting the amazing diversity in the kinases required to maintain tumourigenic phenotypes or permit drug resistance and emphasise the importance that neglected or understudied kinases play in the development and maintenance of a tumour. Thus a major goal of our lab is to identify and elucidate novel kinase drivers that are essential for lung tumour development, required for maintenance of lung tumorigenic phenotypes or play a role in lung cancer therapeutic resistance.

Genetic Drivers of Lung Cancer

Lung cancer patients receive significant benefit from targeted therapies aimed at mutationally activated kinases, such as EGFR or EML4-ALK, where inhibition of the activated kinase suppresses proliferation and promotes cell death. However, despite the significant advances in targeted therapies for lung cancer patients, only approximately 20% of lung cancer patients can be stratified for treatment with targeted therapies. Therefore, it is necessary to elucidate novel signalling pathways that are essential to maintain lung cancer cell survival. The lab utilises three strategies to identify such pathways in lung cancer. In the first approach we use bioinformatic tools to evaluate the functional impact of somatic mutations in novel or understudied kinases identified in lung cancer genomic screens. We use the following bioinformatics applications to assess the functional impact of somatic mutations: PMUT, polyphen2, mutationtaster, snpeffect, SIFT, and SNPs and go. Kinases where a majority of mutations are predicted to be "likely cancer", "pathological" or likely to have a "high" functional impact are further evaluated in the lab. Additionally, we model many of the mutations that score highly in our analysis to determine the structural consequences of the putative driver mutations concomitant with experiments in the lab. We have found that this approach works extremely well in identifying LOF mutations and candidate tumour suppressing kinases.

In a second approach we use genetic dependency screens to identify mutationally activated drivers of lung cancer. Utilising cancer genomic sequencing data from the Sanger Institute we depleted six lung cancer cell lines of all somatically mutated proteins to discover novel genetic dependencies and identify low frequency driver mutations. Targeted genetic dependency screens are an effective way to uncover low frequency oncogenes that can serve as targets for therapeutic intervention for tumours of any origin. Specifically, we identified FGFR4, PAK5, and MLK1 as kinases that harbour novel GOF mutations in lung cancer patients and this results in hyperactivation of the MEK/ERK pathway (Figure 1). The mutation frequency for the genes we identified ranged from 2-10% of lung cancers; given the frequency of lung cancer in the population, these targets could be exploited by pharmaceutical companies for drug discovery development. These types of screening approaches have the potential to identify both therapeutic targets and biomarkers.

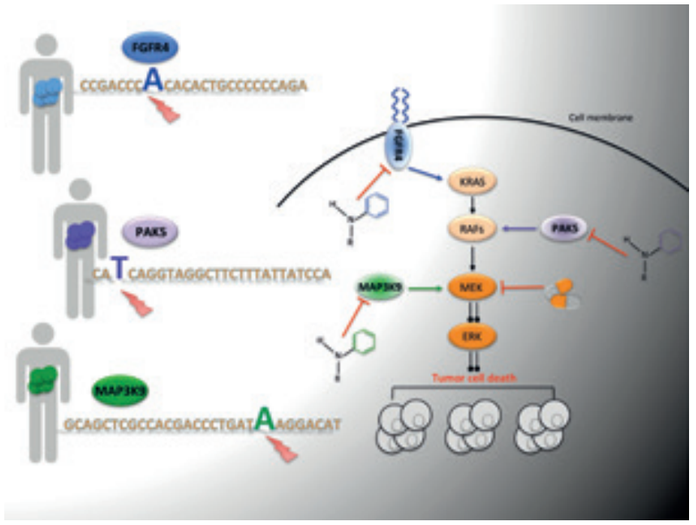

Figure 1
Targeted genetic dependency screen to identify novel actionable mutations; mutated FGFR4, MAP3K9 and PAK5 are illustrated examples.
Patient tumours are exome sequenced and treatment is stratified based upon the mutational profile of each individual. Treatment options include novel pharmacological compounds to specifically inhibit driver oncogenes, and/or targeting downstream the main hyper-activated pro-proliferative signalling pathway, for example inhibition of MEK.

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

Characterisation of mutant kinases

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

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

Kinase mutations in other cancers

In addition, the lab is focused on a novel family of kinases capable of promoting resistance to targeted therapies for melanoma patients. Guided by cancer genomic studies the lab has elucidated a novel family of MEK kinases that can promote resistance to RAF inhibitors in melanoma. Future work will be aimed at understanding the molecular mechanisms that dictate pathway activation downstream of these kinases. Lastly, we have identified a novel tumour suppressing kinase in colon cancer and we aim to elucidate why LOF mutations in this kinase are essential for the development of colon cancer.

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STEM CELL BIOLOGY

www.cruk.manchester.ac.uk/stemcellbiology

Group Leader

Georges Lacaud

Postdoctoral Fellows

Julia Draper
Kiran Batta
Roshana Thambyrajah
Michael Lie-A-Ling
Guilherme Costa[2]
Anne Largeot[1]

Scientific Officer

Rahima Patel

Graduate Students

Milena Mazan[2]
Elli Marinopoulou
Magdalena Florkowska

[1]joined in 2013

[2]left in 2013

Transcription factors bind to specific sequences in DNA and control how genes are transcribed into RNA and, as a consequence, indirectly control the translation of RNA into functional proteins.

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

MOZ regulates the expression of the tumour suppressor p16^{Ink4a} and entry into replicative senescence of neuronal and haematopoietic stem cells

The histone acetyl-transferase MOZ (Monocytic Leukaemia Zinc Finger protein; MYST3, or KAT6A) is a key regulator of haematopoiesis, frequently found translocated in acute myeloid leukaemia. Previous studies have revealed a crucial role of MOZ in controlling haematopoietic stem cells and progenitors' (HSC/Ps) proliferation.

We recently established that this effect is not limited to the haematopoietic compartment, but extends to neuronal stem cells and progenitors (NSC/Ps), suggesting that these two types of cells, HSCs and NSCs, use the same mechanism involving MOZ-driven acetylation in order to maintain their capacity to proliferate. We then demonstrated that the HAT activity of MOZ is critical for silencing expression of the tumour suppressor *p16^{Ink4a}*. In the absence of MOZ HAT activity, expression of *p16^{Ink4a}* is up-regulated in haematopoietic and neuronal progenitor and stem cells, inducing an early entrance into replicative senescence and loss of self-renewal. Silencing of *p16^{Ink4a}* by genetic deletion reverses the proliferative defect in both *Moz$^{HAT-/-}$* haematopoietic and neural progenitors.

Altogether, these results provide new insights into the control of stem and progenitor cell proliferation and identify an unexpected role of MOZ-mediated acetylation in the regulation of *p16^{Ink4a}* expression (Figure 1). We propose that this mechanism could also be critical for the self-renewal of other types of stem cells. These findings also suggest that, in the context of leukaemia, the repressive activity mediated by MOZ acetylation on the expression of the tumour suppressor *p16^{Ink4a}* might be further exacerbated in fusion proteins created upon translocations at the human MOZ locus. As such, these MOZ leukaemic fusion proteins would inhibit the triggering of senescence and favour the development of leukaemia.

Generation of smooth muscle cells by haemangioblast

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

The earliest site of blood cell development in the mouse embryo is the yolk sac where blood islands, consisting of haematopoietic cells surrounded by a layer of angioblasts, develop at approximately day 7.5 of gestation. The parallel development of these two lineages in close association provided the basis for the hypothesis that they arise from a common precursor, a cell called the haemangioblast. A conflicting theory however, associates the first haematopoietic cells to a phenotypically differentiated endothelial cell with haematopoietic potential, i.e. a haemogenic endothelium. Support for the haemangioblast concept was initially provided by the identification during embryonic stem cells differentiation of a clonal precursor, the blast colony-forming cell (BL-CFC), which gives rise after four days of culture to blast colonies with both endothelial, smooth muscle and haematopoietic potential. Recent studies have now provided further evidence for the presence of this tri-potential precursor in vivo. We have recently established a new model of haematopoietic development based on the in vitro differentiation of embryonic stem cells. We demonstrated that haematopoietic cells are generated from the haemangioblast through the formation of a haemogenic endothelium intermediate. During this process, the haemogenic endothelial cells lose their endothelial identity, altering their flat, adherent appearance into the characteristic round shape of mobile haematopoietic precursor cells.

The haemangioblast generates blast colonies containing both haematopoietic, endothelial and smooth muscle cells. These vascular smooth muscle cells represent a major component of the cardiovascular system. During vasculogenesis, newly formed endothelial vessels become rapidly associated with mural cells of the smooth muscle cell lineage. These cells are either referred to as vascular smooth muscle cells if they encircle larger vessels, or as pericytes if they reside within the wall of small vessels, such as capillaries and post-capillary venules. These cells regulate blood flow through contraction and have been proposed to control endothelial cell proliferation and differentiation. Although the role of smooth muscle cells in the pathophysiology of cancer is still unclear, evidence suggests that aberrations in pericyte-endothelial cell signalling networks could contribute to tumour angiogenesis and metastasis.

To further investigate the development of smooth muscle cells, we generated a mouse reporter ES cell line in which the expression of the fluorescent protein, H_2B-VENUS, is driven from the α-SMA (Smooth Muscle Actin) regulatory sequences. We demonstrated that this reporter cell line allows us to efficiently track smooth muscle development during murine ES cell differentiation. The expression of H2B-VENUS was strongly correlated with α-*Sma* expression during the in vitro differentiation of this reporter ES cell line (Figure 2A). In addition, the enrichment for expression of a panel of smooth muscle markers indicated that H_2B-VENUS$^+$ cells accurately represent a smooth muscle cell lineage. Furthermore, we detected, mostly in the H_2B-VENUS sorted cells, transcripts of the long isoform of *Smoothelin-B*, suggesting that our ES differentiation conditions preferentially generate vascular rather than visceral smooth muscle cells. Altogether, these results indicate that H_2B-VENUS detection allows the direct quantification or isolation of smooth muscle cells. With this reporter ES cell line, we then confirmed that clonal BL-CFCs generate smooth muscle cells. To determine if the generation of smooth muscle cell is associated with, or independent from, the emergence of the haemogenic endothelium intermediate cell population, we examined the presence of H_2B-VENUS$^+$ cells in subpopulations containing this precursor. We indeed observed the presence of some H_2B-VENUS$^+$ cells in these haemogenic endothelium populations. However, these cells lacked haematopoietic potential and therefore did not correspond to functional haemogenic endothelium, indicating that smooth muscle cells are largely generated independently from the haemogenic endothelium. Altogether, these findings are consistent with an early separation between haemogenic endothelium and smooth muscle lineages during development (Figure 2B). Whether signalling by smooth muscle contributes to some extent to the generation of blood cells from haemogenic endothelium is still unclear. This study provides new and important insights into haematopoietic and vascular development, which may help drive further progress towards the development of bioengineered vascular grafts, or help discover new opportunities to control tumour angiogenesis and metastasis.

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Figure 1
The Histone Acetyl Transferase (HAT) activity of MOZ regulates proliferation of haematopoietic and neuronal stem cells
MOZ histone acetyl transferase activity prevents entry into early replicative senescence by regulating the expression of the tumour suppressor *p16^{INK4a}*. In the absence of MOZ HAT activity, the levels of *p16^{INK4a}* are significantly increased in both haematopoietic and neural stem cell compartments. Upon *p16^{INK4a}* expression upregulation, these cells leave the cell cycle to become senescent, resulting in severely impaired haematopoietic and neural stem cell self-renewal.

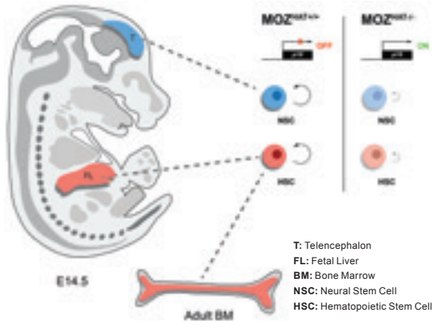
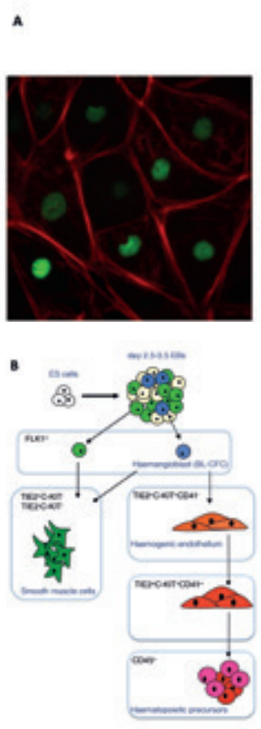


Figure 2
Smooth Muscle cells develop independently from haemogenic endothelium
A. Immmuno-staining of smooth muscle cells generated upon ES cell differentiation (red: SMA Smooth muscle actin, green; H_2B:Venus). B. Model of generation of smooth muscle cells by Flk$_1^+$ cells and haemangioblast.



STEM CELL HAEMATOPOIESIS

www.cruk.manchester.ac.uk/sch

Group Leader

Valerie Kouskoff

Postdoctoral Fellows
Alexia Eliades
Maud Fleury
Eva Garcia-Alegria
Josh Lilly

Scientific Officers
Stella Pearson
Kirstie Wallace

Graduate Students
Sara Cuvertino
Genny Filiciotto[1]
Andrzej Mazan
Sharmin Naaz[2]

[1]joined in 2013

[2]joint with ACBB group

Early events of embryonic development can be recapitulated in vitro upon the differentiation of pluripotent stem cells, such as embryonic stem cells or induced pluripotent stem cells. However, many pluripotent cell lines are notoriously refractory to differentiation toward some of these specific lineages.

Given the therapeutic potential of cell populations generated upon in vitro differentiation, it has become essential to determine how pluripotent stem cells can be directed to specific fates for the generation of lineages such as blood, endothelial or cardiac cells to be used for therapeutic purposes. To date, the molecular mechanisms controlling the specification of mesodermal precursors to each of these fates remain poorly understood.

Specification of mesoderm derivatives

At the early stage of embryonic development, the multipotent epiblast gives rise to the three germ layers which are subsequently specified to form all tissues of the developing organism. Initiation of germ layer formation occurs through a process known as gastrulation, in which cells from the epiblast undergo an epithelial to mesenchymal transition and ingress through the primitive streak to generate mesoderm and endoderm. Cells exiting the posterior part of the primitive streak form the extra-embryonic and lateral plate mesoderms, which later are specified to become blood precursors, endothelial and smooth muscle, forming the vasculature, heart and mesothelium (Figure 1). The molecular mechanisms controlling the specification of mesoderm are just starting to be unravelled, and the overall transcriptional network orchestrating this process is still poorly understood. In particular, how alternate cell fates are regulated in nascent mesoderm is mostly unknown.

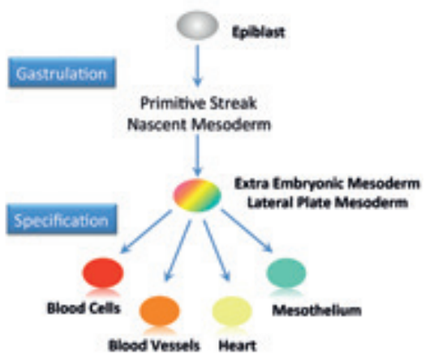


Figure 1
Schematic representation of the cellular steps leading to the formation of mesoderm derivatives.

The specification of blood and endothelial lineages is tightly associated, depending on similar signalling pathways and transcription factors for their emergence. We and others have shown that the FLK1-VEGF signalling axis and the ETS transcription factor ETV2 are essential for the generation of both lineages. However, both FLK1 and ETV2 are widely expressed in nascent mesoderm and are unlikely to be responsible for restricting mesoderm specification to blood and/or endothelium fate. Directly regulated by ETV2, the bHLH transcription factor SCL is required for both haematopoiesis through its control of haemogenic endothelium formation. Interestingly, SCL was recently shown to repress cardiogenesis in prospective haemogenic endothelium, suggesting that even after specification the fate of mesoderm lineages remains plastic and requires active repression to alternative fates.

Specification of the cardiac tissues depends on the transcription factor MESP1, a master regulator of cardiac development expressed in the primitive streak. Upon enforced expression, MESP1 promotes the generation of all cardiac lineages, controlling the downstream cascade of transcriptional regulators. A recent study, however, has challenged this view, implicating MESP1 more widely in the patterning of mesoderm. Depending on specific cell contexts, MESP1 was shown to specify mesoderm toward haematopoietic, cardiac or skeletal muscle lineages. It remains to be determined, however, what this cell context specificity implies and how it impinges on MESP1 activity. Little is known about the specification of mesoderm to the mesothelium that forms an epithelial monolayer lining all body cavities and organs. Apart from its lateral plate mesoderm origin, nothing is known about the molecular control underlying the specification of this lineage that shares a common mesodermal origin with the cardiovascular system.

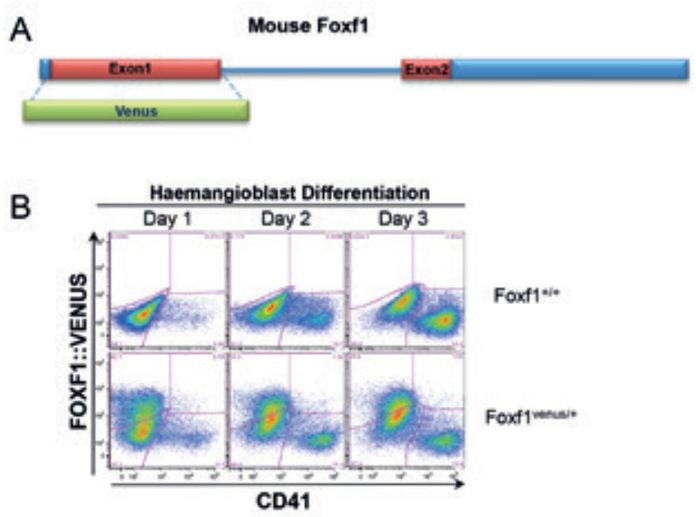


Figure 2
A) Schematic representation of the *foxf1* knock-in construct in which the Venus cassette replaces the first exon of foxf1. A PGK-neomycin resistance cassette was initially present in the construct for selection purpose but was removed via Flp/Frt deletion. B) Wild-type (foxf1$^{+/+}$) and Foxf1::Venus knock-in (foxf1venus/$^{+}$) ES lines were differentiated for 3 days through embryoid body formation; FLK1+ cells were isolated and further differentiated in haemangioblast culture conditions promoting the formation of CD41 haematopoietic progenitors. Flow cytometry analysis for FOXF1::VENUS and CD41 expression was performed at day 1, 2 and 3.

FOXF1, a novel regulator of mesoderm specification

Forkhead box (FOX) transcription factors belong to an evolutionary conserved family of proteins named after the *Drosophila* forkhead gene whose mutation results in a spiked head phenotype. There are 44 FOX proteins in the mouse genome, all characterised by a forkhead DNA-binding motif, and assembled into 19 subgroups based on sequence homologies inside and outside the fork-head domain. FOXF1, one of the two members of the FOXF subgroup, is expressed widely in extra-embryonic and lateral plate mesoderm, and its deletion leads to early embryonic lethality with a complete absence of vasculogenesis and mis-expression of haematopoietic and vascular markers. At a later stage of embryonic development, heterozygosity or mutation in FOXF1 is linked to lung malformation and alveolar capillary dysplasia involving the abnormal development of the vascular system in the lungs. Given its expression pattern and null phenotype, we became interested in the potential role of this transcription factor during mesoderm specification.

In order to characterise in detail its expression, to track and isolate FOXF1-expressing mesoderm, we generated embryonic stem (ES) cells and transgenic mouse lines carrying a *foxf1-venus* knock-in allele (Figure 2A). When FLK1^{+} cells, isolated from in vitro differentiated Foxf1$^{venus/+}$ ES cells, were cultured to generate blood progenitors, we observed the formation of a distinct FOXF1::VENUS^{+} subpopulation. Interestingly, CD41^{+} blood progenitors were not VENUS^{+} (Figure 2B). This observation was further confirmed in gastrulating embryos where FOXF1::VENUS expression was exclusively detected within the yolk sac and did not co-segregate with CD41 expression. Further analysis of E7.5 embryo tissue sections revealed that while FOXF1::VENUS expression was seen in the allantois, the amnion and the mesothelium lining the exocoelomic cavity, it was not detected in the prospective blood islands.

To further assess the relationship between FOXF1 expression and blood specification, we generated an ES cell line in which the expression of *Foxf1* can be induced by doxycycline. Upon culture of in vitro differentiated FLK1^{+} cells, we observed that the induction of FOXF1 expression dramatically compromised the production of blood progenitors. Taken together, our data suggest that FOXF1 expression is incompatible with blood specification and actively represses the specification of mesoderm precursors to haematopoiesis.

The potential role of FOXF1 in cardiac specification was assessed using an ES cell line in which both *Foxf1* alleles were deleted. Upon in vitro differentiation, the absence of FOXF1 expression led to a dramatic increase in cardiogenesis potential, suggesting an active repression of this programme by FOXF1 expression. In line with this observation, explant culture of yolk-sac derived from *Foxf1*-deficient embryos were capable of generating cardiomyocytes, further delineating a role for FOXF1 in the repression of the cardiac programme in mesoderm precursors. Genome wide experiments are currently underway to further understand how, at the molecular level, FOXF1 represses both haematopoiesis and cardiogenesis. Mesothelium appeared to be the only mesoderm derivative in which high expression of FOXF1 is observed. Interestingly, in the adult organism, the mesothelium retains remarkable progenitor characteristics with the ability to differentiate into myofibroblast, smooth muscle and endothelium upon injury or specific signals. It will be important in the future to explore the possible role of FOXF1 in the maintenance of this progenitor potential.

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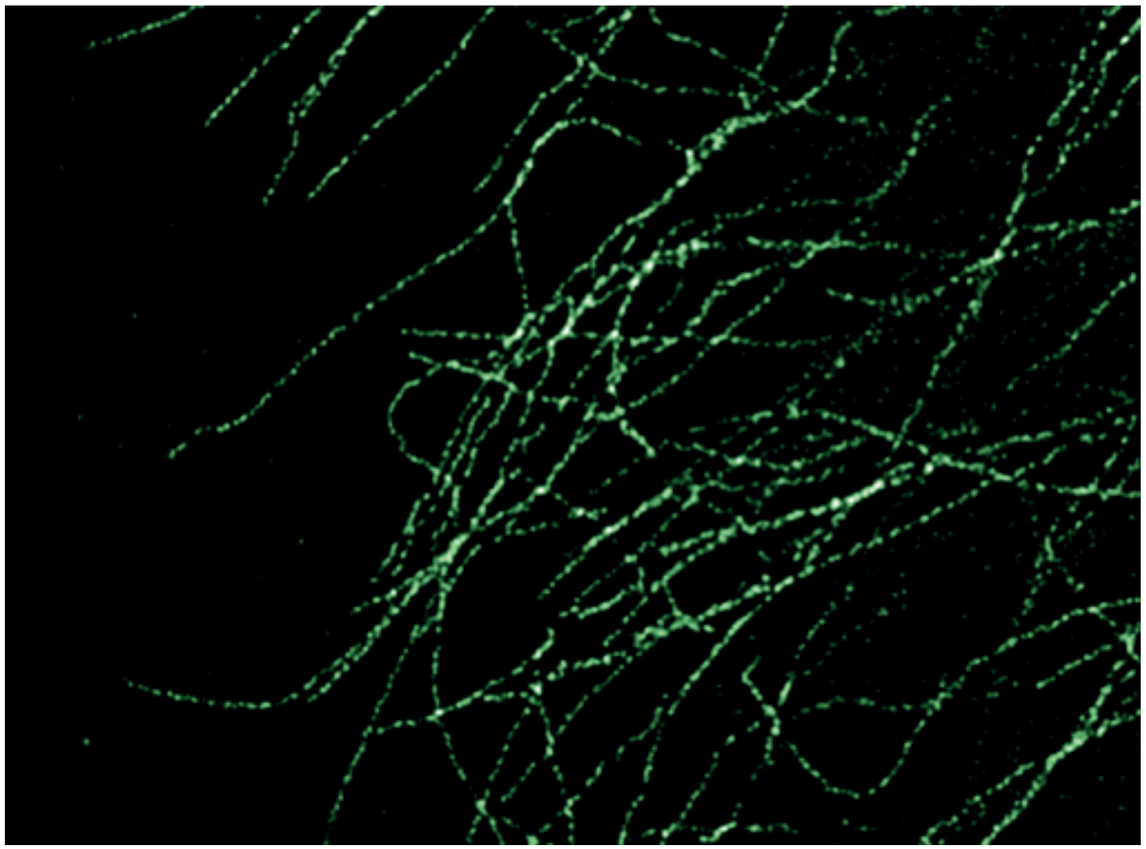

Imaging with gated stimulated emission depletion (gSTED) microscopy. Actin has been labelled with Alexa488-Phalloidin. Each pixel of the image is 19nm in size and the whole image took twelve seconds to capture. After image capture, deconvolution has been applied with the Huygens software resulting in an overall resolution of around 28-30nm. The spotted nature of the image is due to the size of the labelling complex which blocks additional binding sites.

Image supplied by Steve Bagley.

CANCER RESEARCH UK MANCHESTER INSTITUTE

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Chief Laboratory Officer
Stuart Pepper

Towards the end of 2012, HEFCE awarded £8.7 million to the University of Manchester from the UK Research Partnership Investment Fund, to be used for the provision of equipment to support cutting edge cancer research. Most of this equipment will be housed in the existing core facilities within the Institute and, as the sections below indicate, this opportunity is already providing a boost to the range of services available.

Chief Laboratory Officer
Stuart Pepper

Most of the purchasing during 2013 has been to support genomic sequencing, histological analysis and FACS analysis and details are given below of the equipment that has been procured. Extra funding was also provided by the MCRC to support purchase of a gated STED microscope, a truly state of the art imaging system, discussed further under 'Advanced Imaging and Flow Cytometry'.

Given the recent major investment into the service units, this seemed like a good opportunity to undertake a full review of the core facilities to ensure that the services are being developed in a way that closely matches the needs of the researchers. An external panel of experts visited the Institute in November and met with service managers and key users. The outcome was very positive and will help guide the development of the services during 2014.

Advanced Imaging and Flow Cytometry

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

[1]joined in 2013

The facility has experienced substantial growth during 2013, both in data complexity and with an increase in equipment provision, consequently allowing the researcher to study the molecule, the cell, the population and the tissue. In response to the research requirements of the Institute, there was a real need to increase capacity and accelerate workflows, as well as installing systems that provide new techniques to ensure the Institute maintains its cutting edge data collection capabilities. New developments have taken place across all parts of the facility: flow cytometry; whole slide histology scanning; high content scanning; and microscopy.

Within flow cytometry, a BD Aria III and BD Fortessa were purchased to increase sample throughput. The BD Aria has more lasers than the standard off-the-shelf system, allowing greater versatility for future projects, such as sorting Confetti cell populations. The Confetti technique produces a population of cells that stochastically express several fluorescent proteins, which in turn are passed down to the daughter cells, a technique that is heavily utilised in stem cell studies. As the amount of primary samples has increased, the system has been installed in a class II cabinet to ensure purity of sort and operator safety. The BD Fortessa was installed at the same time as the Aria, providing an additional platform for cytometry analysis. The system has automation for high throughput multi-well plate analysis, thus increasing the rate of data collection for the exploration of protein expression within large cell populations.

An Amnis ImageStream X II has been installed in the CEP laboratory, which allows all samples to be run to GCP(L) standardisation. The equipment is a combination of cytometer, microscope and high content system which allows visualisation of every non-adherent cell in a sample, containing up to twelve different labels per cell, at high capture rates (up to 4,000 cells per second) in ten minutes. The nature of the equipment allows for mathematical modelling of the variation that exists in a population of cells, which can be exploited for the analysis of potential biomarkers.

The introduction of the Seahorse XFe allows researchers to measure the extracellular flux of the two major energy pathways of the cell, mitochondrial respiration and glycolysis, in a multi-well plate and in real-time. With few cells, this technology analyses the effects of up to four compounds on cellular metabolism over minutes, hours or days.

Within the histology imaging section of the facility, a new scanner has been installed which permits the sampling of tissue by searching for cells that exhibit user-defined parameters based on their spectral qualities. By utilising a spectrophotometer, immuno-histochemical and fluorescently labelled tissues are imaged, modelled and analysed. This allows for the removal of background auto-fluorescence (which is inherent in all cells and tissues) and the application of multiple labels so that complex questions can be asked of primary tissue. The technique is beneficial when detecting rare cells within a tissue section which exhibit morphological or protein expression differences when compared to the main population.

A new member of staff has been appointed during this year to manage histological imaging, to train and provide assistance to the users, and to develop techniques for the image analysis of tissues. The development of scanning, analysis and modelling is on-going as researchers are asking more involved questions of tissue that requires assessment at the molecular level.

High content screening (HCS) has been undertaken within the facility since 2004 with a variety of microscope systems, however in 2011 the purchase of a HCS solution allowed for a greater level of automation of cellular analysis. Within the Institute, HCS is predominantly used by two large research groups; for the assessment of drug screens and the analysis of circulating tumour cells. This year, an automated loader arm was installed which permits batch processing of up to 80 multi-well plates, consequently allowing a higher throughput of data.

In the microscopy part of the facility, the installation of the two-photon confocal microscope and the gated stimulated emission depletion (gSTED) nano-scope has allowed researchers to visualise cells with a greater clarity than was previously possible. The two-photon microscope images deep into tissue, enabling the visualisation of cell invasion, tumour formation and the interaction of cells with connective tissues. The gSTED nano-scope allows the high resolution study of cells under laser illumination. The system produces a high-resolution image where the relationship between two labelled proteins can be characterised in such a way that each pixel of the image equates to 19nm. This level of resolution is far greater than achieved through use of more traditional systems, for example a confocal microscope, which typically has a resolution of around 200nm. The antibody-label complex used to visualise and provide contrast has an approximate size of 35-45nm, hence the gSTED system produces data that cannot be resolved by the confocal microscope. The gSTED resolution improvement is made possible by side stepping some of the barriers that are inherent in traditional microscope illumination and design.

As demands upon the facility will increase in the coming year with the appointment of new research groups within the Institute and the completion of the Manchester Cancer Research Centre building, a major external review of the facility was undertaken to assess the established structures, workflows, efficiency, management and staffing levels. The review incorporated the comments of Group Leaders at the Institute. In light of the review, several new structures are to be put in place to enable the facility to meet future demands.

Biological Mass Spectrometry Facility

Duncan Smith, Yvonne Connolly, John Griffiths

The role of the facility is to enable research groups to access cutting edge proteomic workflows. The lab is active in routine service provision, project design and data interpretation, through to the bespoke collaborative development of novel workflows designed to answer previously intractable biological questions. The foundations of the facility are based on maximising CRUK MI's research output by balancing high quality service provision with active research and development to evolve new workflows and technology key to delivering a world-class facility.

We have pushed our current Orbitrap technology further than ever with the development of ion trap centric data independent acquisition methods that facilitate more comprehensive analysis of every analyte within a given sample. This year has seen a massive improvement in our ability to both detect and map sites of SUMO modification by bespoke enzymatic digestion, chemical derivitisation and subsequent diagnostic ion generation (Chicooree et al). We have further enhanced this approach towards fully quantitative analysis by utilising mTRAQ labelling

chemistries that facilitate quantitative analysis in both MS and MSMS modes. In the sample preparation area, we have utilised a range of immobilised proteases with enhanced activities in an attempt to enhance proteolysis in workflows where this step is currently performance limiting, such as the analysis of complex protein mixtures. We have also been very active in developing the next generation of data analysis tools, including de novo sequencing and intelligent integration of multiple database search algorithms into a unifying results report.

In 2013, staff members from the facility were authors of six peer reviewed publications. A collaboration with the Cell Division group showcased the power of our comprehensive phosphomapping workflow by helping uncover fundamental aspects of mitotic commitment (Grallert et al).

Biological Resources Unit

The animal facility has been fully occupied throughout 2013, both for Transgenic Production and Experimental Services, with a total of 15 Project Licences in place with primary and secondary availability. The renewal of the Transgenic Service Licence over the summer has ensured production of progeny for the next five years for the scientific programmes.

Transgenic Services

To facilitate expansion of the transgenic service the entire facility is relocating to space in the University Incubator Building. This move has required the rederivation of over 100 lines and it has been a significant challenge to manage this rederivation programme whilst maintaining a fully functional service. As 2013 closes, the rederivation is nearing completion and should be finished in the early stages of 2014. The next step will be an expansion of stock in the new facility, followed by a winding down of the breeding program in the existing facility. Once this move is accomplished, there will be the capacity for a significant expansion from the current level of approximately 2000 cages up to a maximum of 5,500 cages.

Experimental Services

The experimental area has been supported by licensed technicians who have delivered a varied range of non-surgical and surgical procedures for 11 research groups. As always, we have ensured that the highest quality of care has been provided, and that Home Office legislation has been adhered to, by performing daily health and welfare checks along with extensive monitoring for animals under procedure.

A range of techniques have been supported by the facility, including subcutaneous implantation of slow-releasing hormone pellets, parenteral injections, gavage, blood sampling with and without recovery, whole body irradiation, local irradiation to subcutaneous tumour and in vivo imaging. For most of the year the experimental facility has run close to capacity. As the move of the transgenic facility described above progresses, there will be a great opportunity to expand the experimental service. Funds are in place to increase the number of staff and provide new platforms for in vivo imaging.

A significant change to the law took place at the start of the year with the implementation of Directive 2010/63EU across the UK. This has led to some local changes for the management of the Certificate of Designation, now known as the Licence 2C for the establishment. Two new roles have also been created: Training and Competency Officer and Named Information Officer. These two roles will help to ensure that all Personal Licence Holders have access to the most recent information on relevant procedures and will be able to carry out experiments to the highest possible standards.

Histology

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

The past year has once again been extremely busy as the Histology unit continues to develop and expand. The range and complexity of the services offered continues to grow, all of which have seen exceptional demand over the period. The continued professional development and retention of staff, with particular focus on cross training, has ensured that the unit continues to offer a comprehensive and flexible service at all times. The unit specialises in the histological preparation and analysis of a wide range of samples, including tissues from mouse models, whole mouse embryo preps, cell preps, and human biopsies. The samples are in either paraffin or frozen format.

The laboratory was refurbished earlier in the year allowing better use of existing space and thus creating a second clinical sample preparation area. In addition, more space has since been secured and it is hoped that the new immunohistochemistry platform will be housed in this area. The existing automated immunohistochemistry (IHC) platforms continue to offer a high throughput, routine, troubleshooting and antibody validation service. Demand on this service has once again seen a sharp rise with many antibodies being validated. The new joint post between the Histology and Advanced Imaging and Flow Cytometry Facilities, focussing on the numerical analysis of histological data in tissue biomarker expression studies, is proving very successful.

Multispectral imaging has allowed the Histology facility to evaluate the methodologies of truly multiplex immunohistochemistry. This allows researchers to study both the levels of expression of different proteins within the same tumour tissue/cells, and the interactions between proteins, and for the first time on a large-scale to understand how these markers interact. Ultimately, this will allow us to study tumour heterogeneity from a different angle that complements efforts in understanding the genetic/molecular heterogeneity of tumours. We have also facilitated the adoption of other sophisticated multiplex labelling techniques, including multiplex quantum-dot based immunofluorescence and proximity ligation assay for in situ detection of molecular interactions.

As the expansion of tissue biomarkers research continues to gather pace, the construction of tissue microarrays (TMAs), using both the ATA27 and MTA1 platforms, has also increased. High throughput, accurate TMA construction of tumour specific and custom arrays has resulted in the production of extremely high quality TMAs giving true representation. The service is now embedded within the facility with TMAs from disease groups including breast, melanoma, prostate (cores and chips), bladder, lymphoid, small cell and non-small cell lung cancer having all been constructed. In addition, mouse model and cell pellet control microarrays have been constructed.

Today, the nature of oncology research requires more downstream analysis on smaller clinical samples and as a result we are continually looking at developing workflows requiring less material from both laser capture microdissection and macrodissection. Recent work has allowed us to extract both RNA and DNA, sufficient in quantity and quality for Next Generation Sequencing from relatively small amounts of material, whilst still leaving material for future subsequent analysis. Workflows for both nucleic acid and proteomic analysis from the same sample, taking into account tissue heterogeneity, have also been developed and used successfully.

The unit continues to process FFPE and frozen samples for the Manchester Cancer Research Centre Biobank. To date samples from over 5200 patients have been collected. Blood, bone marrow and plasma (at various disease status time points) from over 330 haematological malignancy patients has also been collected. So far, over 64 research projects have been approved. Samples have also been released for method validation studies. Histology and molecular pathology, with the adoption of sophisticated labelling techniques and the availability of new platforms/technologies, is at very exciting point, placing the Histology facility at the forefront of biomarker analysis in

oncology. The addition of a new platform and a new member of staff in early 2014 will allow the unit to focus primarily on its development within these areas.

Laboratory Services

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

During 2012, work had been carried out to improve the laboratory facilities available for Laboratory Services to make media and other solutions, and to provide a new autoclave and other equipment. This work has allowed the team to operate very efficiently during 2013 and provide a highly reliable service. Aside from the main role of providing sterile glass and plasticware, the facility also generates approximately 1200L of sterile water, 600L of PBS, 400L of liquid and up to 3000 agar plates.

The team make daily collections to remove dirty glassware from laboratories and make daily deliveries to the labs and research areas of new sterile items, such as filter tips and microtubes, supplying approximately 1700 non-filter pipette tip boxes, 400 filtered pipette tip boxes and 600 packs of sterile microtubes to laboratories every month.

The Laboratory Services team also provides a critical safety function for the Institute by carrying out Legionella monitoring. Once a month all 400 taps in the building are run to ensure there is no build-up of Legionella in pipes. Alongside these principle tasks the team also maintains the Institute's film processor, and organises the provision of clean lab coats and first aid items throughout the building.

Molecular Biology Core Facility and Cancer Research UK Microarray Service

Stuart Pepper, Chris Clark, Yvonne Hey, Nelson Iley[1], Gill Newton, Leanne Wardleworth[1], John Weightman, Jodie Whittaker[2]

[1]joined in 2013 [2]left in 2013

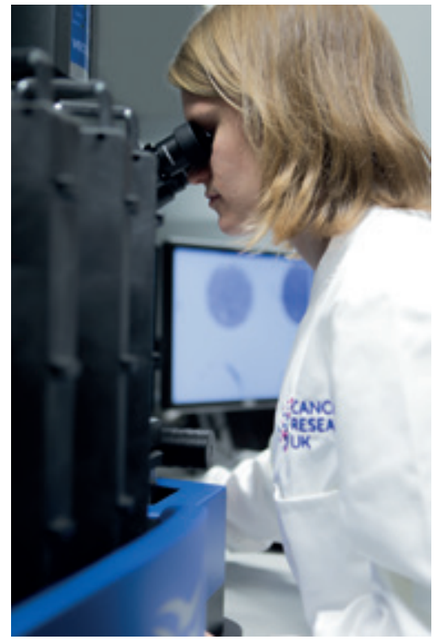
The Molecular Biology Core Facility (MBCF) provides a range of services to support the research groups on site. These include diagnostic services, such as cell line authentication and mycoplasma screening, and research services for expression profiling and DNA sequence analysis.

The core routine services have continued to run smoothly and had constant demand throughout the year. Cell line authentication is now well established in the Institute and all cell lines in use in the building can now be regularly authenticated by Short Tandem Repeat (STR) profiling. This technique is the gold standard method for checking cell line identity but requires some care when interpreting results. The MBCF team has considerable experience in this area to help interpret any unexpected results. Alongside the regular mycoplasma screening, this service gives confidence that all cell lines in use are appropriate for the experiments being carried out.

Demand for the daily Sanger sequencing service has continued to be high over the last year, whereas demand for the automated plasmid DNA extraction has fluctuated. At the start of 2013, we had two new Next Generation Sequencing (NGS) platforms delivered – a HiSeq 2500 and a MiSeq. The team has worked hard to implement the various workflows needed on the new platform and start delivering results. The facility now offers full genome sequencing and exome sequencing for DNA, and has protocols for total RNA sequencing or polyA selected RNA sequencing. The team has also run sequencing on ChIP samples and PCR products. In total, forty data sets have been delivered in the first year of operation, though this is likely to increase considerably next year as demand for the service has increased towards the end of 2013. To help maintain throughput of the service, an automation platform has been purchased to help process larger sample batches. Once fully established this will allow the service to offer a good turnaround time for larger experiments.

For expression profiling of individual genes, rather than entire transcriptomes, the facility has two ABI7900 machines with a choice of 96 or 384 well format. These systems, in conjunction with our Probe Library, continue to be heavily used and provide a very cost effective route for small experiments on small numbers of samples. This year a new platform has been added to support larger qPCR projects. The Fluidigm Biomark HD allows the analysis of up to 96 genes on samples in a single run. Initial data looks very good and this platform will be valuable both for large expression studies, and for analysis of expression variability in single cells.

The CRUK Affymetrix microarray service has also continued to experience significant demand throughout the year. Although many groups have switched to NGS for expression work, there is still demand for microarray analysis to allow comparison of new experiments to legacy data. To date, demand for this service has not significantly dropped and there are already projects ready for processing in 2014.

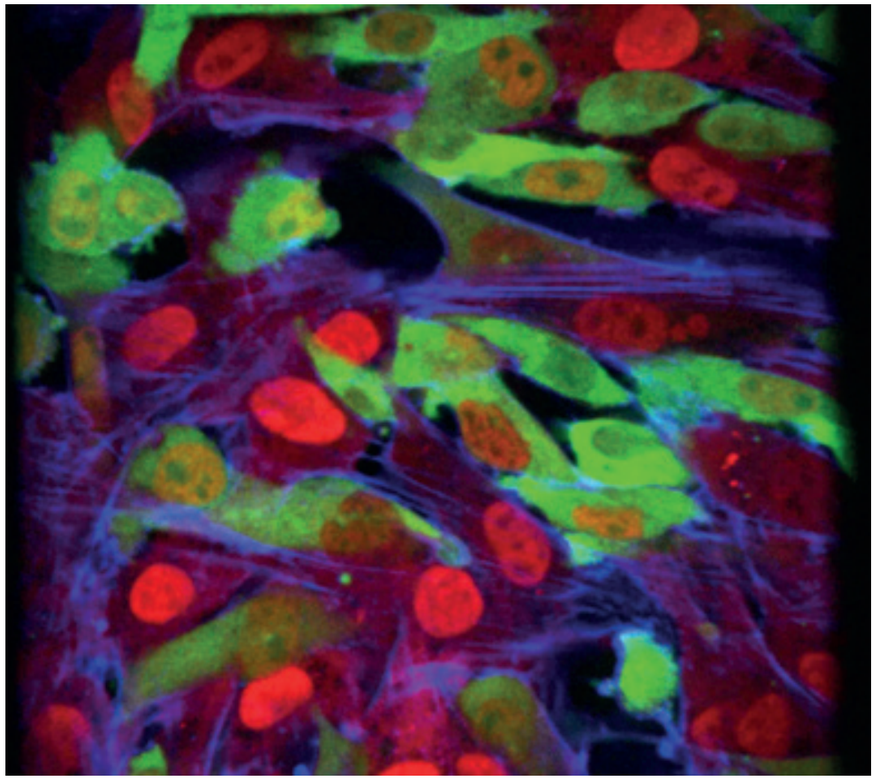


Scientific Computing

Wei Xing, Zhi Cheng Wang

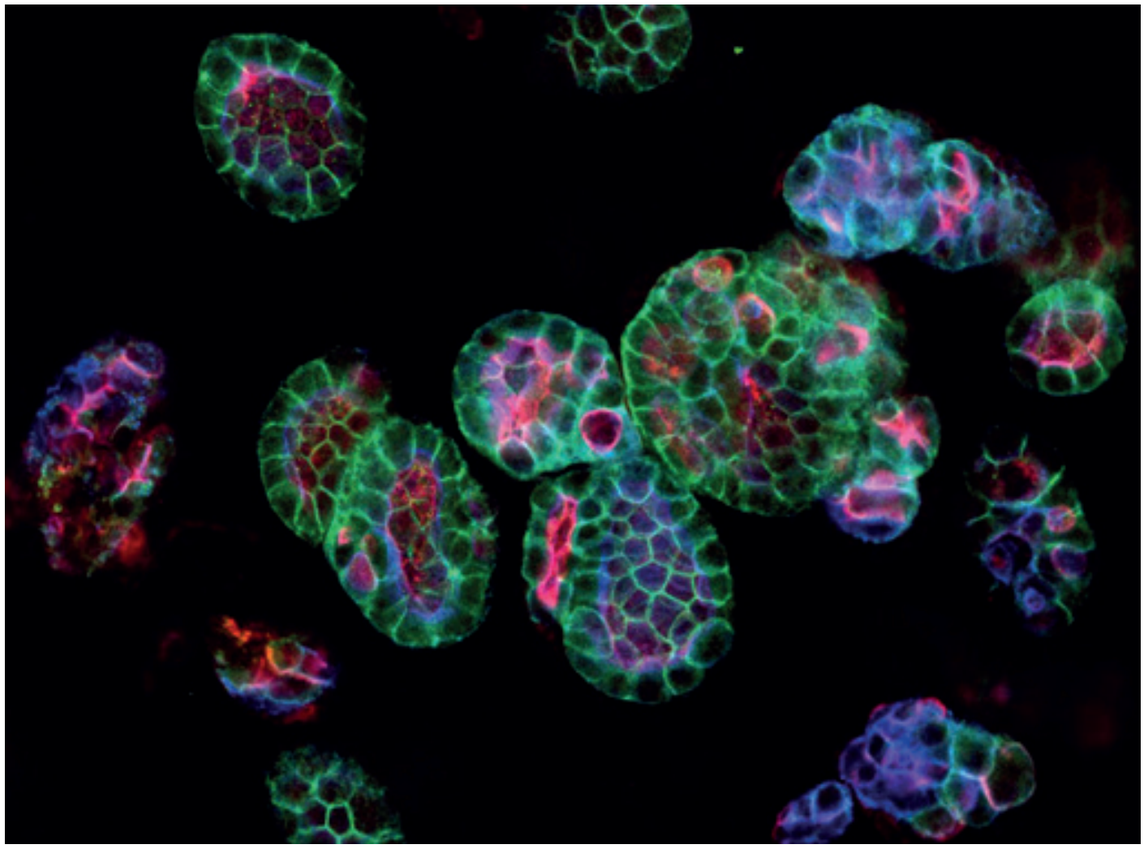
Dr Wei Xing joined us during the summer, from the Institute of Cancer Research in London, to become the Head of Scientific Computing. The newly created department has been established to provide the computational support needed within the Institute to handle the high volumes of genomics and imaging data arising from our deep sequencing, proteomics and microscopy platforms. To do this we are building a High Performance Computing (HPC) facility and data centre, significantly extending our current capabilities. Wei's team will provide the infrastructure and expertise required to provide this essential resource to the Institute. HPC has become a pre-requisite for the proper exploitation of cancer genomics data, in part, a consequence of the high volumes of data produced by recent advances in deep sequencing, imaging, and mass spectrometry. These have the potential to revolutionise translational cancer research and to offer new therapeutic strategies for personalised and stratified medicine.

Wei's primary focus will be to provide the underlying infrastructure needed to support integrated analysis of cancer data sets and intelligent bio-computing platforms, and will be working closely with the Computational Biology Support team to help provide the platforms that support our pipelines for proteomics and genomics data analysis. Zhi Cheng Wang has joined the Scientific Computing department from the Institute's IT team.



Co-culture of breast cancer cells (green), and normal human fibroblasts (non-green). Cancer cells interact with stromal cells, in this case fibroblasts, to influence their gene expression profile. A transcription factor (red) is found to accumulate in the fibroblast nuclei upon co-culturing with cancer cells.

Image provided by Haoran Tang from Molecular Oncology.



MDCK cells forming cysts and other 3D structures in collagen, stained with β-Catenin (a basal marker, green), GP135 (an apical marker, red) and actin (blue).

Image supplied by Andrew Porter, Cell Signalling.

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

RESEARCH PUBLICATIONS

Crispin Miller (page 14)
Applied Computational Biology and Bioinformatics

Refereed Research Papers

Betts GN, Eustace A, Patiar S, Valentine HR, Irlam J, Ramachandran A, Merve A, Homer JJ, Moller-Levet C, Buffa FM, Hall G, Miller CJ, Harris AL, and West CM. (2013)
Prospective technical validation and assessment of intra-tumour heterogeneity of a low density array hypoxia gene profile in head and neck squamous cell carcinoma. ***Eur J Cancer*** 49, 156-165.

Dhani DK, Goult BT, George GM, Rogerson DT, Bitton DA, Miller CJ, Schwabe JW, and Tanaka K. (2013)
Mzt1/Tam4, a fission yeast MOZART1 homologue, is an essential component of the gamma-tubulin complex and directly interacts with GCP3Alp6. ***Mol Biol Cell*** 24, 3337-3349.

Eustace A, Mani N, Span PN, Irlam JJ, Taylor J, Betts GN, Denley H, Miller CJ, Homer JJ, Rojas AM, Hoskin PJ, Buffa FM, Harris AL, Kaanders JH, and West CM. (2013)
A 26-gene hypoxia signature predicts benefit from hypoxia-modifying therapy in laryngeal cancer but not bladder cancer. ***Clin Cancer Res*** 19, 4879-4888.

Fawdar S, Trotter EW, Li Y, Stephenson NL, Hanke F, Marusiak AA, Edwards ZC, Lentile S, Waszkowycz B, Miller CJ, Brognard J. (2013)
Targeted genetic dependency screen facilitates identification of actionable mutations in FGFR4, MAP3K9, and PAK5 in lung cancer. ***Proc Natl Acad Sci USA*** 110(30), 12426-31.

Hall JS, Iype R, Armenoult LS, Taylor J, Miller CJ, Davidson S, de Sanjose S, Bosch X, Stern PL, and West CM. (2013)
Poor prognosis associated with human papillomavirus alpha7 genotypes in cervical carcinoma cannot be explained by intrinsic radiosensitivity. ***Int J Radiat Oncol Biol Phys*** 85, e223-229.

Ramachandran A, Betts G, Bhana S, Helme G, Blick C, Moller-Levet C, Saunders E, Valentine H, Pepper S, Miller CJ, Buffa F, Harris AL, and West CM. (2013)
An in vivo hypoxia metagene identifies the novel hypoxia inducible factor target gene SLCO1B3. ***Eur J Cancer*** 49, 1741-1751.

Karim Labib (page 16)
Cell Cycle

Refereed Research Papers

Foltman M, Evrin C, De Piccoli G, Jones RC, Edmondson RD, Katou Y, Nakato R, Shirahige K, and Labib K. (2013)
Eukaryotic replisome components cooperate to process histones during chromosome replication. ***Cell Rep*** 3, 892-904.

Nkosi PJ, Targosz BS, Labib K, and Sanchez-Diaz A. (2013)
Hof1 and Rvs167 have redundant roles in actomyosin ring function during cytokinesis in budding yeast. ***PLoS One*** 8, e57846.

Sengupta S, van Deursen F, de Piccoli G, and Labib K. (2013)
Dpb2 integrates the leading-strand DNA polymerase into the eukaryotic replisome. ***Curr Biol*** 23, 543-552.

Iain Hagan (page 18)
Cell Division

Refereed Research Papers

Grallert A, Chan KY, Alonso-Nunez ML, Madrid M, Biswas A, Alvarez-Tabares I, Connolly Y, Tanaka K, Robertson A, Ortiz JM, Smith DL, and Hagan IM. (2013)
Removal of centrosomal PP1 by NIMA kinase unlocks the MPF feedback loop to promote mitotic commitment in S. pombe. ***Curr Biol*** 23, 213-222.

Grallert A, Patel A, Tallada VA, Chan KY, Bagley S, Krapp A, Simanis V, and Hagan IM. (2013)
Centrosomal MPF triggers the mitotic and morphogenetic switches of fission yeast. ***Nat Cell Biol*** 15, 88-95.

Tay YD, Patel A, Kaemena DF, and Hagan IM. (2013)
Mutation of a conserved residue enhances the sensitivity of analogue-sensitised kinases to generate a novel approach to the study of mitosis in fission yeast. ***J Cell Sci*** 126, 5052-5061.

Other Publications

Hagan IM and Grallert A. (2013)
Spatial control of mitotic commitment in fission yeast. ***Biochem Soc Trans*** 41, 1766-1771.

Nic Jones (page 20)
Cell Regulation

Refereed Research Papers

Walczynski J, Lyons S, Jones N, and Breitwieser W. (2013)
Sensitisation of c-MYC-induced B-lymphoma cells to apoptosis by ATF2. ***Oncogene*** doi: 10.1038/onc.2013.28.

Angeliki Malliri (page 22)
Cell Signalling

Refereed Research Papers

Castillo-Lluva S, Tan CT, Daugaard M, Sorensen PH and Malliri A. (2013)
The tumour suppressor HACE1 controls cell migration by regulating Rac1 degradation. ***Oncogene*** 32, 1735-1742.

Chicooree N, Connolly Y, Tan CT, Malliri A, Li Y, Smith DL, Griffiths JR. (2013)
Enhanced detection of ubiquitin isopeptides using reductive methylation. ***J Am Soc Mass Spectrom*** 24(3), 421-30.

Chicooree N, Griffiths JR, Connolly Y, Tan CT, Malliri A, Eyers CE, Smith DL. (2013)
A novel approach to the analysis of SUMOylation with the independent use of trypsin and elastase digestion followed by database searching utilising consecutive residue addition to lysine. ***Rapid Commun Mass Spectrom*** 27(1), 127-34.

Dalton LE, Kamarashev J, Barinaga-Rementeria Ramirez I, White G, Malliri A, Hurlstone A. (2013)
Constitutive Rac Activation Is Not Sufficient to Initiate Melanocyte Neoplasia but Accelerates Malignant Progression. ***J Invest Dermatol*** 133(6), 1572-81.

Daugaard M, Nitsch R, Razaghi B, McDonald L, Jarrar A, Torrino S, Castillo-Lluva S, Rotblat B, Li L, Malliri A, Lemichez E, Mettouchi A, Berman JN, Penninger JM, Sorensen PH. (2013)
Hace1 controls ROS generation of vertebrate Rac1-dependent NADPH oxidase complexes. ***Nat Commun*** 4, 2180.

Caroline Dive (page 24)
Clinical and Experimental Pharmacology

Refereed Research Papers

Adamski J, Price A, Dive C, and Makin G. (2013)
Hypoxia-induced cytotoxic drug resistance in osteosarcoma is independent of HIF-1Alpha. ***PLoS One*** 8, e65304.

Bouranis L, Sperrin M, Greystoke A, Dive C, and Renehan AG. (2013)
The interaction between prognostic and pharmacodynamic biomarkers. ***Br J Cancer*** 109, 1782-1785.

Cawthorne C, Burrows N, Gieling RG, Morrow CJ, Forster D, Gregory J, Radigois M, Smigova A, Babur M, Simpson K, Hodgkinson C, Brown G, McMahon A, Dive C, Hiscock D, Wilson I, and Williams KJ. (2013)
[18F]-FLT positron emission tomography can be used to image the response of sensitive tumors to PI3-kinase inhibition with the novel agent GDC-0941. ***Mol Cancer Ther*** 12, 819-828.

Cummings J, Morris K, Zhou C, Sloane R, Lancashire M, Morris D, Bramley S, Krebs M, Khoja L, and Dive C. (2013)
Method validation of circulating tumour cell enumeration at low cell counts. ***BMC Cancer*** 13, 415.

Gibb A, Greystoke A, Ranson M, Linton K, Neeson S, Hampson G, Illidge T, Smith E, Dive C, Pettitt A, Lister A, Johnson P, and Radford J. (2013)
A study to investigate dose escalation of doxorubicin in ABVD chemotherapy for Hodgkin lymphoma incorporating biomarkers of response and toxicity. ***Br J Cancer*** 109, 2560-2565.

Greystoke A, Harris G, Jenkins M, Goonetilleke D, Moore D, Lancashire M, Ranson M, Hughes A, Clack G, and Dive C. (2013)
Assessment of diurnal changes and confounding factors that affect circulating cell death biomarker levels: a short communication. ***J Pharm Biomed Anal*** 84, 184-188.

Honeychurch J, Dive C, and Illidge TM. (2013)
Synchronous apoptosis in established tumors leads to the induction of adaptive immunity. ***Oncoimmunology*** 2, e24501.

Horsley L, Cummings J, Middleton M, Ward T, Backen A, Clamp A, Dawson M, Farmer H, Fisher N, Halbert G, Halford S, Harris A, Hasan J, Hogg P, Kumaran G, Little R, Parker GJ, Potter P, Saunders M, Roberts C, Shaw D, Smith N, Smythe J, Taylor A, Turner H, Watson Y, Dive C and Jayson GC. (2013)
A phase 1 trial of intravenous 4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide (GSAO) in patients with advanced solid tumours. ***Cancer Chemother Pharmacol*** 72, 1343-1352.

Linnik IV, Scott ML, Holliday KF, Woodhouse N, Waterton JC, O'Connor JP, Barjat H, Liess C, Ulloa J, Young H, Dive C, Hodgkinson CL, Ward T, Roberts D, Mills SJ, Thompson G, Buonaccorsi GA, Cheung S, Jackson A, Naish JH, Parker GJ. (2013)
Noninvasive tumor hypoxia measurement using magnetic resonance imaging in murine U87 glioma xenografts and in patients with glioblastoma. ***Magn Reson Med*** doi: 10.1002/mrm.24826.

Melis MH, Simpson KL, Dovedi SJ, Welman A, MacFarlane M, Dive C, Honeychurch J, Illidge TM. (2013)
Sustained tumour eradication after induced caspase-3 activation and synchronous tumour apoptosis requires an intact host immune response. ***Cell Death Differ*** 20, 765-773.

Polanski R, Hodgkinson C, Fusi A, Nonaka D, Priest L, Kelly P, Trapani F, Bishop P, White A, Critchlow SE, Smith PD, Blackhall FH, Dive C, Morrow CJ. (2013)
Activity of the Monocarboxylate Transporter 1 inhibitor AZD3965 in Small Cell Lung Cancer. ***Clin Cancer Res*** Nov 25. [Epub ahead of print]

Simpson KL, Cawthorne C, Zhou C, Hodgkinson CL, Walker MJ, Trapani F, Kadirvel M, Brown G, Dawson MJ, MacFarlane M, Williams KJ, Whetton AD, Dive C. (2013)
A caspase-3 'death-switch' in colorectal cancer cells for induced and synchronous tumor apoptosis in vitro and in vivo facilitates the development of minimally invasive cell death biomarkers. ***Cell Death Dis*** 4, e613.

Stovold R, Meredith SL, Bryant JL, Babur M, Williams KJ, Dean EJ, Dive C, Blackhall FH, White A. (2013)
Neuroendocrine and epithelial phenotypes in small-cell lung cancer: implications for metastasis and survival in patients. ***Br J Cancer*** 108, 1704-1711.

Other publications

Fusi A, Metcalf R, Krebs M, Dive C, Blackhall F. (2013)
Clinical utility of circulating tumour cell detection in non-small-cell lung cancer. ***Curr Treat Options Oncol*** 14, 610-622.

Ivan Ahel (page 28)

DNA Damage Response

Refereed Research Papers

Barkauskaite E, Brassington A, Tan ES, Warwicker J, Dunstan MS, Banos B, Lafite P, Ahel M, Mitchison TJ, Ahel I, Leys D. (2013)
Visualization of poly(ADP-ribose) bound to PARG reveals inherent balance between exo- and endo-glycohydrolase activities. ***Nat Commun*** 4, 2164.

Sharifi R, Morra R, Appel CD, Tallis M, Chioza B, Jankevicius G, Simpson MA, Matic I, Ozkan E, Golia B, Schellenberg MJ, Weston R, Williams JG, Rossi MN, Galehdari H, Krahn J, Wan A, Trembath RC, Crosby AH, Ahel D, Hay R, Ladurner AG, Timinszky G, Williams RS, Ahel I. (2013)
Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. ***EMBO Journal*** 32, 1225–1237.

Other Publications

Barkauskaite E, Jankevicius G, Ladurner AG, Ahel I, Timinszky G. (2013)
The recognition and removal of cellular poly(ADP-ribose) signals. ***FEBS J*** 280, 3491-3507.

Tallis M, Morra R, Barkauskaite E, Ahel I. (2013)
Poly(ADP-ribosyl)ation in regulation of chromatin structure and the DNA damage response. ***Chromosoma*** Oct 27. [Epub ahead of print]

Zaja R, Mikoc A, Barkauskaite E, and Ahel I. (2013)
Molecular Insights into Poly(ADP-ribose) Recognition and Processing. ***Biomolecules*** 3, 1-17.

Donald Ogilvie (Page 30)

Drug Discovery

Refereed Research Papers

Raoof A, Depledge P, Hamilton NM, Hamilton NS, Hitchin JR, Hopkins GV, Jordan AM, Maguire LA, McGonagle AE, Mould DP, Rushbrooke M, Small HF, Smith KM, Thomson GJ, Turlais F, Waddell ID, Waszkowycz B, Watson AJ, Ogilvie DJ. (2013)
Toxoflavins and deazaflavins as the first reported selective small molecule inhibitors of tyrosyl-DNA phosphodiesterase II. ***J Med Chem*** 56, 6352-6370.

Thomson G, Watson A, Caldecott K, Denneny O, Depledge P, Hamilton N, Hopkins G, Jordan A, Morrow C, Raoof A, Waddell I, Ogilvie D. (2013)
Generation of assays and antibodies to facilitate the study of human 5'-tyrosyl DNA phosphodiesterase. ***Anal Biochem*** 436, 145-150.

Thomson GJ, Hamilton NS, Hopkins GV, Waddell ID, Watson AJ, Ogilvie DJ. (2013)
A fluorescence-based assay for the apurinic/apyrimidinic-site cleavage activity of human tyrosyl-DNA phosphodiesterase 1. ***Anal Biochem*** 440, 1-5.

Belot A, Kasher PR, Trotter EW, Foray AP, Debaud AL, Rice GI, Szynkiewicz M, Zabot MT, Rouvet I, Bhaskar SS, Daly SB, Dickerson JE, Mayer J, O'Sullivan J, Juillard L, Urquhart JE, Fawdar S, Marusiak AA, Stephenson N, Waszkowycz B, W Beresford M, Biesecker LG, C M Black G, René C, Eliaou JF, Fabien N, Ranchin B, Cochat P, Gaffney PM, Rozenberg F, Lebon P, Malcus C, Crow YJ, Brognard J, Bonnefoy N. (2013)
Protein kinase cdelta deficiency causes mendelian systemic lupus erythematosus with B cell-defective apoptosis and hyperproliferation. ***Arthritis Rheum*** 65, 2161-2171.

Fawdar S, Trotter EW, Li Y, Stephenson NL, Hanke F, Marusiak AA, Edwards ZC, Ientile S, Waszkowycz B, Miller CJ, Brognard J. (2013)
Targeted genetic dependency screen facilitates identification of actionable mutations in FGFR4, MAP3K9, and PAK5 in lung cancer. ***Proc Natl Acad Sci USA*** 110, 12426-12431.

Hitchin JR, Blagg J, Burke R, Burns S, Cockerill MJ, Fairweather E E, Hutton C, Jordan AM, McAndrew C, Mirza A, Mould D, Thomson GJ, Waddell I, Ogilvie DJ. (2013)
Development and evaluation of selective, reversible LSD1 inhibitors derived from fragments. ***Med Chem Commun*** 4(11), 1513-1522.

Lynch JT, Cockerill MJ, Hitchin JR, Wiseman DH, Somervaille TC. (2013)
CD86 expression as a surrogate cellular biomarker for pharmacological inhibition of the histone demethylase lysine-specific demethylase 1. ***Anal Biochem*** 442, 104-106.

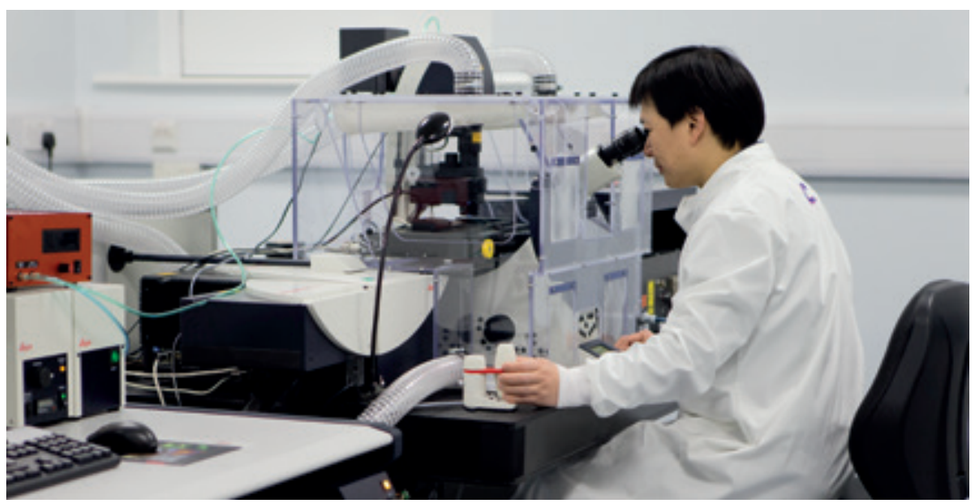
Morley AD, Pugliese A, Birchall K, Bower J, Brennan P, Brown N, Chapman T, Drysdale M, Gilbert IH, Hoelder S, Jordan A, Ley SV, Merritt A, Miller D, Swarbrick ME, Wyatt PG. (2013)
Fragment-based hit identification: thinking in 3D. ***Drug Discov Today*** 8(23-24), 1221-7.

Peter Stern (page 32)

Immunology

Refereed Research Papers

Bosch FX, Broker TR, Forman D, Moscicki AB, Gillison ML, Doorbar J, Stern PL, Stanley M, Arbyn M, Poljak M, Cuzick J, Castle PE, Schiller JT, Markowitz LE, Fisher WA, Canfell K, Denny LA,



Franco EL, Steben M, Kane MA, Schiffman M, Meijer CJ, Sankaranarayanan R, Castellsagué X, Kim JJ, Brotons M, Alemany L, Albero G, Diaz M, Sanjosé S. (2013)
Comprehensive control of human papillomavirus infections and related diseases. ***Vaccine*** 31 Suppl, 7:H1-H31.

Nullin Divecha (page 34)
Inositide Laboratory

Refereed Research Papers
Elouarrat D, van der Velden YU, Jones DR, Moolenaar WH, Divecha N, Haramis AP. (2013)
Role of phosphatidylinositol 5-phosphate 4-kinase alpha in zebrafish development. ***Int J Biochem Cell Biol*** 45, 1293-1301.

Gupta A, Toscano S, Trivedi D, Jones DR, Mathre S, Clarke JH, Divecha N, Raghu P. (2013)
Phosphatidylinositol 5-phosphate 4-kinase (PIP4K) regulates TOR signaling and cell growth during Drosophila development. ***Proc Natl Acad Sci USA*** 110, 5963-5968.

Keune WJ, Sims AH, Jones DR, Bultsma Y, Lynch JT, Jirström K, Landberg G, Divecha N. (2013)
Low PIP4K2B expression in human breast tumors correlates with reduced patient survival: A role for PIP4K2B in the regulation of E-cadherin expression. ***Cancer Res*** 73(23), 6913-25.

Jones DR, Foulger R, Keune WJ, Bultsma Y, Divecha N. (2013)
PtdIns5P is an oxidative stress-induced second messenger that regulates PKB activation. ***FASEB J*** 27, 1644-1656.

Jones DR, Ramirez IB, Lowe M, Divecha N. (2013)
Measurement of phosphoinositides in the zebrafish Danio rerio. ***Nat Protoc*** 8, 1058-1072.

van den Bout I, Jones DR, Shah ZH, Halstead JR, Keune WJ, Mohammed S, D'Santos CS, Divecha N. (2013)
Collaboration of AMPK and PKC to induce phosphorylation of Ser413 on PIP5K1B resulting in decreased kinase activity and reduced PtdIns(4,5)P2 synthesis in response to oxidative stress and energy restriction. ***Biochem J*** 455, 347-358.

Other Publications
Keune WJ, Jones DR, Divecha N. (2013)
PtdIns5P and Pin1 in oxidative stress signaling. ***Adv Biol Regul*** 53, 179-189.

Shah ZH, Jones DR, Sommer L, Foulger R, Bultsma Y, D'Santos C, Divecha N. (2013)
Nuclear phosphoinositides and their impact on nuclear functions. ***FEBS J*** 280(24), 6295-310.

Tim Somervaille (page 36)
Leukaemia Biology

Refereed Research Papers
Alimam SM, Mamat MK, Kulkarni S, Somervaille TC. (2013)
Sudden onset bilateral deafness as a presentation of chronic myeloid leukaemia. ***Br J Haematol*** 160, 3.

Greystoke BF, Huang X, Wilks DP, Atkinson S, Somervaille TC. (2013)
Very high frequencies of leukaemia-initiating cells in precursor T-acute lymphoblastic leukaemia may be obscured by cryopreservation. ***Br J Haematol*** 163, 538-541.

Huang X, Spencer GJ, Lynch JT, Ciceri F, Somerville TD, Somervaille TC. (2013)
Enhancers of Polycomb EPC1 and EPC2 sustain the oncogenic potential of MLL leukemia stem cells. ***Leukemia*** Oct 29. doi: 10.1038/leu.2013.316.

Lynch JT, Cockerill MJ, Hitchin JR, Wiseman DH, Somervaille TC. (2013)
CD86 expression as a surrogate cellular biomarker for pharmacological inhibition of the histone demethylase lysine-specific demethylase 1. ***Anal Biochem*** 442, 104-106.

Lynch JT, Somerville TD, Spencer GJ, Huang X, Somervaille TC. (2013)
TTC5 is required to prevent apoptosis of acute myeloid leukemia stem cells. ***Cell Death Dis*** 4, e573.

White DJ, Unwin RD, Bindels E, Pierce A, Teng HY, Muter J, Greystoke B, Somerville TD, Griffiths J, Lovell S, Somervaille TC, Delwel R, Whetton AD, Meyer S. (2013)
Phosphorylation of the leukemic oncoprotein EVI1 on serine 196 modulates DNA binding, transcriptional repression and transforming ability. ***PLoS One*** 8, e66510.

Williams MS, Ali N, Nonaka D, Bloor AJ, Somervaille TC. (2013)
Fatal invasive aspergillosis of the larynx. ***Eur J Haematol*** 90, 354.

Other Publications
Wiseman DH, Greystoke BF, Somervaille TC. (2013)
The variety of leukemic stem cells in myeloid malignancy. ***Oncogene*** doi: 10.1038/onc.2013.269.

Richard Marais (page 38)
Molecular Oncology

Refereed Research Papers
Escuin-Ordinas H, Atefi M, Fu Y, Cass A, Ng C, Huang RR, Yashar S, Comin-Anduix B, Avramis E, Cochran AJ, Marais R, Lo RS, Graeber TG, Herschman HR, Ribas A. (2013)
COX-2 inhibition prevents the appearance of cutaneous squamous cell carcinomas accelerated by BRAF inhibitors. ***Mol Oncol*** S1574-7891(13)00163-4 doi: 10.1016/j.molonc.2013.11.005.

Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, Desjardins L, Turajlic S, Piperno-Neumann S, de la Grange P, Roman-Roman S, Stern MH, Marais R. (2013)
SF3B1 mutations are associated with alternative splicing in uveal melanoma. ***Cancer Discov*** 3(10), 1122-9.

Falck Miniotis M, Arunan V, Eykyn TR, Marais R, Workman P, Leach MO, Beloueche-Babari M. (2013)
MEK1/2 inhibition decreases lactate in BRAF-driven human cancer cells. ***Cancer Res*** 73(13), 4039-49.

Furney SJ, Turajlic S, Stamp G, Nohadani M, Carlisle A, Thomas JM, Hayes A, Strauss D, Gore M, van den Oord J, Larkin J, Marais R. (2013)
Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma. ***J Pathol*** 230(3), 261-9.

Niculescu-Duvaz D, Niculescu-Duvaz I, Suijkerbuijk BM, Ménard D, Zambon A, Davies L, Pons JF, Whittaker S, Marais R, Springer CJ. (2013)
Potent BRAF kinase inhibitors based on 2,4,5-trisubstituted imidazole with naphthyl and benzothiophene 4-substituents. ***Bioorg Med Chem*** 21(5), 1284-304.

Pedersen M, Küsters-Vandevelde HV, Viros A, Groenen PJ, Sanchez-Laorden B, Gilhuis JH, van Engen-van Grunsven IA, Renier W, Schieving J, Niculescu-Duvaz I, Springer CJ, Küsters B, Wesseling P, Blokx WA, Marais R. (2013)
Primary melanoma of the CNS in children is driven by congenital expression of oncogenic NRAS in melanocytes. ***Cancer Discov*** 3(4), 458-69.

Smith MP, Ferguson J, Arozarena I, Hayward R, Marais R, Chapman A, Hurlstone A, Wellbrock C. (2013)
Effect of SMURF2 targeting on susceptibility to MEK inhibitors in melanoma. ***J Natl Cancer Inst*** 105(1), 33-46.

Girotti MR, Pedersen M, Sanchez-Laorden B, Viros A, Turajlic S, Niculescu-Duvaz D, Zambon A, Sinclair J, Hayes A, Gore M, Lorigan P, Springer C, Larkin J, Jorgensen C, Marais R. (2013)
Inhibiting EGF receptor or SRC family kinase signaling overcomes BRAF inhibitor resistance in melanoma. ***Cancer Discov*** 3(2), 158-67.

Maertens O, Johnson B, Hollstein P, Frederick DT, Cooper ZA, Messiaen L, Bronson RT, McMahon M, Granter S, Flaherty K, Wargo JA, Marais R, Cichowski K. (2013)
Elucidating distinct roles for NF1 in melanomagenesis. ***Cancer Discov*** 3(3), 338-49.

Viros A, Hayward R, Martin M, Yashar S, Yu CC, Sanchez-Laorden B, Zambon A, Niculescu-Duvaz D, Springer C, Lo RS, Marais R. (2013)
Topical 5-fluorouracil elicits regressions of BRAF inhibitor-induced cutaneous squamous cell carcinoma. ***J Invest Dermatol*** 133(1), 274-6.

Other Publications
Martin M, Marais R. (2013)
Braking BRAF: AMPK leaves ERK stranded in the desert. ***Mol Cell*** 52(2), 155-6.

Marais R, Sellers W, Livingston D, Mihich E. (2013)
Twenty-fourth annual Pezcoller symposium: Molecular basis for resistance to targeted agents. ***Cancer Res*** 73, 1046-1049.

Sanchez-Laorden B, Viros A, Marais R. (2013)
Mind the IQGAP. ***Cancer Cell*** 23(6), 715-7.

Girotti MR, Marais R. (2013)
Deja Vu: EGF receptors drive resistance to BRAF inhibitors. ***Cancer Discov*** 3(5), 487-90.

Zambon A, Niculescu-Duvaz D, Niculescu-Duvaz I, Marais R, Springer CJ. (2013)
BRAF as a therapeutic target: a patent review (2006 – 2012). ***Expert Opin Ther Pat*** 23(2), 155-64.

Rebocho AP, Marais R. (2013)
ARAF acts as a scaffold to stabilize BRAF:CRAF heterodimers. ***Oncogene*** 32(26), 3207-12.

John Brognard (page 40)
Signalling Networks in Cancer

Refereed Research Papers
Belot A, Kasher PR, Trotter EW, Foray AP, Debaud AL, Rice GI, Szynkiewicz M, Zabot MT, Rouvet I, Bhaskar SS, Daly SB, Dickerson JE, Mayer J, O'Sullivan J, Juillard L, Urquhart JE, Fawdar S, Marusiak AA, Stephenson N, Waszkowycz B, W Beresford M, Biesecker LG, C M Black G, René C, Eliaou JF, Fabien N, Ranchin B, Cochat P, Gaffney PM, Rozenberg F, Lebon P, Malcus C, Crow YJ, Brognard J, Bonnefoy N. (2013)
Protein kinase cdelta deficiency causes mendelian systemic lupus erythematosus with B cell-defective apoptosis and hyperproliferation. ***Arthritis Rheum*** 65, 2161-2171.

Fawdar S, Trotter EW, Li Y, Stephenson NL, Hanke F, Marusiak AA, Edwards ZC, Ientile S, Waszkowycz B, Miller CJ, Brognard J. (2013)
Targeted genetic dependency screen facilitates identification of actionable mutations in FGFR4, MAP3K9, and PAK5 in lung cancer. ***Proc Natl Acad Sci USA*** 110, 12426-12431.

Other Publications
Belot A, Brognard J, Crow YJ, Bonnefoy N. (2013).
Author reply. ***Arthritis Rheum*** doi: 10.1002/art.38234.

Fawdar S, Edwards ZC, Brognard J. (2013)
Druggable drivers of lung cancer. ***Oncotarget*** 4, 1334-1335.

Active Patents
Kozikowski AP, Dennis PA, Brognard J, and Sun H: Akt Inhibitors, Pharmaceutical Compositions, and uses Thereof. US Patent: 7,378,403, 2008. (WO/2004/022569)

Newton AC, GaoT, Brognard J: Compositions and Methods for Treating Diseases Associated with PHLPP. US Patent Pending 60/667,709, filed: March 31, 2006. (WO/2006/105490)

Georges Lacaud (page 42)
Stem Cell Biology

Refereed Research Papers
Stefanska M, Costa G, Lie-A-Ling M, Kouskoff V, Lacaud G. (2013)
Smooth muscle cells largely develop independently of functional hemogenic endothelium. ***Stem Cell Res*** 12, 222-232.

Perez-Campo FM, Costa G, Lie-A-Ling M, Stifani S, Kouskoff V, Lacaud G. (2013)
MOZ-mediated repression of p16INK4a is critical for the self-renewal of neural and hematopoietic stem cells. ***Stem Cells***. 2013 Dec 4. doi: 10.1002/stem.1606.

Other Publications
Perez-Campo FM, Costa G, Lie-a-Ling M, Kouskoff V, Lacaud G. (2013)
The MYSTerious MOZ, a histone acetyltransferase with a key role in haematopoiesis. ***Immunology*** 139, 161-165.

Valerie Kouskoff (page 44)
Stem Cell Haematopoiesis

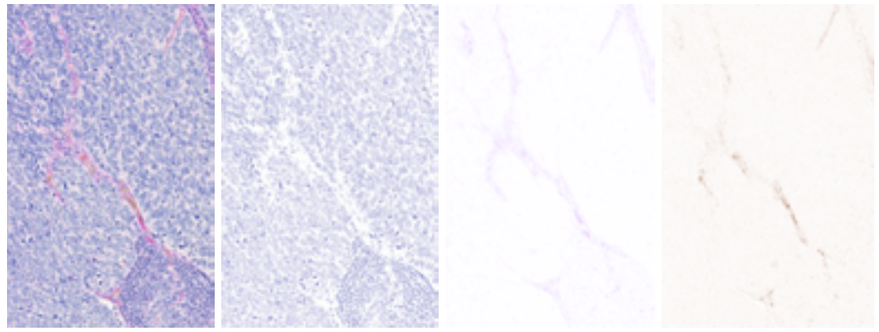
Refereed Research Papers
Stefanska M, Costa G, Lie-A-Ling M, Kouskoff V, Lacaud G. (2013)
Smooth muscle cells largely develop independently of functional hemogenic endothelium. ***Stem Cell Res*** 12, 222-232.

Perez-Campo FM, Costa G, Lie-A-Ling M, Stifani S, Kouskoff V, Lacaud G. (2013)
MOZ-mediated repression of p16INK4a is critical for the self-renewal of neural and hematopoietic stem cells. ***Stem Cells***. 2013 Dec 4. doi: 10.1002/stem.1606.

Other Publications
Perez-Campo FM, Costa G, Lie-a-Ling M, Kouskoff V, Lacaud G. (2013)
The MYSTerious MOZ, a histone acetyltransferase with a key role in haematopoiesis. ***Immunology*** 139, 161-165.

Geoff Margison

Refereed Research Papers
Bonello N, Sampson J, Burn J, Wilson IJ, McGrown G, Margison GP, Thorncroft M, Crossbie P, Povey



Vasculogenic Mimicry (VM) describes the ability of aggressive tumour cells to mimic properties of endothelial cells that enable de novo generation of tumour-derived vascular networks and provide microcirculation independently of the host. CD31 (right most panel) staining is combined with PAS staining (second from right), to identify endothelial cells and determine the basement membranes of blood vessels and tumour cells in which any structure containing CD31-positive immunoreactivity will be defined as a blood vessel, while VM structures will be strictly defined as CD31-negative PAS-positive structures (the images are from small cell lung cancer and are supplied by Francesca Trapani, Clinical and Experimental Pharmacology).

AC, Santibanez-Koref M, Walters K. (2013)
Bayesian inference supports a location and neighbour-dependent model of DNA methylation propagation at the MGMT gene promoter in lung tumours. ***J Theor Biol*** 336, 87-95.

Bosch FX, Broker TR, Forman D, Moscicki AB, Gillison ML, Doorbar J, Stern PL, Stanley M, Arbyn M, Poljak M, Cuzick J, Castle PE, Schiller JT, Markowitz LE, Fisher WA, Canfell K, Denny LA, Franco EL, Steben M, Kane MA, Schiffman M, Meijer CJ, Sankaranarayanan R, Castellsagué X, Kim JJ, Brotons M, Alemany L, Albero G, Diaz M, Sanjosé S. (2013)
Comprehensive control of human papillomavirus infections and related diseases. ***Vaccine*** 31 Suppl, 7:H1-H31.

Crosbie PA, Harrison K, Shah R, Watson AJ, Agius R, Barber PV, Margison GP, Povey AC. (2013)
Topographical study of O(6)-alkylguanine DNA alkyltransferase repair activity and N7-methylguanine levels in resected lung tissue. ***Chem Biol Interact*** 204, 98-104.

Senthong P, Millington CL, Wilkinson OJ, Marriott AS, Watson AJ, Reamtong O, Eyers CE, Williams DM, Margison GP, Povey AC. (2013)
The nitrosated bile acid DNA lesion O6-carboxymethylguanine is a substrate for the human DNA repair protein O6-methylguanine-DNA methyltransferase. ***Nucleic Acids Res*** 41, 3047-3055.

Zhang F, Tsunoda M, Suzuki K, Kikuchi Y, Wilkinson O, Millington CL, Margison GP, Williams DM, Czarina Morishita E, Takénaka A. (2013)
Structures of DNA duplexes containing O6-carboxymethylguanine, a lesion associated with gastrointestinal cancer, reveal a mechanism for inducing pyrimidine transition mutations. ***Nucleic Acids Res*** 41, 5524-5532.

Akira Orimo

Refereed Research Papers
Polanska UM, Orimo A. (2013)
Carcinoma-associated fibroblasts: non-neoplastic tumour-promoting mesenchymal cells. ***J Cell Physiol*** 228, 1651-1657.

Other Publications
Togo S, Polanska UM, Horimoto Y, Orimo A. (2013)
Carcinoma-associated fibroblasts are a promising therapeutic target. ***Cancers (Basel)*** 5, 149-169.

Duncan Smith

Refereed Research Papers
Hálová L, Du W, Kirkham S, Smith DL, Petersen J. (2013)
Phosphorylation of the TOR ATP binding domain by AGC kinase constitutes a novel mode of TOR inhibition. ***J Cell Biol*** 203(4), 595-604.

Chicooree N, Griffiths JR, Connolly Y, Smith DL. (2013)
Chemically facilitating the generation of diagnostic ions from SUMO(2/3) remnant isopeptides. ***Rapid Commun Mass Spectrom*** 27, 2108-2114.

Weekes MP, Tan SY, Poole E, Talbot S, Antrobus R, Smith DL, Montag C, Gygi SP, Sinclair JH, Lehner PJ. (2013)
Latency-associated degradation of the MRP1 drug transporter during latent human cytomegalovirus infection. ***Science*** 340(6129), 199-202.

THESES

Filippo Ciceri

Filippo Ciceri
Leukaemia Biology Group
Studies on the proliferation of AML blasts

Emily Holmes

Emily Holmes
Cell Regulation
The identification of novel targets of the fission yeast Sty1 MAPK

Leila Khoja
CEP
Clinical Significance and Utility of Circulating Tumour Cells in Pancreatic Carcinoma and Melanoma

Andrzej Mazan

Georgi Marinov
Immunology
Trafficking and functional interactions of oncofoetal trophoblast glycoprotein 5T4

Andrzej Mazan
Stem Cell Haematopoiesis
Elucidating the role of Sox7 in the generation of haemogenic endothelium

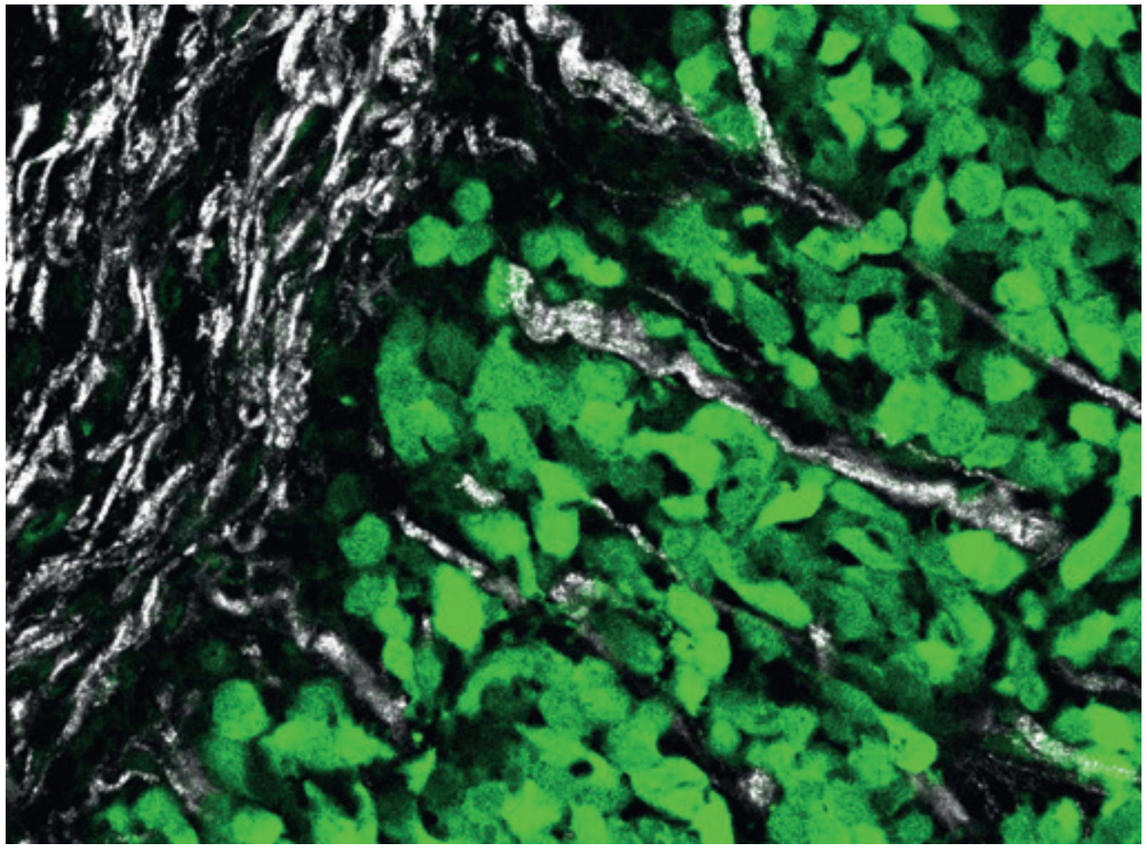
Milena Mazan

Milena Mazan
Stem Cell Biology
Exploring the role of GFI1 and GFI 1B in early haematopoiesis

Sharmin Naaz

Sharmin Naaz
ACBB
The role of non-coding RNAs at the onset of haematopoietic commitment

Monika Stefanska (nee Antkiewicz)
Stem Cell Biology
Investigating the origin of primitive erythrocytes and vascular smooth muscle cells



Tumour-stroma interface. GFP expressing tumour cells (green) re-arrange collagen bundles (grey) perpendicularly to the tumour edge to facilitate tumour growth and invasion. In contrast, collagen bundles (grey) in the adjacent normal tissue run parallel to the tumour edge acting as a physical barrier to limit tumour progression.

Image provided by Haoran Tang (Molecular Oncology)

EXTERNAL SEMINAR SPEAKERS 2013

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. 2013 was another successful year for scientific interaction with an excellent set of internationally renowned speakers visiting the Institute. In its fifth year, The Breakthrough Breast Cancer Research Unit seminar series continues to produce an outstanding range of speakers. The postdoctoral researchers at the Institute also give weekly seminars which are very well attended and help to integrate the entire cancer research efforts of the Institute.

Chris Bakal
Institute of Cancer Research, *London*

Nick Cross
National Genetics Reference Laboratory, *Wessex*

Bill Earnshaw
Wellcome Trust Centre for Cell Biology, University of Edinburgh

Oskar Fernandez-Capetillo
Spanish National Cancer Research Center, *Madrid*

Greg Findley
Samuel Lunenfeld Research Institute, *Ontario*

Margaret Frame
Edinburgh Cancer Research Centre

David Glover
University of Cambridge

Mary J.C. Hendrix
Lurie Children's Research Center, Northwestern University, *Illinois*

Martin Humphries
Wellcome Trust Centre for Cell-Matrix Research, University of Manchester

Tony Hunter
The Netherlands Cancer Institute, *Amsterdam*

Claus Jorgensen
Institute of Cancer Research, *London*

Stefan Knapp
Nuffield Department of Medicine, *Oxford*

Richard Lamb
Liverpool Cancer Research UK Centre

Oliver Maddocks
The Beatson Institute for Cancer Research, *Glasgow*

Chris Maher
London Research Institute, *London*

Matthias Merkenschlager
MRC Clinical Sciences Centre, Imperial College, *London*

Sebastian Nijman
CeMM Research Center for Molecular Medicine, *Vienna*

Christian Ottensmeier
University of Southampton

Luca Pellegrini
Department of Biochemistry, Cambridge University

Rickard Sandberg
Karolinska Institutet, *Stockholm*

Owen Sansom
The Beatson Institute for Cancer Research, *Glasgow*

Stephen Taylor
Institut Gustave Roussy, *Paris*

Johannes Zuber
Institute of Molecular Pathology, *Vienna*

Breakthrough Breast Cancer Research Unit Seminar Series 2013

Alan Ashworth
Institute of Cancer Research, *London*

Cathrin Brisken
École Polytechnique Fédérale de Lausanne, *Lausanne*

Ferruccio Galbiati
University of Pittsburgh School of Medicine, *Pittsburgh*

Beatrice Howard
Breakthrough Research Centre, *London*

Rama Khokha
Ontario Cancer Institute, *Ontario*

Jeffrey Rosen
Baylor College of Medicine, *Texas*

John J. Wysolmerski
Yale School of Medicine, *Connecticut*

POSTGRADUATE EDUCATION

www.cruk.manchester.ac.uk/education

Julie Edwards
Postgraduate
Education Manager

Ian Waddell
Postgraduate Tutor

The Cancer Research UK Manchester Institute (CRUK MI) offers a range of graduate degrees for students interested in a career involving cancer research. The Institute considers education of both research and clinical scientists to be a major investment in the future of cancer research, and has an excellent track record of launching careers in basic, translation and clinical research.

As part of this commitment, we have an active postgraduate programme that provides excellent students and clinical research fellows the opportunity to study for cancer-related PhD or MD degrees. This is achieved through a training programme that aims to improve effectiveness in research, provide professional and management skills and enhance career development. Ninety-nine percent (99%) of our students in the past eight years have found employment after graduation; half of these are in American or European laboratories, while 20% continue to progress in their clinical careers in the NHS. Students leave the CRUK MI with excellent career prospects across the world.

In 2013, we welcomed three graduate students to our PhD programme, working in a variety of fields from drug discovery, to stem cells and leukaemia biology. It was also particularly gratifying to see that, over the past twelve months, eleven of the Institute's publications had students as first authors in journals as diverse as Melanoma Research, Nature Communications and Stem Cell Research. During the course of the year, a total of five PhD students and one Clinical Fellow were awarded their PhDs.

The Cancer Research UK Manchester Graduate Programme

We aim for each student to receive high quality training in scientific research through an intellectually demanding but achievable research programme. Each project is peer-reviewed in advance and monitored throughout the course of their studies via a mixture of oral presentations, written reports, and progress meetings. These modes of assessment are designed not only to provide formal points at which progress (of both the student and the project) can be monitored, but also to help develop the presentation skills that are so fundamental to the majority of careers in science and elsewhere. Graduate training is monitored by an Education Committee, which features CRUK core-funded Group Leaders, scientists and student representatives (see below). A main supervisor and a second or co-supervisor is nominated for each student, who is able to provide additional advice and consultation on both academic and non-academic matters. Each student is also assigned an advisor (similar to a personal tutor on an undergraduate programme) whose role is to provide impartial support and advice, while further support is also available individually from the Education Committee chair, Postgraduate Tutor, Postgraduate Manager, or collectively as the Education Committee Administration Group.

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

The annual CRUK MI Colloquium, held in September, is an excellent opportunity for our new intake of students to meet other established PhD students, members of the Institute, including Group Leaders, Postdoctoral Fellows, and Scientific Officers. This forum communicates up to date science in the form of oral presentations given by Group Leaders and second year PhD students as well as poster presentations from a range of scientists across the Institute. Poster prizes are awarded, including the Lizzy Hitchman Prize for the best poster presented by a PhD student or Clinical Fellow. In 2013, Daniel Wiseman from the Leukaemia Biology was the recipient of the Lizzy Hitchman Prize for his work describing a potential test to predict relapse in Acute Myeloid Leukaemia.

PhD studentships

All of our CRUK core funded studentships are of four years duration, and consist of an approved research project in one of our research groups. Some students have joint supervisors in different groups, fostering important collaborations and providing exposure to different disciplines. Recruitment is highly competitive, with hundreds of applicants competing for around four to eight places each year. Interviews are typically conducted over a two-day period in early January.

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

Fellowships in Clinical Pharmacology Research

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

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

Education Committee 2013

The Education Committee acts for postgraduate students based within CRUK core funded research groups and consists of Senior Group Leaders, the Chief Operating Officer and the Postgraduate Education Manager of CRUK MI.

Our goal is for every student to have a project that is both achievable and intellectually stimulating and demanding. Projects and students are monitored by the Education Committee which makes sure that the proposed plan of research is suitable, and that progress is made consistently throughout the course of the studentship. Various assessments throughout the studentship, including regular talks, progress meetings and written reports, are key to ensuring successful completion of the PhD programme. Such assessments not only monitor progress, but also help to develop performance and presentations skills.

Valerie Kouskoff
(Chair, Education Committee)
Julie Edwards
Karim Labib
(until July)
Angeliki Malliri
Donald Ogilvie
Tim Somervaille
Ian Waddell
(Postgraduate Tutor from October)
Caroline Wilkinson
Richard Marais - Ex-Officio Member
(from February)

Student Representatives

Hadir Marei (until December)
Danish Memon (from November)
Alekh Thapa (from December)

OPERATIONS

Caroline Wilkinson
Chief Operating Officer

Stuart Pepper
Chief Laboratory Officer

Margaret Lowe
Head of Finance

Rachel Powell
Head of Human Resources

The Operations Department provides the necessary infrastructure and services to facilitate the running of the Institute. During 2013, Caroline Wilkinson and Stuart Pepper were appointed Chief Operating Officer and Chief Laboratory Officer respectively. Finance and purchasing, as well as Estates and Logistics, fall under the leadership of Margaret Lowe while Stuart Pepper oversees IT as well as Health and Safety; Rachel Powell is head of HR and Caroline Wilkinson is responsible for all aspects of Scientific Administration and acts as the primary point of operational contact within the Institute for both The University of Manchester and Cancer Research UK.

This year, the Operations team welcomed Ekram Aidaros and David Stanier to the Administration Department; Lewis Parkinson joined Estates and Neepa Begum was recruited to the Finance team. Hong Mach joined IT to provide specialist support for Apple devices while Tom Bolton was recruited to the position of Web Developer.

A major project for all operational staff during the year involved the Institute's change of name which came into effect at the beginning of October. This involved a great deal of work leading up to the event including replacing signage and the design and development of a new website.

Administration Department

Ruth Perkins, Ekram Aidaros, Steven Morgan, David Stanier

The Administration department has expanded this year with the addition of David Stanier as Administration Assistant who provides support to Caroline Wilkinson, Chief Operating Officer; to the HR department; and cover for Steve Morgan on Reception. Ruth Perkins took up the position of Executive Assistant to the Director when Siana Peters left the Institute in May and she is now supported by Ekram Aidaros, Administrative Services Coordinator.

The department has been incredibly busy this year with arrangements for the Institute name change that took effect on the 1st October. It was an exciting time for us and we launched a twitter feed @CRUK_MI and Facebook page under our new name. The department has assisted with the organisation of several events over the course of the year, including the Institute Colloquium and quinquennial/mid-term reviews. Administrative support is provided for the external seminar series, which has been a great success in 2013, and the 2014 programme is already in place. We aim to provide a varied programme of national and international speakers, serving to foster collaboration and encourage positive interaction within the wider scientific community. Details can be found at www.cruk.manchester.ac.uk/seminars

Estates

Steve Alcock, Graham Hooley, Lewis Parkinson, Tony Woollam

The past year has seen our team expand with the arrival of Lewis Parkinson who has been appointed as an Apprentice Building Services Engineer. He will gain great benefit from over a hundred years of combined experience in the Estates team.

During 2013, the Estates team have carried out a number of small works' projects to adapt laboratories and offices for the arrival of new groups and the relocation of others. The Estates team endeavour to identify sustainable solutions when putting new schemes together to help reduce the Institute's carbon footprint whenever possible. Lighting is one of the main users of energy in a building and the team is hoping to reduce our energy usage by replacing the existing luminaires with LED lighting.

The team has been proactive throughout the year, attending to many legislative requirements; examples include Legionella best practices and fire alarm testing. Team members have attended relevant courses to improve skills and keep their knowledge up to date with current working practices and changing legislation; the team attended an asbestos awareness training course in December.

Finance & Purchasing

Margaret Lowe, Neepa Begum[1], David Jenkins, Denise Owen, Muhammad Raja, Debbie Trunkfield

[1]joined in 2013

The Institute Finance Team supports the Director with the management of the Institute's £21m budget, which is devolved to the various groups and service units. The team provides a comprehensive service to the Institute, which covers all areas of Procurement and Finance, ensuring we comply with the University financial regulations and procedures.

The University has upgraded the Oracle system in 2013. This has involved close liaison with the Project Team to ensure as little disruption as possible within the Institute. There have inevitably been a few minor issues but the effect on the scientists has been minimal.

The Institute has been successful in securing several new external grant awards that will be activated in 2014 and we are also awaiting the outcome of several other applications. The team supports the research groups by providing effective and efficient professional advice when costing new research proposals and administering existing grants.

Towards the end of 2012, we were advised that an application to UK Research Partnership Investment Fund (UKRPIF) had been successful. This has resulted in the purchase of several significant pieces of equipment during 2013 with further purchases planned throughout 2014.

Health & Safety

Colin Gleeson

During the year, an increased emphasis on the importance of reporting accidents and near misses led to an increase in the number of reported incidents that more accurately reflect the situation in the Institute. We achieved a doubling in the number of reported near misses, which, it is suspected, have been historically under-reported. There was only one RIDDOR reportable incident in 2013; this involved an escape of liquid nitrogen from a brand new vessel. As a result, we reviewed our arrangements for liquid nitrogen safety resulting in the provision of personal low-oxygen monitors and the repeat of a cryogenic training session.

Other health and safety management system performance indicators were encouraging. A survey to assess the effectiveness of the processes for capturing new starters for induction training indicated that it was very effective, as all new starters in the previous eight months had been inducted. Furthermore, surveys showed that statutory required performance testing of all fume cupboard and safety cabinets had been completed. Other site-wide initiatives included compliance checks on stocks held of chemical weapons, desensitised explosives, dangerous pathogens and toxins, Euratom materials and radioactive waste disposal records.

Some of the Health and Safety initiatives undertaken in 2013 involved the Institute's core facilities. To increase protection when sorting live cells, bespoke microbiological safety cabinets were selected and installed in the Flow Cytometry facility. Accordingly, all live cell sorting can be contained within safety cabinets, whose performance can be validated by KI containment testing. In the Biological Resources Unit, monitoring was undertaken to assess the levels of airborne concentrations of animal allergens. The results indicated that in most of the facility, allergen levels were undetectable or very low indeed. Allergen was detected in the cage cleaning area and action is being undertaken to improve matters and so reduce the possibility of exposure to as low as reasonably practicable. Respiratory protective equipment testing was also carried out.

Health and Safety training has been delivered including Induction training as well as during specialist sessions for working with biological agents and clinical material, working with hazardous chemicals and completing risk assessments. These sessions were well attended. As the new MCRC building nears completion, equipment acquisition and formalising relationships with stakeholders has begun.

Human Resources

Rachel Powell, Laura Jones, Julie Jarratt, Lisa Rumsey[1]

[1]joined in 2013

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

During 2013, 44 individuals were successfully appointed who will endeavour to complement and enhance the work of the Institute. The department has continued its drive for efficiency by evaluating where we advertise our vacancies. This is to ensure that we are attracting the best candidates to the deliver the aims and objectives of the Institute and to ensure that we remain a leading cancer research establishment.

We have continued our joint partnership working with the unions which has resulted in the revision of many policies such as the Career Path for Scientists, Special Leave Policy and the Career Break Policy. We are committed to developing an environment where staff are able to fully contribute to the service while feeling valued and respected. These values are reinforced by the Institute with the revision and re-launch of the Respect at Work Policy.

Over the next year, the main focus for the HR department will be the successful recruitment of new groups to the Institute and the implementation of the new online probationary system.

Information Technology

Malik Pervez, Hong Mach[1], Brian Poole, Steve Royle, Zhi Cheng Wang[2], Matthew Young

[1]joined in 2013

[2] Moved to Scientific Computing team

The CRUKMI IT team have continued to maintain IT services to the high standards that are expected by service users as well as providing increased functionality where required. The team support and maintain more than 500 PC and Apple based desktops as well as over 100 mobile devices including Blackberry, iPads and mobile tablets, therefore it is vital that front-end and back-end systems are continuously updated and upgraded to ensure system security and stability.

During 2013, the on-going need for additional data storage has meant we have increased our storage capability to over 200TB to meet the requirements of our staff and accommodate the scientific needs for the coming year. A review of the storage strategy will be undertaken in the New Year to take into account the research undertaken at the CRUK MI as well as the new MCRC building. The IT team are looking forward to the challenge of supporting the new MCRC building which will have a diverse customer base resulting in complex interactions in terms of IT systems. The team will be looking to devise creative solutions to ensure that the Institute extends the high standards of IT, that current users have become accustomed to, across to the new MCRC building.

The recent separation of the high performance cluster into a dedicated new department has led the IT department to focus their attention on the core IT challenges of the Institute's infrastructure. As a consequence of the rise in the number of Apple desktop systems, IT support for Apple devices has been doubled. The new name for the Institute necessitated the rebranding of all IT systems which was achieved in a seamless manner.

Logistics

Maurice Cowell, Edward Fitzroy, Sedia Fofana, Stephen Keane[1], Andrew Lloyd, Jonathan Lloyd

[1]joined in 2013

The Logistics facility plays a vital role in supporting the research carried out at the Institute. This includes the receipting, checking, booking in and distribution of goods ordered by staff as well as the collection and removal of waste. The team also provides assistance with moving equipment and supplies and therefore helps facilitate internal rearrangements and the arrival of new groups. Some larger liquid nitrogen vessels are now in operation, increasing the usage of nitrogen and its transportation to the labs. This is performed three times a week with dry ice deliveries taking place twice a week. During 2013, the Institute used approximately 90 litres of liquid nitrogen in storage vessels. Gas cylinders are also monitored and replaced as necessary. We have been able to make savings by assessing our gas cylinders and to cutting down on those in less demand thus decreasing rental charges. We have also been able to make significant savings of just over £4,000 this year by setting up orders that cover multiple deliveries from a single company with a number of suppliers.

Researchers can order central stores stock items via the intranet which are then distributed by the Logistics team who also ensure that adequate stock levels are maintained at all times. Included in this system are the enzymes and media stored in the Institute freezers (Sigma, Invitrogen, Roche, Promega, New England Biolabs, Fisher kits and Qiagen). The department works closely with all the research groups and helps out where necessary, for example by tracing and confirming delivery of goods with suppliers. The team also manages the moving of heavy equipment or furniture, and the reconfiguration of meeting rooms for numerous events which this year has included various fundraising events, QQRs and events for the Manchester Science Festival.

Scientific Operations

Caroline Wilkinson, Tom Bolton[1,2], Gillian Campbell

[1]joined in 2013

[2]joint with MCRC

Scientific administration is overseen by the Chief Operating Officer, Caroline Wilkinson, who provides support to the Director in order to facilitate the day-to-day running of the Institute. The team is also responsible for producing a variety of scientific communications for the Institute including publications such as the Annual Scientific Report, the Institute's Newsletter, writing material for the intranet and external website and for the Institute's social media presence. Talks and tours are also provided for a packed programme of fundraisers' events throughout the year. During 2013, Tom Bolton was recruited to the position of Web Developer and took on the re-design and development of the external website to coincide with the rebranding of the Institute.

In addition to receiving core funding from CRUK, our researchers also apply for external awards to extend the portfolio of research that we can undertake. All grant applications submitted by our researchers are screened for the appropriate ethical approvals, as well as the ability of the Institute to accommodate the proposed programme of work. We also have a rigorous internal peer review process for grant applications which is organised by Gill Campbell, the Institute's Grants Advisor. Gill also provides support to the Institute's researchers through the grant preparation and submission process. This year, saw an increase in the number of submissions including a successful

application to Prostate Cancer UK, for the award of a Movember Prostate Cancer Centre of Excellence (in collaboration with Queen's University Belfast).

In September, the team organised a one day meeting between the Institute's scientists and those working on cancer-related aspects at The University of Manchester's Faculty of Life Sciences. This was highly successful and we are planning to make this a regular event.

During the year, we modified the Institute's new staff induction process to include information regarding research integrity. In addition, Gill Campbell was appointed as the Institute's Research Integrity Officer to help promote and maintain the highest standards of rigour and integrity in all aspects of our research.

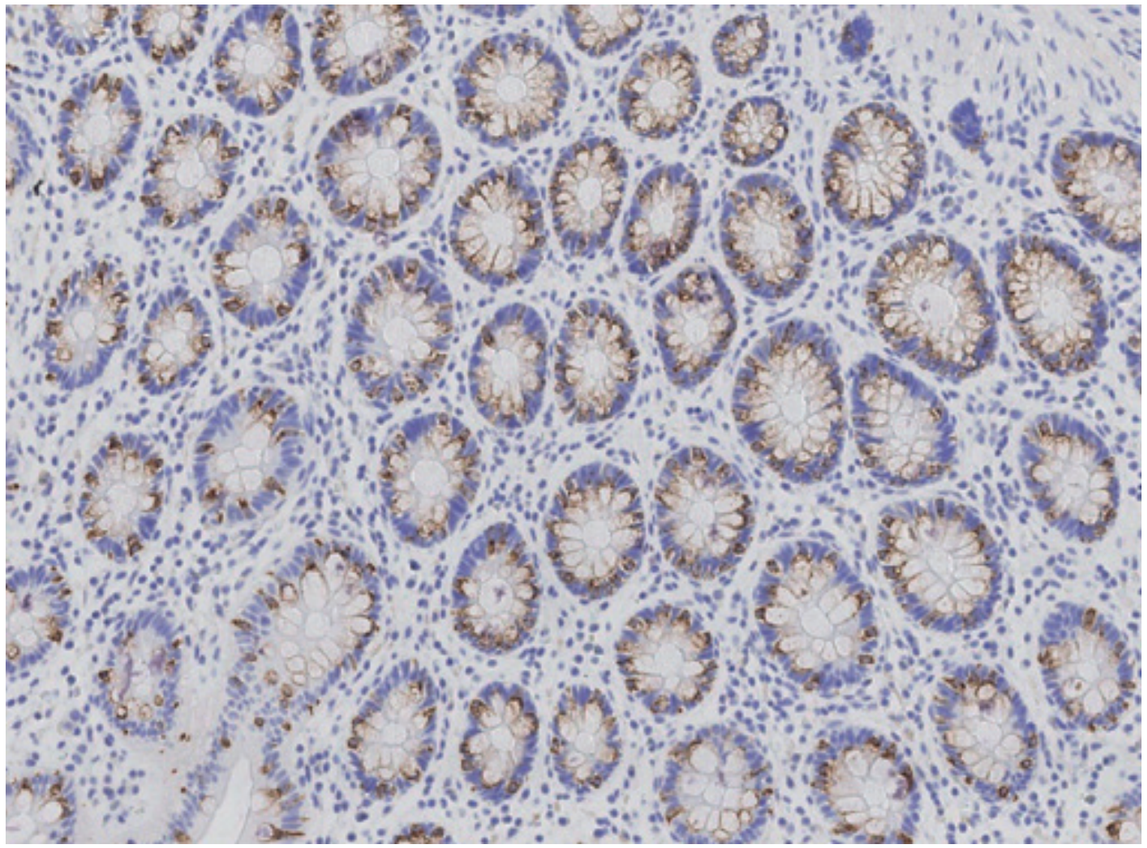
Cancer Research Technology

Martyn Bottomley

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

At CRT, we bridge the fundamental gap between cutting edge academic research and industrial development of cancer therapeutics and diagnostics. We achieve this by working closely with prestigious international research institutes, such as the Cancer Research UK Manchester Institute and funding bodies to develop, protect and commercialise oncology related discoveries. Core activities of business development and drug discovery are supported by specialists, integrated in the business with expertise in patents, legal, finance and marketing. Our exclusive focus in oncology provides an unrivalled depth of knowledge and experience in cancer-specific translational development and commercialisation. We now also have access to the CRT Pioneer Fund; this £50m fund has been established with Cancer Research Technology (CRT) and the European Investment Fund (EIF) to bridge the investment gap between cancer drug discovery and early development. It will take potential cancer drugs, primarily discovered by Cancer Research UK, from discovery through to entry to Phase II clinical trials before partnering with pharmaceutical and biotechnology companies.

By arrangement with The University of Manchester, CRT owns and is responsible for the development and commercialisation of intellectual property arising from Cancer Research UK funded research at The University of Manchester (including the Cancer Research UK Manchester Institute). Our relationship with the Cancer Research UK Manchester Institute reflects the specific requirements of the scientist, Cancer Research UK Manchester Institute, Cancer Research UK and the individual project. To effectively facilitate these requirements and interactions, Martyn Bottomley, a CRT Business Manager, is based on-site at the Cancer Research UK Manchester Institute and is dedicated to working closely with the staff at the Institute and also The University of Manchester. Martyn is here to offer access to oncology-focused expertise in technology evaluations, patent applications and management, funding for development, commercialisation, drug discovery, market intelligence, and project management. He also works closely with UMIP, The University of Manchester technology transfer organisation. CRT continues to work very closely with the Drug Discovery Laboratories based at the Cancer Research UK Manchester Institute to facilitate the development of small molecules drug therapies to satisfy the unmet clinical needs of cancer patients. In the year to date, we have negotiated four collaboration agreements with commercial partners for the Drug Discovery Laboratories, including AstraZeneca, GSK and HitGen. CRT is also currently actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. We look forward to building on our success and continuing to work closely with the Cancer Research UK Manchester Institute to advance discoveries to beat cancer in the years ahead.



Normal human colon stained with an antibody to the marker MUC2 (brown). MUC2 is used as a marker of specialised Goblet cells and is deregulated in several tumour types. The tissue has been counterstained with haematoxylin to show the nuclei of cells (blue).

Image provided by Darren Roberts, Immunology.

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Cancer Research UK's Research Engagement Manager

Eve Hart[1]

Cancer Research UK's Research Engagement Manager

James Dunphy[2]

[1] joined in 2013

[2] left in 2013

The Cancer Research UK Manchester Institute has had a fantastic year of supporting fundraisers, thanking them and joining them in a range of activities.

More than 1000 charity supporters, twice as many as in 2012, have visited the Institute this year and taken part in a range of activities, from a film screening to lab tours and interactive hands-on science taster sessions. Many more have joined us outside the Institute for events and activities, including science festivals and projects with local schools, and in total there have been more than 260 events in which Institute scientists interacted face-to-face with the public who fund our work.

Before and after. The Cancer Research UK Manchester Institute's Drug Discovery team tackle Movember. In the first photo, Stuart is the control subject. The team raised a brilliant total of £1366.

Institute scientists themselves have also taken part in a series of fundraising activities, raising tens of thousands of pounds for research. These range from the most challenging 40 mile Keswick to Barrow walk and 24 hour Relay for Life, to running through clouds of paint in the Colour Run, practising yoga poses for Stand up to Cancer, growing a series of stylish moustaches during Movember, and even representing scientific research through the medium of cake during the charity's inaugural Science Cakes competition.

The range of visitors to the Institute this year has been wider than ever before. In September, we piloted an open afternoon for local businesses to encourage them to support our work, an event to be repeated twice in the first half of 2014. In the same month, more than 30 people affected by cancer visited us as part of a network of patient engagement events taking place around the UK at the same time. The events, which included a live webcast by CRUK Chief Executive Harpal Kumar, will help the charity to develop a new strategy to better involve people affected by cancer in research. Colette Hughes attended with her sister Diane. She said 'We are a family who carry the BRCA2 gene and my sister is on personalised treatment [an early phase clinical trial at the Christie NHS Foundation Trust]. Getting more survivors to talk to the scientists and seeing what is being done from both viewpoints would be just brilliant'.

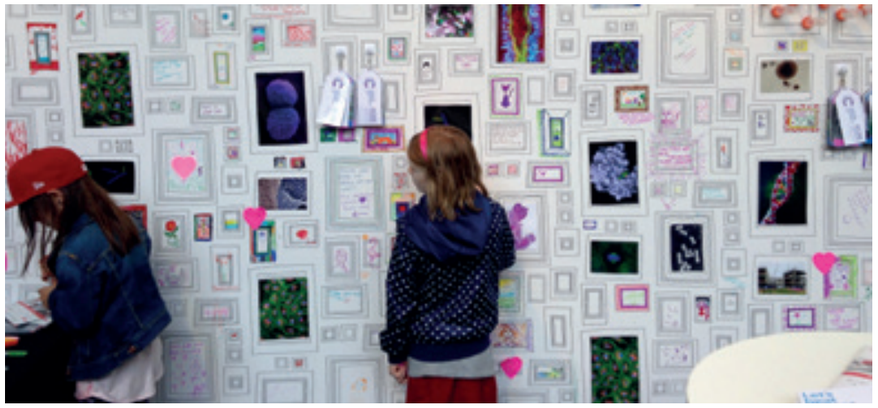
Outside the Institute, a new audience were exposed to our work and encouraged to support us when we established a surprising 'Gallery of Research' as part of the Buy Art Fair in central Manchester. Thousands of visitors saw Steve Bagley's images hung alongside original paintings and artworks, telling the story of cancer research from DNA to drug discovery. The gallery even had its own catalogue in the form of image flashcards that related each picture to its place in the research journey. Visitors were invited to add their messages of support to the gallery walls in return for a charity donation. Buy Art Fair's founder Thom Hetherington said 'Cancer Research UK's involvement really brought something to the fair this year, and hopefully it generated profile and opportunity for you'.

In 2014, we hope to build on the success of 2013, including the Manchester Science Festival, which takes a look at the past, present and future of cancer research as part of Manchester Histories Festival and cross-disciplinary partnership projects with schools involved with the University of Manchester's SUPI (School and University Partnership) initiative. The Institute's scientists have many fundraising plans, such as running the Manchester Marathon, the Great Manchester Run and climbing Scafell Pike.

It is unlikely that our 40,000 supporters in Greater Manchester would make the contribution to our work that they do without the passion and enthusiasm of the Institute's scientists, who are always keen and willing to share their work in new and creative ways.

Diane Hughes shares ideas of how patients could better inform Cancer Research UK's research policy and progress during September's Patient Engagement event at the Cancer Research UK Manchester Institute

Nelson Iley talks about the work of the Molecular Biology Core Facility before one of our visitors get hands on with a pipette

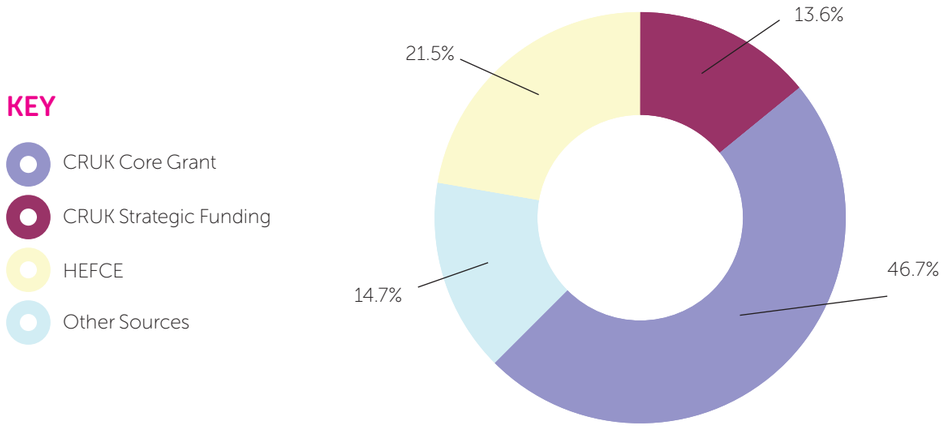


The Cancer Research UK Gallery at Buy Art Fair 2013, Spinningfields, Manchester

ACKNOWLEDGEMENT FOR FUNDING OF THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2013 was £20.8m. The major source of this funding was awarded by Cancer Research UK (CRUK) via a core grant of £10.1m, plus additional strategic funding of £3.7m. This funding enables the various scientific groups and service units within the Institute to carry out their research.

CRUK MANCHESTER INSTITUTE FUNDING 2013



The infrastructure of the CRUK Manchester Institute is funded by HEFCE generated income at a cost of £2.4m. This was further enhanced by £2.3m from the UK Research Partnership Investment Fund (UKRPIF).

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

These sources are as follows:

- AstraZeneca
- European Commission
- European Research Council
- BBSRC
- Leukaemia & Lymphoma Research Fund
- Abbott Laboratories
- Christie Hospital NHS Foundation Trust
- Association for International Cancer Research
- Medical Research Council
- Kay Kendal Leukaemia Fund
- Wellcome Trust
- Parsortix
- Pancreatic Cancer Research Fund
- Academy of Medical Sciences
- Immunogen Inc

We are immensely grateful to all our sponsors.

CAREER OPPORTUNITIES AT THE CANCER RESEARCH UK MANCHESTER INSTITUTE

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

The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular services, next generation sequencing, real-time PCR, a microarray platform, proteomics, flow cytometry, histology, advanced imaging, and the production of knock-in/knock-out animal models. Details of all groups and facilities are given in this report, and can guide interested parties to the appropriate contacts.

Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates with a first or 2.1 honours degree in a biological science can apply each year to train for a four-year PhD in one of our research laboratories. The University of Manchester offers a wide range of training for new and existing students which provides opportunities to acquire skills that will complement the research programme and help achieve personal and career development goals. At the Institute, we also ensure that postgraduate students are provided with high quality, relevant and appropriate training alongside development opportunities. The Institute also has a well-developed process for ensuring excellent pastoral care and mentoring for all students.

Postdoctoral applicants of high calibre are regularly sought. Although Postdoctoral Fellows will be encouraged to apply for their own fellowships, funded positions are available for outstanding candidates. Interested applicants should contact the Group Leaders directly, with details of their research interests and recent experience.

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

Specific vacancies can be found on our web pages (www.cruk.manchester.ac.uk/Jobs/), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

CONTACT DETAILS

Cancer Research UK Manchester Institute
Director: Professor Richard Marais
Tel: +44(0) 161 446 3156
Fax: +44(0) 161 446 3109

Address
Cancer Research UK Manchester Institute
Wilmslow Road
Manchester
M20 4BX
United Kingdom

e-mail: enquiries@cruk.manchester.ac.uk
website: www.cruk.manchester.ac.uk

Tel +44(0) 161 446 3156

Electronic version of this report can be found at:
www.cruk.manchester.ac.uk/About/

Cancer Research UK
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Registered address: Angel Building, 407 St John Street, London, EC1V 4AD.

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

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Edited by: Caroline Wilkinson
Gillian Campbell

Cancer Research UK Manchester Institute
Wilmslow Road
Manchester
M20 4BX
United Kingdom
Telephone +44(0) 161 446 3156

www.cruk.manchester.ac.uk