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Cover image
PhD student Michaela Mrschik and post-doc
Alice Baudot from Kevin Ryan's group.

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The Beatson Institute for Cancer
Research building

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Professor Karen Vousden
CBE, FRS, FRSE, FMedSci
Director of The Beatson
Institute for Cancer Research

The past year has seen some exciting projects nearing completion, as we consolidate and build on existing programmes within the Beatson Institute, while also extending our collaborations with the University of Glasgow.

At the Beatson Institute itself, we are continuing to develop in our two key areas of research strength – cancer cell growth, metabolism and survival, and cancer cell invasion and metastasis. In collaboration with our clinical imaging colleagues within the NHS, including the PET team at the Beatson West of Scotland Cancer Centre and the chemists within the Radionuclide Dispensary, we have established preclinical PET/SPECT/CT imaging and a small team to help support this technology. This capability will complement our existing fluorescence imaging programme, and our work on tissue specific expression of fluorescent proteins will be a powerful aid in the development of radiolabelled probes and therapeutic agents. Further investment into the metabolomics facility has expanded our ability to analyse metabolite levels and metabolic flux in experimental models. In addition, we have appointed a facility manager, **Gillian Mackay** to oversee this expanding service. Finally, the biological services unit was completed this year and is now fully operational and open for business. With the increased capacity we are now able to support cancer scientists from across Glasgow.

A number of reviews in 2012 have allowed us to take stock of our progress. Our research services – which include significant investment into microscopy, proteomics/metabolomics and preclinical models – were assessed by an external panel of experts who were overwhelmingly positive, and particularly impressed by the collegiate environment of the Institute and the professionalism of its staff. Reviews of our scientific groups were also highly successful, and our Science Advisory Group visited, providing us with some very valuable input into our work. All the reviewers were extremely impressed with the Institute's recent progress.

Our collaborations with the University of Glasgow have also expanded and consolidated. We have increased the number of our Beatson Associates and were delighted to welcome **Daniel Murphy**, whose work focuses on how oncogene activation may result in tumour specific vulnerabilities, and how these might be exploited for therapy. Most obvious building work has been the completion of the Translational Cancer Research Centre (TCRC) next to the Beatson, with the move of both the Institute of Cancer Sciences and the Glasgow Polyomics facility into the new space ongoing as I write this. The TCRC will be led by **Professor Andrew Biankin**, recruited from the Garvan Institute in Melbourne, Australia. Andrew is a surgeon and scientist specialising in pancreatic cancer, and he plans to develop a personalised medicine strategy focusing on pancreatic cancer in close collaboration with many of us at the Beatson. We are extremely fortunate that Andrew has chosen to join us, his arrival will expand our ability to integrate basic research, preclinical studies and clinical trials. We are also very pleased to welcome **Iain McNeish** who has been appointed as Professor of Gynaecology Oncology. Iain's arrival further increases our critical mass of clinician scientists, and he has also agreed to take on a role as mentor to our clinical research fellows.

We were delighted with the number of our group leaders receiving external recognition this year. **Laura Machesky** was elected to both EMBO and the American Society for Cell Biology Council, while **Owen Sansom** was elected as a Fellow of the Royal Society of Edinburgh and awarded the CR-UK Future Leaders in Cancer Research Prize. **Kurt Anderson** was the first ever winner of the Royal Microscopical Society Medal for Life Sciences, while **Kevin Ryan** was awarded the Tenovus Medal for 2012 by the University of Glasgow.

Our postdoctoral scientists continue to do well, with a number taking up independent group leader positions – this includes **Simon Calaminus**, **Christian Frezza** and **Paul Timpson** who have moved to Hull, Cambridge and Sydney, Australia, respectively in the last year. In addition, **Marta Dozynkiewicz**, a former student with Jim Norman, was awarded the Joseph Black Medal and Alan Hird Prize for her PhD thesis. As in previous years, our students and post-docs were very actively involved in this year's conference and workshop – both events were a great success thanks to their input, that of the organisers and also that of all of the participants who joined us from around the world.

As always, we are grateful and encouraged by the generous support of so many people who help to raise the funds for our work year after year. These generous individuals include the staff at Clyde Travel, the members of the Lanarkshire Women's Bowling Association and the pupils at Mosshead Primary School.

Of course, we would not exist without the continuing support of our parent charity, Cancer Research UK. CR-UK rebranded this year and we have also changed our logo to reflect our close links. We are extremely happy to promote and advertise the close relationship between us, and the tremendous work the charity is doing towards beating cancer.

Recent arrival Dr Daniel Murphy.



This section features some of the key research findings made by scientists at the Beatson Institute and Institute of Cancer Sciences in the past year.

The Beatson Institute for Cancer Research

Chaneton B, Hillmann P, Zheng L, Martin AC, Maddocks OD, Chokkathukalam A, Coyle JE, Jankevics A, Holding FP, Vousden KH, Frezza C, O'Reilly M, Gottlieb E.

Serine is a natural ligand and allosteric activator of pyruvate kinase M2. **Nature** 2012; 491: 458-62

Cancer cells exhibit several unique metabolic phenotypes that are critical for cell growth and proliferation. Specifically, they overexpress the M2 isoform of the tightly regulated enzyme pyruvate kinase (PKM2), which controls glycolytic flux, and are highly dependent on *de novo* biosynthesis of serine and glycine. In this paper, the authors describe a new rheostat-like mechanistic relationship between PKM2 activity and serine biosynthesis. They show that serine can bind to and activate human PKM2, and that PKM2 activity in cells is reduced in response to serine deprivation. This reduction in PKM2 activity shifts cells to a fuel-efficient mode in which more pyruvate is diverted to the mitochondria and more glucose-derived carbon is channelled into serine biosynthesis to support cell proliferation.

Cordero JB, Stefanatos RK, Scopelliti A, Vidal M*, Sansom OJ*.

Inducible progenitor-derived Wingless regulates adult midgut regeneration in *Drosophila*. **EMBO J** 2012; 31: 3901-17

*corresponding authors

The ability to regenerate following stress is a hallmark of self-renewing tissues. However, little is known about how regeneration differs from homeostatic tissue maintenance. In this paper, the authors study the role and regulation of Wingless (Wg)/Wnt signalling during intestinal regeneration using the *Drosophila* adult midgut. They show that Wg is produced by the intestinal epithelial compartment upon damage or stress and it is exclusively required for intestinal stem

cell (ISC) proliferation during tissue regeneration. Reducing Wg or downstream signalling components from the intestinal epithelium blocked tissue regeneration. Importantly, they demonstrate that Wg from the undifferentiated progenitor cell, the enteroblast, is required for Myc-dependent ISC proliferation during regeneration. Similar to young regenerating tissues, ageing intestines required Wg and Myc for ISC hyperproliferation. Unexpectedly, their results demonstrate that epithelial but not mesenchymal Wg is essential for ISC proliferation in response to damage, while neither source of the ligand is solely responsible for ISC maintenance and tissue self-renewal in unchallenged tissues. Therefore, fine-tuning Wnt results in optimal balance between the ability to respond to stress without negatively affecting organismal viability.

Dou H, Buetow L, Hock A, Sibbet GJ, Vousden KH, Huang DT.

Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. **Nat Struct Mol Biol** 2012; 19: 184-92

Cbls are RING ubiquitin ligases that attenuate receptor tyrosine kinase (RTK) signal transduction. Cbl ubiquitination activity is stimulated by phosphorylation of a linker helix region (LHR) tyrosine residue. To elucidate the mechanism of activation, the authors determined the structures of human CBL, a CBL-substrate peptide complex and a phosphorylated-Tyr371-CBL-E2-substrate peptide complex, and compared them with the known structure of a CBL-E2-substrate peptide complex. Structural and biochemical analyses show that CBL adopts an auto-inhibited RING conformation, where the RING's E2-binding surface associates with CBL to reduce E2 affinity. Tyr371 phosphorylation activates CBL by inducing LHR conformational changes that eliminate auto-inhibition, flip the RING domain

and E2 into proximity of the substrate-binding site and transform the RING domain into an enhanced E2-binding module. This activation is required for RTK ubiquitination. The results present a mechanism for regulation of c-Cbl's activity by auto-inhibition and phosphorylation-induced activation.

Dou H, Buetow L, Sibbet GJ, Cameron K, Huang DT.

BIRC7-E2 ubiquitin conjugate structure reveals the mechanism of ubiquitin transfer by a RING dimer. **Nat Struct Mol Biol** 2012; 19: 876-83

Certain RING ubiquitin ligases (E3s) dimerise to facilitate ubiquitin (Ub) transfer from ubiquitin-conjugating enzyme (E2) to substrate, but structural evidence on how this process promotes Ub transfer is lacking. In this paper, the authors report the structure of the human dimeric RING domain from BIRC7 in complex with the E2 Ubch5B covalently linked to Ub (Ubch5B~Ub). The structure reveals extensive non-covalent donor Ub interactions with Ubch5B and both subunits of the RING domain dimer that stabilise the globular body and C-terminal tail of Ub. Mutations that disrupt these non-covalent interactions or RING dimerisation reduce Ubch5B~Ub binding affinity and ubiquitination activity. Moreover, NMR analyses demonstrate that BIRC7 binding to Ubch5B~Ub induces peak-shift perturbations in the donor Ub consistent with the crystallographically observed Ub interactions. Our results provide structural insights into how dimeric RING E3s recruit E2~Ub and optimise the donor Ub configuration for transfer.

Gao M, Patel R, Ahmad I, Fleming J, Edwards J, McCracken S, Sahadevan K, Seywright M, Norman J, Sansom O, Leung HY.

SPRY2 loss enhances ErbB trafficking and PI3K/AKT signalling to drive human and mouse prostate carcinogenesis. **EMBO Mol Med** 2012; 4: 776-90

Loss of SPRY2 and activation of receptor tyrosine kinases are common events in prostate cancer (PC). However, the molecular basis of their interaction and clinical impact remains to be fully examined. SPRY2 loss may functionally synergise with aberrant cellular signalling to drive PC and to promote treatment-resistant disease. In this paper, the authors report evidence for a positive feedback regulation of the ErbB-PI3K/AKT cascade by SPRY2 loss in *in vitro* as well as pre-clinical *in vivo* models and clinical PC. Reduction in SPRY2 expression resulted in hyper-activation of PI3K/AKT signalling to drive proliferation and invasion by enhanced internalisation of EGFR/HER2 and

their sustained signalling at the early endosome in a PTEN-dependent manner. This involved p38 MAPK activation by PI3K to facilitate clathrin-mediated ErbB receptor endocytosis. Finally, *in vitro* and *in vivo* inhibition of PI3K suppressed proliferation and invasion, supporting PI3K/AKT as a target for therapy particularly in patients with PTEN-haploinsufficient, low SPRY2 and ErbB expressing tumours. In conclusion, SPRY2 is an important tumour suppressor in PC since its loss drives the PI3K/AKT pathway via functional interaction with the ErbB system.

Jamieson T, Clarke M, Steele CW, Samuel MS, Neumann J, Jung A, Huels D, Olson MF, Das S, Nibbs RJ, Sansom OJ.

Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. **J Clin Invest** 2012; 122: 3127-44

The chemokine receptor CXCR2 is a key mediator of neutrophil migration that also plays a role in tumour development. However, CXCR2 influences tumours through multiple mechanisms and might promote or inhibit tumour development depending on context. In this paper, the authors used several mouse models of spontaneous and inflammation-driven neoplasia to define indispensable roles for CXCR2 in benign and malignant tumours. CXCR2-activating chemokines were part of the secretome of cultured primary benign intestinal adenomas (ApcMin/+) and highly expressed by all tumours in all models. CXCR2 deficiency profoundly suppressed inflammation-driven tumorigenesis in skin and intestine as well as spontaneous adenocarcinoma formation in a model of invasive intestinal adenocarcinoma (AhCreER;Apcfl/+;Ptenfl/fl mice). Pepducin-mediated CXCR2 inhibition reduced tumorigenesis in ApcMin/+ mice. Ly6G+ neutrophils were the dominant source of CXCR2 in blood, and CXCR2 deficiency attenuated neutrophil recruitment. Moreover, systemic Ly6G+ cell depletion purged CXCR2-dependent tumour-associated leukocytes, suppressed established skin tumour growth and colitis-associated tumorigenesis, and reduced ApcMin/+ adenoma formation. CXCR2 is thus a potent pro-tumorigenic chemokine receptor that directs recruitment of tumour-promoting leukocytes into tissues during tumour-inducing and tumour-driven inflammation. Similar leukocyte populations were also found in human intestinal adenomas, which suggests that CXCR2 antagonists may have therapeutic and prophylactic potential in the treatment of cancer.

Maddocks ODK, Berkers CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH. Serine starvation induces stress and p53 dependent metabolic remodeling in cancer cells. *Nature* doi: 10.1038/nature11743, published online 16 Dec 2012

Cancer cells acquire distinct metabolic adaptations to survive stress associated with tumour growth and to satisfy the anabolic demands of proliferation. The tumour suppressor protein p53 (also known as TP53) influences a range of cellular metabolic processes, including glycolysis, oxidative phosphorylation, glutaminolysis and anti-oxidant response. In contrast to its role in promoting apoptosis during DNA-damaging stress, p53 can promote cell survival during metabolic stress, a function that may contribute not only to tumour suppression but also to non-cancer-associated functions of p53. In this paper, the authors show that human cancer cells rapidly use exogenous serine and that serine deprivation triggered activation of the serine synthesis pathway and rapidly suppressed aerobic glycolysis, resulting in an increased flux to the tricarboxylic acid cycle. Transient p53-p21 (also known as CDKN1A) activation and cell cycle arrest promoted cell survival by efficiently channelling depleted serine stores to glutathione synthesis, thus preserving cellular anti-oxidant capacity. Cells lacking p53 failed to complete the response to serine depletion, resulting in oxidative stress, reduced viability and severely impaired proliferation. The role of p53 in supporting cancer cell proliferation under serine starvation was translated to an *in vivo* model, indicating that serine depletion has a potential role in the treatment of p53-deficient tumours.

Rainero E, Caswell PT, Muller PA, Grindlay J, McCaffrey MW, Zhang Q, Wakelam MJ, Vousden KH, Graziani A, Norman JC. Diacylglycerol kinase alpha controls RCP-dependent integrin trafficking to promote invasive migration. *J Cell Biol* 2012; 196: 277-95

Inhibition of $\alpha v \beta 3$ integrin or expression of oncogenic mutants of p53 promote invasive cell migration by enhancing endosomal recycling of

$\alpha 5 \beta 1$ integrin under control of the Rab11 effector Rab-coupling protein (RCP). In this paper, the authors show that diacylglycerol kinase α (DGK- α), which phosphorylates diacylglycerol to phosphatidic acid (PA), was required for RCP to be mobilised to and tethered at the tips of invasive pseudopods and to allow RCP-dependent $\alpha 5 \beta 1$ recycling and the resulting invasiveness of tumour cells. Expression of a constitutively active mutant of DGK- α drove RCP-dependent invasion in the absence of mutant p53 expression or $\alpha v \beta 3$ inhibition, and conversely, an RCP mutant lacking the PA-binding C2 domain was not capable of being tethered at pseudopod tips. These data demonstrate that generation of PA downstream of DGK- α is essential to connect expression of mutant p53s or inhibition of $\alpha v \beta 3$ to RCP and for this Rab11 effector to drive the trafficking of $\alpha 5 \beta 1$ that is required for tumour cell invasion through three-dimensional matrices.

Veltman DM, King JS, Machesky LM, Insall RH. SCAR knockouts in Dictyostelium: WASP assumes SCAR's position and upstream regulators in pseudopods. *J Cell Biol* 2012; 198: 501-8

Under normal conditions, the Arp2/3 complex activator SCAR/WAVE controls actin polymerisation in pseudopods, whereas Wiskott-Aldrich syndrome protein (WASP) assembles actin at clathrin-coated pits. The authors show that, unexpectedly, *Dictyostelium discoideum* SCAR knockouts could still spread, migrate, and chemotax using pseudopods driven by the Arp2/3 complex. In the absence of SCAR, some WASP relocated from the coated pits to the leading edge, where it behaved with similar dynamics to normal SCAR, forming split pseudopods and travelling waves. Pseudopods co-localised with active Rac, whether driven by WASP or SCAR, though Rac was activated to a higher level in SCAR mutants. Members of the SCAR regulatory complex, in particular PIR121, were not required for WASP regulation. The authors thus show that WASP is able to respond to all core upstream signals and that regulators coupled through the other members of SCAR's regulatory complex are not essential



for pseudopod formation. They conclude that WASP and SCAR can regulate pseudopod actin using similar mechanisms.

Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futo K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrady B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM. N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. *J Cell Biol* 2012; 199: 527-44

Metastasising tumour cells use matrix metalloproteases, such as the transmembrane collagenase MT1-MMP, together with actin-based protrusions, to break through extracellular matrix barriers and migrate in dense matrix. In this paper, the authors show that the actin nucleation-promoting protein N-WASP (Neural Wiskott-Aldrich syndrome protein) is upregulated in breast cancer, and has a pivotal role in mediating the assembly of elongated pseudopodia that are instrumental in matrix degradation. Although a role for N-WASP in invadopodia was known, the authors now show how N-WASP regulates invasive protrusion in three-dimensional matrices. In actively invading cells, N-WASP promoted trafficking of MT1-MMP into invasive pseudopodia, primarily from late endosomes, from which it was delivered to the plasma membrane. Upon MT1-MMP's arrival at the plasma membrane in pseudopodia, N-WASP stabilised MT1-MMP via direct tethering of its cytoplasmic tail to F-actin. Thus, N-WASP is crucial for extension of invasive pseudopods into which MT1-MMP traffics and for providing the correct cytoskeletal framework to couple matrix remodeling with protrusive invasion.

Institute of Cancer Sciences

Liu L, Ulbrich J, Müller M, Wüstefeld T, Aeberhard L, Kress TR, Muthalagu N, Moll R, Kempa S, Zender L, Eilers M, Murphy DJ. Deregulated MYC expression induces dependence upon AMPK-related kinase 5. *Nature* 2012; 483: 608-12

Deregulated expression of the MYC oncoprotein contributes to the genesis of many human tumours, yet strategies to exploit this for a rational tumour therapy are scarce. MYC promotes cell growth and proliferation, and alters cellular metabolism to enhance the provision of precursors for phospholipids and cellular macromolecules. In this paper, the authors show in human and murine cell lines that oncogenic levels of MYC establish a dependence on AMPK-related kinase 5 (ARK5; also known as NUAK1) for maintaining metabolic homeostasis and for cell survival. ARK5 is an upstream regulator of AMPK and limits protein synthesis via inhibition of the mammalian target of rapamycin 1 (mTORC1) signalling pathway. ARK5 also maintains expression of mitochondrial respiratory chain complexes and respiratory capacity, which is required for efficient glutamine metabolism. Inhibition of ARK5 leads to a collapse of cellular ATP levels in cells expressing deregulated MYC, inducing multiple pro-apoptotic responses as a secondary consequence. Depletion of ARK5 prolongs survival in MYC-driven mouse models of hepatocellular carcinoma, demonstrating that targeting cellular energy homeostasis is a valid therapeutic strategy to eliminate tumour cells that express deregulated MYC.

BACKGROUND

The name Beatson used in our title is in recognition of the early work of Sir George Beatson who in 1912 established a research department at the cancer hospital in Glasgow. This department became independent from the hospital in 1967 when The Beatson Institute for Cancer Research was founded by the then Director, Dr John Paul. Dr Paul also raised sufficient funds to move the Institute in 1976 to our present location at Garscube Estate in Glasgow.

In 1990 Glasgow University researchers moved to adjacent refitted accommodation. More recently, other teams with University affiliations have moved here to share laboratory facilities and, when more space is available in new buildings, this will continue. The resulting Institute of Cancer Sciences provides a cutting edge research environment situated in the beautiful, leafy green Garscube Estate on the north-western edge of Glasgow.

Sir George Beatson
1848 - 1933



The Beatson Institute for
Cancer Research



REGULATION OF CANCER CELL GROWTH METABOLISM AND SURVIVAL

THE BEATSON INSTITUTE FOR CANCER RESEARCH

David Gillespie - Checkpoints and Cell Cycle Control
Eyal Gottlieb - Apoptosis and Tumour Metabolism
Danny Huang - Ubiquitin Signalling
Hing Leung - Prostate Cancer Biology
Kevin Ryan - Tumour Cell Death
Karen Vousden - Tumour Suppression

CHECKPOINTS AND CELL CYCLE CONTROL

www.beatson.gla.ac.uk/david_gillespie



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²until October

³until November

⁴joint with Peter Adams

DNA damage is a key factor both in the evolution and treatment of cancer. Genomic instability is rampant in cancer cells, fuelling accumulation of oncogenic mutations and therapy resistance. Conversely, DNA-damaging radiation and genotoxic chemotherapies are the mainstays of conventional cancer treatments. Although imperfect, and often associated with undesirable side effects, such therapies seem likely to remain in use for the foreseeable future. As a result, we want to understand how normal and tumour cells respond to DNA damage and whether DNA damage responses (DDR) can be exploited for therapy.

DNA damage activates a complex, interacting web of DNA repair and cell cycle checkpoint processes that collaborate to protect cells from genetic damage and mutation. These offer a diverse range of potentially 'druggable' targets that includes multiple protein kinases such as ATM, ATR, Chk1, Chk2 and Wee1, and enzymes involved in DNA repair *per se* such as PARP-1 and MGMT. Numerous DDR inhibitor drugs targeting these and other components have been developed and are currently in pre-clinical or clinical trials, both as single agents and in combination with radiation and genotoxic chemotherapies, however validated therapeutic concepts for the rational deployment of these agents remain limited.

Checkpoints are molecular alarm mechanisms that signal the presence of damaged or unreplicated DNA and trigger cellular responses that minimise the risk of lethal or permanent genetic damage. Checkpoints control many diverse cellular processes, ranging from cell cycle progression, DNA replication and chromosome segregation, to cell senescence and survival. Checkpoints are acutely activated by cancer radio- and chemotherapies, where they favour tumour cell survival, however incipient cancer cells are also thought to experience spontaneous genotoxic stress arising from oncogene activation early during their evolution. In this situation, checkpoints may suppress tumorigenesis by promoting

cancer cell senescence or apoptosis. The role of checkpoints and DNA damage signalling in the evolution and therapy of cancer is therefore complex and context-dependent (Fig. 1).

Chk1 is a serine/threonine kinase that is activated in response to DNA damage and replication arrest, and plays a key role in multiple cell cycle checkpoint responses in tumour cells. Chk1-deficient tumour cells lack the capacity to arrest in G2 in response to ionising radiation, even at very high doses that induce massive amounts of DNA damage (Zachos *et al.*, EMBO J. 2003; 22: 713). Chk1 is also essential for the replication checkpoint functions that stabilise stalled replication forks, inhibit replication origin firing, and delay the onset of mitosis (the S/M checkpoint) when DNA synthesis is blocked. Failure of this latter checkpoint impels Chk1-deficient cells to enter mitosis prematurely with unreplicated DNA when replication is blocked, resulting in mitotic catastrophe and cell death (Zachos *et al.*, Mol. Cell. Biol. 2005; 25: 563, Zachos *et al.*, Dev. Cell 2007; 12: 247).

Importantly, Chk1 also plays a vital role in determining whether tumour cells are killed by exposure to radiation and anti-cancer drugs. Thus, Chk1-deficient tumour cells are extremely sensitive to ionising radiation (Zachos *et al.*, EMBO J. 2003; 22: 713) and replication-inhibiting anti-metabolites such as 5-fluorouracil (Robinson *et al.*, Oncogene 2006;

Figure 1 - DNA damage and cancer.

Natural ultraviolet (UV) and background radiation, endogenous reactive oxygen species (ROS), and genotoxic carcinogens in the environment are known to initiate carcinogenesis by damaging DNA and inducing mutations of oncogenes and tumour suppressor genes. Conversely, radiation and diverse genotoxic agents are used to induce DNA damage intentionally in the course of cancer therapy. Finally, oncogenes can induce spontaneous DNA damage early in the evolution of cancer. This is thought to play a role in suppressing malignant progression by diverting incipient cancer cells to terminal fates such as apoptosis or senescence. The nature of the DNA damage responses evoked in normal and tumour cells plays a key role in determining these distinct biological outcomes.

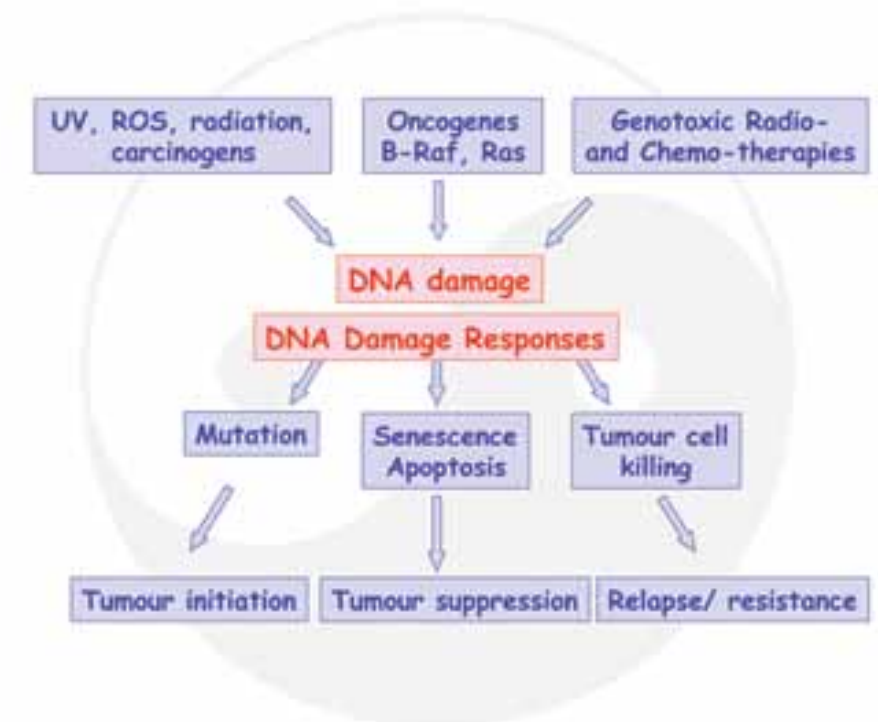
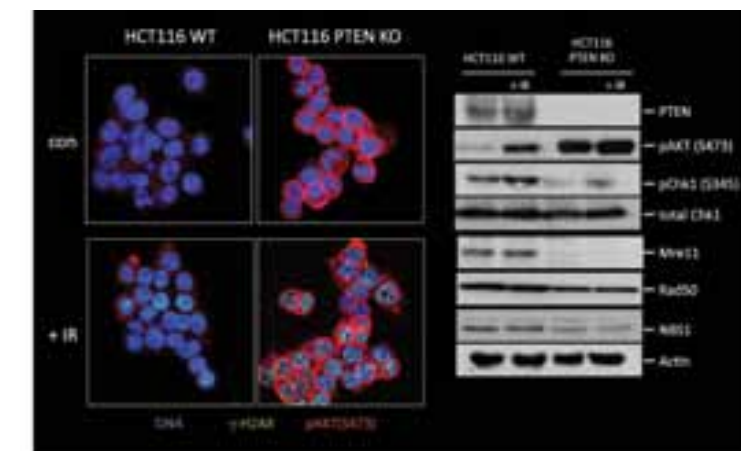


Figure 2 - AKT signalling inhibits Chk1 activation and DNA repair protein expression.

HCT116 colon carcinoma cells deleted for the PTEN tumour suppressor (HCT116 PTEN KO) exhibit very high basal levels of active, phosphorylated AKT (visualised in red) compared to the isogenic wild-type HCT116 parent cells (left panel). After irradiation (+ IR) similar levels of γ -H2AX foci (visualised in green), indicative of DNA damage, are induced in both cell types, however activation of the key checkpoint effector Chk1 is strongly suppressed as judged by induction of Chk1 S345 phosphorylation (right panel). HCT116 PTEN KO cells also show marked downregulation of Mre11 and to a lesser extent Rad50 and NBS1. These proteins form the MRN complex that is crucial for the recognition and repair of radiation-induced DNA damage.



25: 5359). Inhibition of Chk1 also enhances the potency of microtubule directed agents such as taxol, perhaps through its ability to regulate the critical spindle checkpoint kinase, Aurora B (Zachos *et al.*, J. Cell Biol. 2011; 195: 449). Importantly, newly-developed pharmacological Chk1 inhibitor drugs, some of which are currently in clinical trials (Smith *et al.*, Adv. Cancer Res. 2010; 189: 73), can reproduce these effects and enhance tumour cell killing by radiation and diverse chemotherapy agents.

Chk1 inhibition can also exert an inherent anti-cancer effect. Genetic ablation of Chk1 suppresses the formation of skin tumours induced by chemical carcinogens *in vivo* (Tho *et al.*, Oncogene 2012; 31: 1366), most likely because the keratinocyte stem cells of origin are crucially dependent on Chk1 for their survival or proliferation (Tho *et al.*, Oncogene 2012; 31: 1366). Evidence suggests that a particular subpopulation of proliferative stem cells, marked by expression of LGR5, gives rise to a

significant proportion of these tumours. Chk1 is also essential for the proliferation and survival of melanocytes and melanoma cells.

Interestingly, other biochemical pathways that are frequently deregulated in cancer cells, such as Cdk kinases and PI3K-AKT signalling, also impact on DNA damage responses at multiple levels (Xu *et al.*, J. Cell Biol. 2010; 190: 297, Xu *et al.*, Oncogene 2012; 31: 1086). In particular, deregulation of the PI3K-AKT pathway can both suppress Chk1 activation in response to DNA damage and inhibit the expression of important DNA repair proteins such as Mre11 (Fig. 2). Although speculative, inhibition of DNA damage responses by oncogenic signalling pathways could contribute to the remarkably high levels of mutation and genetic instability that are a cardinal, although as yet mechanistically unexplained, feature of the malignant state (Xu *et al.*, J. Oncol. 2012; 2012: 95124).

Publications listed on page 83



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¹ funded by Janssen
Pharmaceutica

² funded by CR-UK
Discovery Committee
and Astra Zeneca

³ funded by The Italian
Association for Cancer
Research

⁴ funded by CR-UK West
of Scotland Cancer Centre

Cancer cells must duplicate their genome, synthesise proteins and lipids, and assemble these components to form daughter cells. These activities require increased uptake of nutrients to be used as biosynthetic precursors and energy. However, rapid tumour growth surpasses the required blood supply and exposes cancer cells to conditions of metabolic stress. Therefore, cancer cells undergo many metabolic changes that support growth and survival. My laboratory combines analytical chemistry (metabolomics), cell biology, and biochemical approaches to study metabolic transformation. These technologies are not only important for understanding the basic biochemistry of cancer cells but they can inform us on future clinical management of cancer and may lead to new therapeutic approaches to target cancer-specific metabolic pathways.

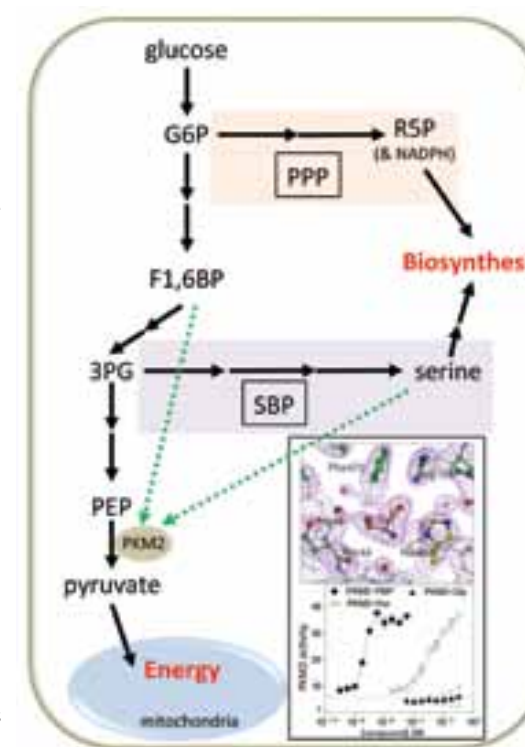
A novel regulatory link between glucose catabolism and serine biosynthesis

Cancer cell metabolism is exemplified by high glucose consumption and lactate production. Specifically, they overexpress the M2 isoform of the tightly regulated enzyme pyruvate kinase (PKM2), which controls the glycolytic flux (Chaneton and Gottlieb, Trends Biochem Sci 2012; 37: 309). Furthermore, cancer cells are highly dependent on *de novo* biosynthesis of serine and glycine from glucose (Possemato *et al.*, Nature 2011; 476: 346). PKM2, which catalyses the last step of glycolysis, has emerged as a potential regulator of several metabolic traits of cancer cells. When PKM2 is fully active the glycolytic rate is high, respiration is partially suppressed, and most of the pyruvate is rapidly converted to lactate. This normally occurs under glucose-rich conditions when the level of fructose 1,6, bisphosphate (FBP), the major activator of PKM2, is high (Fig. 1A). On the other hand, the reduction in PKM2 activity leads to a decrease in lactate production, which is associated with the accumulation of glycolytic intermediates upstream to PKM2, such as 3-phosphoglycerate (3PG), glyceraldehyde 3-phosphate (GA3P) and glucose 6-phosphate (G6P). Consequently, there is an increase in the

synthetic pathways branching from these metabolites (Fig. 1B). Lower PKM2 activity also increases respiration, and with it reactive oxygen species (ROS) are produced. However, high flux via the pentose phosphate pathway (PPP) provides anti-oxidants that counteract the mitochondria-generated ROS (Fig. 1B). Therefore, low PKM2 activity may be beneficial for rapidly growing cells, where glucose-derived carbons are diverted to anabolic processes required for cell growth and proliferation. Many allosteric regulators and post-translational modifications of PKM2 keep the enzyme under tight control and are required to support tumour growth. However, the mechanisms by which PKM2 coordinates high energy requirements with high anabolic activities, and supports cancer cell proliferation, are still not completely understood. In collaboration with Astex Pharmaceuticals (Cambridge, UK), we identified a novel rheostat-like mechanistic relationship between PKM2 activity and serine biosynthesis. We showed that serine can directly bind to and activate PKM2, even in the absence of FBP (Chaneton *et al.*, Nature 2012; 491: 458). Importantly, following serine deprivation, the intracellular activity of PKM2 is diminished leading to a change in energy metabolism to a

Figure 1 - Serine controls its own biosynthesis by directly activating PKM2.

The highly regulated M2 isoform of pyruvate kinase is expressed in cancer cells and helps balance energetic and biosynthetic demands. Abundance of glucose leads to high levels of the glycolytic intermediate fructose 1,6 bisphosphate (F1,6BP), which maintains active PKM2. However, PKM2 can be deactivated under growth conditions that require glucose-derived biosynthetic processes such as the pentose phosphate pathway (PPP) or the serine biosynthetic pathway (SBP). When serine is abundant, it is bound to PKM2 and keeps it fully active allowing for a quick glycolytic flux. However, when the steady state levels of serine drop below a critical point, PKM2 activity decreases and a shuttling of glucose-derived carbon to serine biosynthesis compensates for serine depletion. Insert: crystal structure of L-serine bound to the amino acid binding pocket of PKM2 and biochemical assays that demonstrated that both serine and F1,6BP are potent activators of PKM2 *in vitro*.



'fuel-efficient' mode where more pyruvate is diverted to the mitochondria for oxidative phosphorylation enabling more glucose derived carbon to be channelled into serine biosynthesis to support cell proliferation (Fig. 1B).

Glutamine catabolism regulates mTORC1 activity

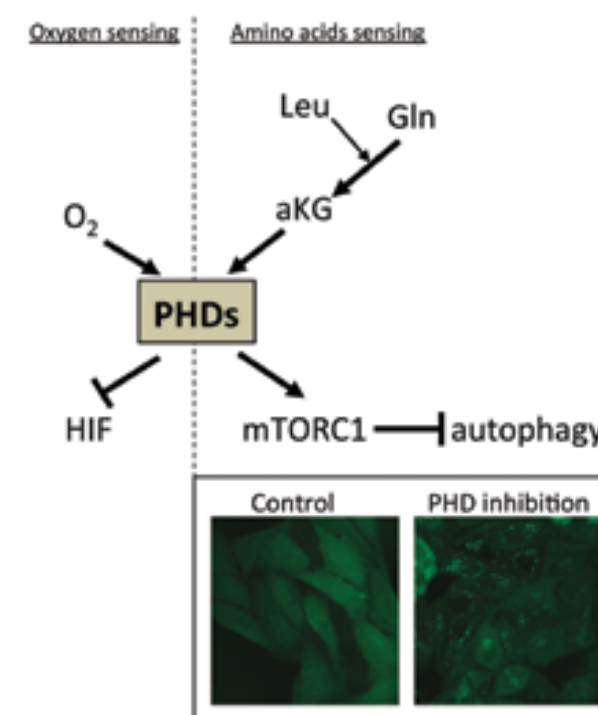
The serine/threonine kinase mTOR complex 1 (mTORC1) functions as a regulator of cellular metabolism. It integrates different stimuli emanating from growth factor signals, oxygen availability, energetic status and amino acid levels, and controls cell growth. Whereas the

mechanisms by which growth factors, oxygen and energetic status control mTORC1 activity are relatively well understood, the pathway by which amino acids activate mTORC1 is still under intensive investigation. Highly proliferating cells have an elevated demand for nutrients, especially glucose and glutamine. Glutamine is catabolised through glutaminolysis, the double de-amination of glutamine to alpha-ketoglutarate (aKG). We demonstrated that leucine and glutamine activate mTORC1 by increasing aKG production through glutaminolysis (Duran *et al.*, Mol Cell 2012; 47: 349). Furthermore, we showed that cell size and autophagy, two processes controlled by mTORC1, are also regulated by glutaminolysis. In addition, we mechanistically connected the glutaminolytic pathway to mTORC1 activity via the regulation of intracellular aKG levels. The prolyl hydroxylase domain proteins (PHDs) are oxygen and aKG dependent enzymes that function as cellular oxygen sensors. However, we now demonstrated that PHD activity also depends on aKG availability and that amino acid starvation (particularly of glutamine and leucine) leads to aKG depletion and to PHD inactivation (Duran *et al.*, Oncogene 2012; in press). Furthermore, pharmacologic or genetic inhibition of PHDs induced autophagy and prevented mTORC1 activation by amino acids. Therefore, PHDs sense not only oxygen but also respond to amino acids, constituting a broad intracellular nutrient-sensing network. We therefore linked amino acid availability and mTORC1 activity via aKG production and PHD activation (Fig. 2).

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Figure 2 - Glutamine-derived alpha-ketoglutarate (aKG) mediates mTORC1 response to amino acids.

Prolyl hydroxylases (PHDs) are the oxygen sensors of cells that mediate the oxygen-induced degradation of hypoxia inducible transcription factor (HIFalpha). However, their activity is also dependent on aKG availability. Glutamine is the major source of aKG and glutaminolysis is controlled by leucine. Hence, either leucine or glutamine deprivation leads to PHD inhibition even when oxygen is abundant. We further demonstrated that PHDs are required to maintain mTORC1 activity in response to glutamine and leucine, and the inhibition of PHDs decreases mTORC1 activity and induces autophagy (measured by LC3-GFP puncta) even in the presence of amino acids (Insert).





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Post-translational modification of ubiquitin (Ub) initiated by sequential actions of Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3) regulates diverse cellular processes, including signal transduction, cell cycle progression, apoptosis and gene transcription. Deregulation in the Ub pathway is often associated with human pathogenesis, including cancer. Our group uses X-ray crystallography and biochemical approaches to study the enzymes in the Ub pathway to understand their regulation, mechanistic functions and mutation-induced deregulation. We anticipate that the knowledge gained from our structural studies will assist in the development of selective therapeutic targets within the Ub pathway.

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes, namely E1, E2 and E3 (Fig. 1). E1 initiates the cascade by adenylating Ub's C-terminus in the presence of Mg²⁺ and ATP, followed by the formation of a covalent thioester intermediate with Ub. E1 then recruits an E2 and transfers the thioesterified Ub to the E2's catalytic cysteine. E3 plays a pivotal role in determining substrate fate. In general, E3 consists of an E2-binding module (HECT, RING or U-box domain) and a protein-protein interaction domain that confers substrate specificity. With this configuration, E3 recruits E2 thioesterified with Ub and substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, the Ub pathway consists of two E1s, ~30–40 E2s and ~600 E3s that collectively ubiquitinate thousands of different substrates. The Ub pathway has emerged as the target for therapeutic intervention. Velcade, a proteasome inhibitor, is the best example and is currently used for treating patients with multiple myeloma and relapsed mantle cell lymphoma. Recent studies demonstrated that inhibitors of NEDD8 E1 (Soucy *et al.*, Nature 2009; 458: 732) and E2 Cdc34 (Ceccarelli *et al.*, Cell 2011; 145: 1075) induced apoptosis and suppressed proliferation of human cancer cells, and are currently in

clinical trials. In addition, several E3 inhibitors have been developed. Our group is interested in understanding the regulation and mechanistic functions of RING E3s with particular focus on RING E3s that have been linked to cancer.

Cbl proteins and receptor tyrosine kinase signalling

Activation of receptor tyrosine kinase (RTK) signalling cascades is important for cellular processes such as cell proliferation, differentiation, migration and survival. Prolonged or aberrant activation of RTKs is commonly associated with cancer. Cbl proteins (Cbls), c-Cbl, Cbl-b and Cbl-c are RING E3s that negatively regulate RTKs, tyrosine kinases and a host of other proteins by promoting their ubiquitination and subsequent degradation by the proteasome or via endocytosis. Independent of their E3 activity, Cbls also function as adaptor proteins through interactions with a variety of proteins involved in diverse biological processes. In RTK signalling cascades, Cbls act as both positive and negative regulators: they propagate signals downstream of activated RTKs as adaptors and simultaneously ubiquitinate and promote degradation of the same RTKs as E3s. Recent studies showed that c-Cbl mutations are found in human patients with myeloproliferative

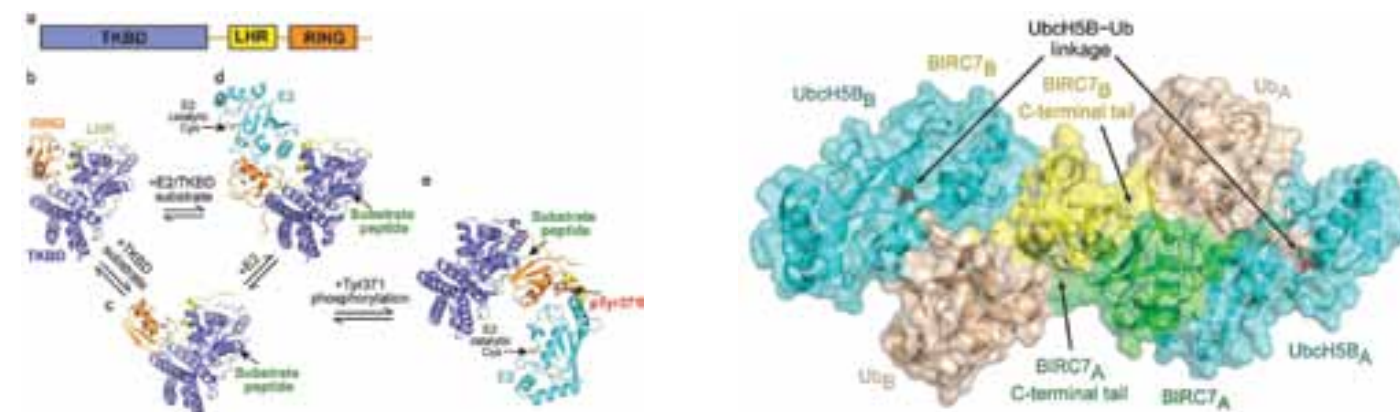


Figure 2 - c-Cbl conformational changes upon substrate binding, E2 recruitment and tyrosine phosphorylation.

(a) The conserved Cbl's N-terminal domain containing TKBD, LHR and RING domain. (b) Structure of native c-Cbl. (c) Structure of c-Cbl bound to a TKBD substrate peptide. (d) Structure of c-Cbl bound to an E2 and a TKBD substrate peptide (Zheng *et al.*, Cell 2000; 102: 533). (e) Structure of Tyr371 phosphorylated c-Cbl bound to an E2 and a TKBD substrate peptide. All structures are coloured as in (a). E2 is in cyan and TKBD substrate peptide is in green.

Figure 3 -Surface representation of BIRC7 RING dimer-UbcH5B-Ub structure.

The two crystallographic heterotrimers are differentiated with A and B subscripts.

diseases and these mutations abrogate E3 ligase activity and induce cell transformation (reviewed in Kale *et al.*, Cancer Res. 2010; 70: 4789). It remains elusive how Cbls are regulated and how these mutations could contribute to oncogenicity.

All Cbls share a highly conserved N-terminal SH2-containing tyrosine kinase-binding domain (TKBD), a linker helix region (LHR) and a RING domain followed by a variable proline-rich region (PRR) (Fig. 2a). The TKBD mediates substrate specificity by binding to proteins containing phosphotyrosine motifs commonly found in RTKs or tyrosine kinases whereas the PRR recruits proteins containing an SH3 domain. The LHR and RING domain play central roles in recruiting E2s and in mediating target ubiquitination. To gain insights into the regulation of Cbls, we determined three new crystal structures of c-Cbl: native c-Cbl, c-Cbl bound to a TKBD substrate peptide and c-Cbl phosphorylated at Tyr371 in complex with an E2 and a TKBD substrate peptide. Our structures and the existing structure of c-Cbl bound to an E2 and a TKBD substrate peptide (Zheng *et al.*, Cell 2000; 102: 533) reveal dramatic conformational changes in the LHR and RING domain (Fig. 2). We showed that in the unphosphorylated state, c-Cbl adopts an auto-inhibited conformation where its E2-binding surface on the RING domain is occluded in a competitive manner to reduce E2 binding, thereby attenuating c-Cbl's activity (Fig. 2b-d). We found that Tyr371 phosphorylation enhances c-Cbl's catalytic efficiency by 1400-fold. Tyr371 phosphorylation activates c-Cbl ligase activity by inducing dramatic LHR conformational changes that (1) enhance overall E2 binding affinity by eliminating auto-inhibition and forming a new phosphoTyr371-induced E2-binding surface; and (2) place the RING domain and E2 in proximity of the substrate-binding site (Fig. 2e). We showed that phosphoTyr371-induced conformational transition is required for EGFR ubiquitination. Together these results demonstrate how Tyr371 phosphorylation could transiently switch on Cbl's ligase activity to attenuate RTK signalling.

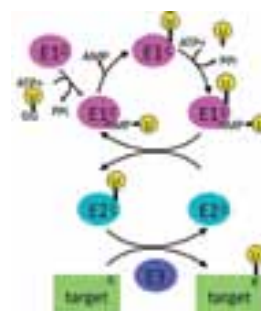
Tyr371 is one of the mutational hot spots (Dou *et al.*, Nat. Struct. Mol. Biol. 2012; 19: 184). We are currently investigating on how c-Cbl mutations could alter c-Cbl's conformational transitions and contribute to oncogenicity.

Mechanism of Ub transfer by RING E3

RING E3s recruit E2 thioesterified with Ub (E2~Ub) and substrate to facilitate the Ub transfer from E2 to the amino group of a substrate lysine. How RING E3s promote Ub transfer is unclear. To gain insight into RING domain-dependent ubiquitination, we determined the crystal structure of the human dimeric RING domain from BIRC7 in complex with the E2 UbcH5B covalently linked to Ub at its active site (UbcH5B~Ub) (Fig. 2). We stabilised the UbcH5B~Ub linkage by mutating UbcH5B's catalytic cysteine to serine, thereby forming a more stable oxyester linkage. Mutation of a conserved asparagine to alanine near the E2's active site further stabilised the UbcH5B~Ub complex in the presence of the BIRC7 RING domain. The structure reveals extensive noncovalent donor Ub interactions with UbcH5B and both subunits of the BIRC7 RING dimer. Notably the C-terminal tails of the BIRC7 dimer contact the donor Ub of the adjacent heterotrimer to stabilise the Ub configuration (Fig. 3). We found that this interaction is critical in optimising both k_{cat} and K_m for Ub transfer, explaining the importance of RING domain dimerisation. A conserved RING domain arginine residue coordinates the C-terminal tail of Ub to UbcH5B's active site cleft, where UbcH5B's active site residues prime the Ub's C-terminus for transfer. This work provided structural insights into how dimeric RING E3s recruit E2~Ub and optimise the donor Ub configuration for transfer (Dou *et al.*, Nat. Struct. Mol. Biol. 2012; 19: 876). Several BIRC7 RING domain residues that participate in UbcH5B~Ub binding are conserved in other RING E3s suggesting that other RING E3s may utilise a similar mechanism in optimising E2~Ub for transfer.

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Figure 1 - Enzymatic cascade for Ub modifications.





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Prostate cancer remains a major global health issue and a major cause of morbidity and mortality in men worldwide. Our laboratory continues to carry out cutting edge translational research aimed at developing new cancer medicines by combining cancer biology studies, novel pre-clinical *in vitro/in vivo* model systems and relevant strategies in developing novel therapies (Fig. 1).

Our research programme examines aspects of molecular control of cell survival (aberrant signalling involving activation of receptor tyrosine kinases and epigenetic silencing of Sprouty2), cell death (agents that may synergise microtubule binding chemotherapeutic agent such as docetaxel) and tumour invasion (MEK5-ERK5 signalling). In this report, we will highlight our recent findings that form the basis of our future strategy in developing personalised targeted therapy in prostate cancer.

Linking SPRY2 loss to castrate resistant disease via cytokine signalling

Even in castrate resistant prostate cancer (CRPC), androgen receptor continues to be important and indeed, recent development of novel anti-androgen therapy has resulted in modest positive impact on patient survival. Nonetheless, resistant disease quickly evolves and hence there remains an urgent need to develop additional novel effective treatments. Large-scale genomic analyses have identified lesions, such as sprouty2 (SPRY2) deficiency, which are frequently associated with treatment or CRPC. Using an integrated approach involving human genetic analysis, *in vitro* and *in vivo* functional studies, and murine cancer genetic modelling we found that SPRY2 deficiency drives development of CRPC by enhancing HER2 mediated signalling. Mechanistically, HER2 activation in turn drives the expression of IL6, which intriguingly can upregulate key steroid enzymes involved in androgen biosynthesis (via autocrine and paracrine actions). Furthermore, in surgically castrated mice bearing SPRY2 deficient tumour, enhanced levels of IL6 systemically associated with SPRY2 loss results in diminished visceral adipose tissue and increased intra-tumoral

androgen levels. Thus, IL6 supports the growth and survival of SPRY2 deficient tumours in a dual manner: (i) by providing cholesterol for *de novo* steroid biogenesis and (ii) by increasing the expression of steroid biosynthetic enzymes. Further, IL6 or IL6 receptor inhibition rendered SPRY2 deficient tumours sensitive to hormone ablation. Together, we identify the IL6 cytokine axis as a potential therapeutic target in host-tumour interactions supporting the 'parasitic' nature of treatment resistant cancer.

Investigation of interacting events in prostate carcinogenesis

Mutations in the tumour suppressor PTEN has been associated with the development and progression of clinical prostate cancer. Transgenic mice carrying homozygous deletion in *Pten* develop prostate cancer after a long latency (> 6 months) suggesting that additional genetic 'events' are required. Our hypothesis is that clinically relevant genetic events that cooperate with PTEN loss to drive prostate cancer can be identified using a functional forward genetic screen in a transgenic mouse model. We are currently performing a transposon-based insertional mutagenesis screen in mice. Mice expressing Cre recombinase specifically within the prostate epithelia are used to study the tumorigenic effects of simultaneous mutagenic mutations and homozygous deletion of *Pten*. Macroscopically visible primary prostate tumours and metastases will be dissected for histological verification. Sequencing of transposon integration sites in tumours will be performed and common insertion sites will be defined, thereby identifying genes and pathways implicated in prostate carcinogenesis. Subsequently, *in vitro* and *in vivo* assays as well

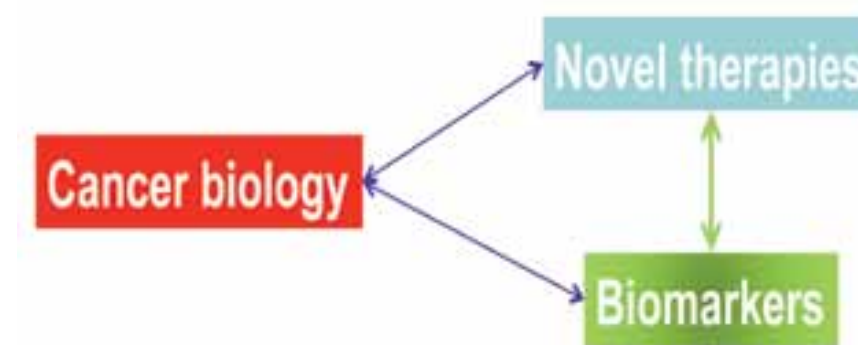


Figure 1
Illustrates the three key components of our group's research strategy.

as human prostate cancer specimens will be studied to investigate the significance of selected candidate tumour suppressor genes (or genetic events).

Developing pre-clinical translational models

We have also developed a robust protocol for performing primary culture of prostate tissue from our mouse models (Ahmad *et al.*, PNAS 2011; 108: 16392, Gao *et al.*, EMBO Mol Med 2012; 4: 776). Primary cultures and established cell lines from the selected mouse models with deletion of PTEN (or combined loss of PTEN and SRPY2) were carried out. This is an invaluable tool to validate candidate genes implicated in cancer progression as well as to carry out detailed biochemical and functional analysis (following manipulation of the target genes by genetic or chemical inhibitors as appropriate). Furthermore, we have successfully implanted murine prostate cancer derived cells directly into the prostate of mice as orthografts, with varying metastatic capabilities including the formation of lymph node and liver lesions. These orthografts will model primary and metastatic disease for both treatment naïve and treatment resistant scenarios, where treatment can be either androgen ablation or docetaxel treatment (Fig. 2). In addition, novel agents in the context of targeted therapy guided by the

underlying biology can also be incorporated. In this way, we will generate a panel of primary and metastatic lesions from the orthografts to model treatment naïve and treatment resistant states.

Nkx3.1^{CreERT2} inducible system

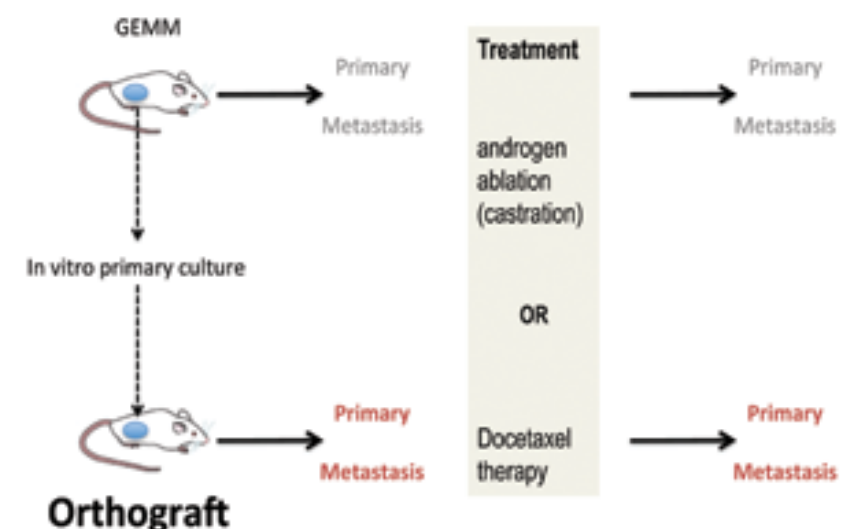
An important *in vivo* model for our lab is the *Nkx3.1^{CreERT2}* inducible system. This system allows us to choose the time point at which Cre recombinase is expressed, as its expression is placed under the control of a tamoxifen-inducible *Nkx3.1* promoter. This, in turn, enables inducible deletion of floxed alleles, such as the tumour suppressor gene *Pten*, or inducible activation of mutations in genes, such as the *β-catenin exon 3* mutation, in mice. An additional benefit of this system is that it enables a specific subpopulation of cells, castrate-resistant Nkx-expressing cells (CARNs) (Wang *et al.*, Nature 2009; 461: 495), to be studied in different prostate cancer models. Castration, prior to induction, will allow us to model CARN-specific changes in genes of interest associated with prostate cancer. In our lab, the inducible protocol has been optimised using *Nkx3.1^{CreERT2/+}; RFP/+* and *Nkx3.1^{CreERT2/+}; Catnb^{Δex3/+}* models. Recombination of genes in both models was assessed two weeks after induction. We are now applying this elegant model to study the interplay with abnormal signalling and the functional impact of the androgen receptor.

Taken together, we are making excellent progress in our understanding of important molecular events in aggressive (and treatment resistant) prostate cancer. Through this new knowledge and these novel model systems, we are now in the position to consider new therapeutic strategies based these events.

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Figure 2

Shows an overview of the use of transgenic mouse models to generate useful cell lines for orthograft studies, producing both primary and metastatic lesions. Following treatment (androgen ablation or chemotherapy), relapsed tumours will provide the basis for studying treatment resistant primary and metastatic lesions.





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The aim of our group is to understand the factors regulating cell viability in cancer. Since it is known that inhibition of cell death mechanisms is a common event in tumour development, this poses problems for many forms of chemotherapy that utilise cell death pathways, leading to drug resistance. We are investigating both known cell death regulators as well as searching for novel proteins and pathways that control cell viability and chemosensitivity. We envisage that the knowledge gained from our studies will be translated and lead to the improvement of existing clinical regimens or new targets for therapeutic intervention.

Autophagy in cell death and cancer

Multiple mechanisms within normal and tumour cells determine whether they live or die. One process that contributes to these cell fate decisions is called macroautophagy (referred to hereafter as autophagy). When translated from Greek, autophagy means 'self-eating' and is an evolutionarily conserved, membrane trafficking process that serves to deliver cellular constituents to lysosomes for degradation. Autophagy is characterised by the formation of cellular structures termed autophagosomes that encapsulate cytoplasmic cargoes such as misfolded proteins and damaged organelles (Fig. 1). Autophagosomes ultimately fuse with lysosomes to form another organelle termed an autolysosome in which the contents of the autophagosome are degraded into their constituent parts and delivered back into the cytoplasm where they can be either further catabolised or used in biosynthetic pathways.

Autophagy is active in the majority, if not all, cells at a basal rate as a mechanism to monitor and degrade misfolded proteins and damaged organelles thereby acting as a critical process to preserve cellular integrity. Depending on the context and in response to external and internal cues, the rate of autophagy, as well as the cargoes destined for degradation, can change to bring about selective effects within the cell. This is no more obvious than with the ways in which autophagy controls cell viability and

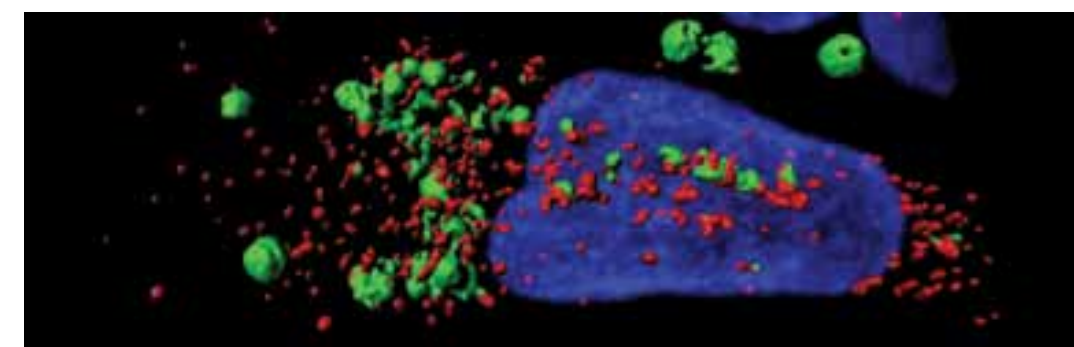
tumour development. In response to catabolic defects or nutrient deprivation, autophagy can be induced to degrade cellular components that can then be used to fuel further catabolic processes for the generation of essential energy. Since many tumours contain areas that are nutrient deprived this may be one mechanism by which autophagy promotes the survival of tumour cells and contributes to cancer progression. In contrast to these clear roles in cell survival, autophagy has also been shown to be a contributing factor to pro-death mechanisms in multiple settings. Although its role in pro-death mechanisms is not always clear, it may be one that contributes to the accumulating evidence that autophagy is a tumour suppressive mechanism in certain contexts.

Autophagy is required for tumour cell invasion

Since the role of autophagy in cancer appears to be context-specific, we have been interested in understating how the process contributes to the different facets and stages of tumour development. One critical step in the development of epithelial cancer is the ability of tumour cells to invade surrounding tissue, a characteristic that is considered to be one of the first stages in the development of metastatic disease. Through collaboration with Kurt Anderson (Beatson Institute) and Andrew Thorburn (University of Colorado), we examined

Figure 1 - Imaris software was used to generate a three-dimensional visualisation of autophagosomes (red) and lysosomes (green) from images of cells in which autophagy had been induced.

Cell nuclei were stained with DAPI. The image was generated with the help of Margaret O'Prey and Ewan McGhee of the BAIR.



the role of autophagy during tumour cell invasion in a three-dimensional organotypic model. To do this, we used a glioma cell line that contains a switchable short-hairpin RNA that targets the essential autophagy gene Atg12. Activation of the short-hairpin RNA in these cells results in autophagy-incompetent cells within a few hours. Using this line, we found that inhibition of autophagy did not affect the viability or growth of the glioma cells. Similarly, loss of autophagy did not affect cell migration in two dimensions. However, when cellular invasion into fibroblast-contracted collagen matrices was measured in three dimensions, it was clear that autophagy-deficient cells had a much lower capacity for invasion when compared to their wild type counterparts (Fig. 2). This indicates, therefore, that autophagy may have an additional critical role in cancer as the disease begins to spread throughout the body.

Analysing the role of autophagy in tumour development

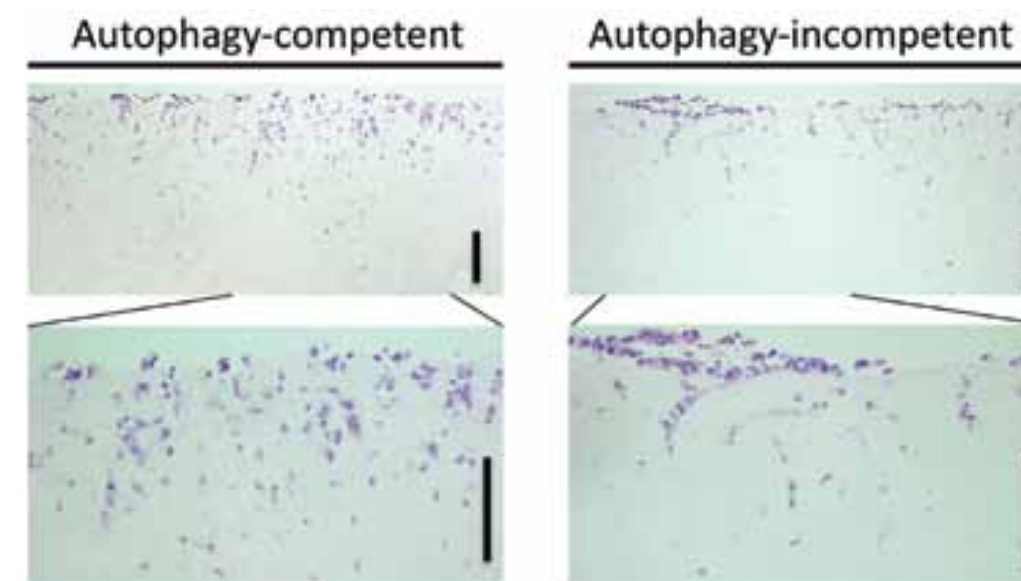
Our studies on the role of autophagy in processes connected to tumour development such as cell death and tumour cell invasion have naturally caused us to examine the role played by autophagy in mouse models of cancer that mirror the human disease. We consider that only with the information gained from these models

can we know where and when autophagy is either oncogenic or tumour suppressive such that autophagy-directed therapeutics can be successfully applied. Through collaboration with Owen Sansom (Beatson Institute), we have been analysing the role played by autophagy during tumour development in a variety of mouse models of cancer including pancreatic cancer and melanoma. To facilitate these studies, however, it was critical to establish an assay to analyze autophagy in tissues samples *ex vivo*. To do this we optimised a staining protocol for the autophagy marker LC3 using autophagy-competent and autophagy-incompetent cells that had been embedded in paraffin to mimic the state in which tissues are prepared for staining. Once optimised, this protocol was applied to *ex vivo* tissue and we could easily distinguish regions containing autophagosomes from those that did not. Moreover, we could also detect changes in autophagosome number following treatment with autophagic stimuli in a highly significant manner. The development of this assay will therefore be an extremely valuable tool in our ongoing studies to understand the role of autophagy in tumour development and therapeutic responses *in vivo*.

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Figure 2 - Glioma cells containing a switchable shRNA targeting the essential autophagy gene Atg12 were assayed for invasion into a fibroblast-contracted three-dimensional matrix.

Autophagy-deficient cells had impaired invasive potential when compared to autophagy-competent cells despite similar rates of migration in two dimensions.





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Our studies this year have focused on how wild type p53 can help cells to adapt to metabolic stress and how mutant p53 promotes invasion and metastasis. These studies are beginning to reveal interesting and sometimes unexpected activities for both wild type and mutant p53 that will be essential to understand if we are to target the p53 pathway for cancer therapy.

A new function for TIGAR in lowering ROS and cell death during hypoxia

p53 has well established functions in promoting apoptosis or programmed cell death in tumour cells exposed to genotoxic stress. Several years ago, we identified PUMA as a p53 target gene, showing that it plays a critical role in mediating this death signal. However, recent studies have revealed that p53 may also help to promote cell survival under some conditions. We identified TIGAR as a p53-inducible protein that functions to regulate glycolysis and allow for the generation of antioxidant capacity to help cell survival. TIGAR functions as a fructose-2,6-bisphosphatase (Fru-2,6-BPase), and through promotion of the pentose phosphate pathway increases NADPH production to help limit reactive oxygen species (ROS). This year, we have shown that under hypoxia or in response to HIF1α activation, a fraction of TIGAR protein re-localised to mitochondria (Fig. 1). The mitochondrial TIGAR formed a complex with hexokinase 2 (HK2), resulting in an increase in HK2 activity. The mitochondrial localisation of TIGAR was dependent on mitochondrial HK2 and hypoxia-inducible factor 1 (HIF1α) activity. Interestingly, this new function of TIGAR was independent of its Fru-2,6-BPase activity. However, both of these activities were able to contribute to the full function of TIGAR in limiting mitochondrial ROS levels and protecting from cell death during hypoxia, which could be essential to support tumour survival in an hypoxic environment. TIGAR therefore has several functions that allow it to control oxidative stress, and we will continue to explore how its functions can contribute to tumour suppression by p53, or whether it can also help to support tumour development.

Serine starvation induces stress and p53 dependent metabolic remodelling in cancer cells

While the role of p53 in promoting cell death during genotoxic stress has been extensively studied, the role of p53 in promoting cell survival during milder forms of stress (e.g. metabolic stress) has not been well explored. The pro-survival functions of p53 are likely to represent an important component of p53 function, not only in the regulation of cancer development but also in other aspects of health and disease, e.g. metabolic homeostasis, ageing and diabetes. Previous work showed that the presence of wild type p53 allowed cells to survive under conditions of glucose limitation, and we found over the past year that lack of p53 makes cells much more vulnerable to death in response to serine depletion (Fig. 2).

We showed that cancer cells rapidly utilise exogenous serine, significant amounts of which are used in the synthesis of glutathione and nucleotides. Serine deprivation triggered activation of the serine synthesis pathway (SSP) in both wild type p53 expressing and p53 null cells. In collaboration with Eyal Gottlieb's group, we found that serine activates PKM2 and maintains glycolytic flux. Reduction in intracellular serine levels resulted in a rapidly suppressed aerobic glycolysis, resulting in increased flux to the TCA cycle. These initial responses to serine starvation were not dependent on p53. However, serine depletion resulted in an activation of the p53-p21 response. The subsequent cell cycle arrest was found to be necessary for the promotion of cell survival, efficiently channelling depleted serine stores to glutathione synthesis and preserving cellular anti-oxidant capacity. Cells lacking p53

Figure 1 - Co-localisation of TIGAR with Tom20, a mitochondrial marker, in cells treated with DPCA to stabilise HIF1α. Cheung EC, Ludwig RL, Vousden KH. Mitochondrial localisation of TIGAR under hypoxia stimulates HK2 and lowers ROS and cell death. PNAS 2012; 109: 20491-6.

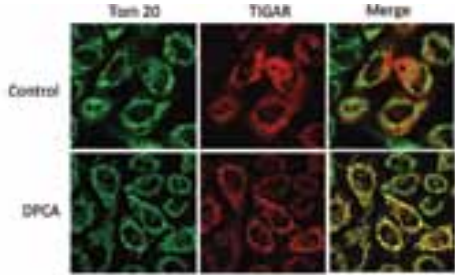
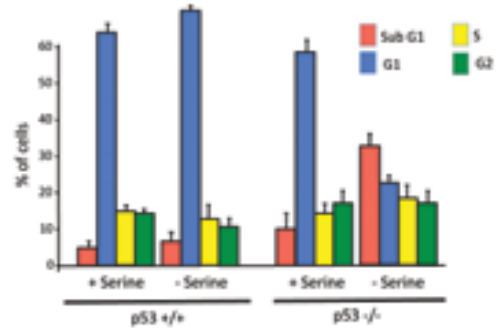


Figure 2 - Survival of p53 -/- but not p53+/+ HCT116 cells is compromised under conditions of serine depletion. Cells were fed or starved of serine for 48 hours, and cell cycle analysed by PI staining and FACS.



failed to complete the response to serine depletion, resulting in oxidative stress, reduced viability and severely impaired proliferation. The role of p53 in supporting cancer cell proliferation under serine starvation was translated to an *in vivo* model, where tumour cells lacking p53 grew significantly slower than p53-proficient tumours in mice on a serine-depleted diet. Our studies also demonstrated that serine uptake supports the Warburg effect, and that a serine deficient diet significantly enhanced the survival of tumour bearing mice. These results therefore suggest that serine depletion may have a potential role in anti-tumour therapy, particularly in cells lacking p53. Future studies will focus on examining the effects of serine starvation on the development of cancer in various genetically modified models.

Mutant p53 enhances MET trafficking and signalling to drive cell scattering and invasion

In previous years, we showed that tumour-derived mutant p53 proteins can enhance the ability of cells to migrate and invade, reflecting a gain of function of mutant p53s in promoting cancer metastasis. We have shown that this activity of mutant p53 reflects, in part, the ability to enhance Rab coupling protein (RCP) dependent cell surface receptor recycling. We previously found that mutant p53 can enhance the recycling and signalling from the epidermal growth factor receptor (EGFR). This year, we have extended these studies by identifying another cell surface growth factor receptor, MET, as an RCP-binding protein. MET is the receptor for HGF, and enhanced MET activity has been strongly linked to metastasis, and the ability of cells to scatter or disperse in cell culture models. Our studies confirmed that, as with the EGFR, mutant p53 expression promoted MET recycling. Mutant p53

expressing cells were more sensitive to HGF, leading to enhanced MET signalling, invasion and cell scattering that was dependent on both MET and RCP. Mutant p53 functions, at least in part, by binding a related protein, Tap63, and inhibition of Tap63 also led to cell scattering and MET-dependent invasion. However, in cells that express very low levels of Tap63, mutant p53 retained the ability to promote MET-dependent cell scattering. Taken together, our results show that mutant p53 can enhance MET signalling to promote cell scattering and invasion through both Tap63 dependent and independent mechanisms. Importantly, we were able to show that the expression of mutant p53 in cancers *in vivo* is associated with higher signalling through downstream effectors of the receptors. MET plays a predominant role in metastatic progression and the identification of mechanisms through which mutations in p53 can drive MET signalling may help to identify and direct therapy. However, our data also suggest that mutant p53 may promote the recycling of other cell surface receptors, and we will apply unbiased approaches to identify additional targets of RCP that may be modulated in mutant p53 expressing cells.

While we have shown that the ability to drive receptor recycling plays an important role in the gain of function activities shown by mutant p53, it is also clear that other pathways can be perturbed by mutant p53. In collaboration with Gerry Melino (MRC Toxicology Unit, Leicester), we showed that inhibition of p63, which is one consequence of mutant p53 expression, leads to the downregulation of expression of miR-205, thereby inhibiting the expression of markers of EMT such as ZEB1 and vimentin, and resulting in enhanced invasion. Importantly, we found that loss of p63/miR-205 components was associated with higher metastasis and worse prognosis in patients with prostate cancer.

Another collaboration with Jayantha Gunaratne and David Lane (A*STAR, Singapore) led us to the identification of Nardilysin as a mutant p53 binding partner, with specificity to binding the 273H form of mutant p53. This interaction promoted an invasive response towards HB-EGF that was not dependent on RCP or p63. Taken together, our work suggests that mutant p53 can function to promote invasion and metastasis through multiple mechanisms, and that each of these may provide novel targets for cancer therapy.

Publications listed on page 91



REGULATION OF CANCER CELL INVASION AND METASTASIS

THE BEATSON INSTITUTE FOR CANCER RESEARCH

Kurt Anderson - Tumour Cell Migration
Jeff Evans - Translational Cancer Therapeutics
Robert Insall - Cell Migration and Chemotaxis
Laura Machesky - Migration, Invasion and Metastasis
Jim Norman - Integrin Cell Biology
Michael Olson - Molecular Cell Biology
Owen Sansom - Colorectal Cancer and Wnt Signalling
Marcos Vidal - Drosophila Approaches to Cancer
Sara Zanivan - Vascular Proteomics



Group Leader
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Scientific Officer
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Graduate Students
Zahra Erami Rud Majani
Max Nobis
Meg Pajak

Our work is focused on development of fluorescence microscopy approaches to study the cellular and molecular dynamics of metastasis *in vitro* and *in vivo*. Metastasis is linked to mortality in most types of cancer, and a matter of intense investigation around the world. Metastatic invasion is challenging to study because it occurs randomly over large scales of time and space, and sensitively depends on features of the local tumour microenvironment. Our goal is to develop mechanistic read-outs of cell migration and apply them to mouse cancer models including pancreatic ductal adenocarcinoma, melanoma and breast. We were the first group to apply fluorescence recovery after photo-bleaching (FRAP) in mice to study the dynamics of the cell adhesion molecule eCadherin in tumour cell migration. More recently, we have demonstrated the first *in vivo* use of fluorescence lifetime imaging (FLIM) to study the activation of the small GTPase Rho during mutant p53 driven invasion of pancreatic cancer cells. This year, we expanded our imaging repertoire to include the pre-clinical use of PET, SPECT and CT.

Use of FRAP to characterise eCadherin dynamics in mouse tumours

We have previously used FRAP to show that the amount of eCadherin-GFP (eCad-GFP) stably incorporated into cell-cell adhesions inversely correlates with the level of cell migration: the greater the immobile fraction of eCad-GFP the lower the level of cell migration. We also demonstrated that *in vivo* FRAP of eCad-GFP could be used to characterise drug response at the molecular level. On the basis of these results and in collaboration with Douglas Strathdee we have generated a transgenic mouse capable of tissue specific expression of eCad-GFP, which will allow us to assess eCadherin dynamics in a variety of transgenic cancer models including pancreas, colon and melanoma. Preliminary data shows that eCad-GFP localises as expected to cell-cell junctions in healthy pancreatic tissue, primary KPC (pancreatic) tumours and epidermis. We

have begun to analyse the dynamics of eCad-GFP in primary pancreatic tumours using *ex vivo* analysis, and are working on the use of chambers to permit longitudinal *in vivo* analysis.

FLIM of Rho activation during mutant p53 driven invasion of pancreatic cancer cells

Pancreatic cancer is one of the most lethal forms of human cancer, with an overall five-year survival rate of less than 5%. Initiating Kras mutations occur in approximately 90% of human pancreatic ductal adenocarcinoma (PDAC), while p53 mutations arise in 50-75% of human pancreatic cancer. Previous work has demonstrated that p53 mutation, rather than loss, can drive metastasis in a mouse model of pancreatic cancer. There is evidence to suggest that the small GTPase RhoA may act downstream of p53 to drive invasion. Fluorescence resonance energy transfer (FRET) biosensors based on the Raichu model have

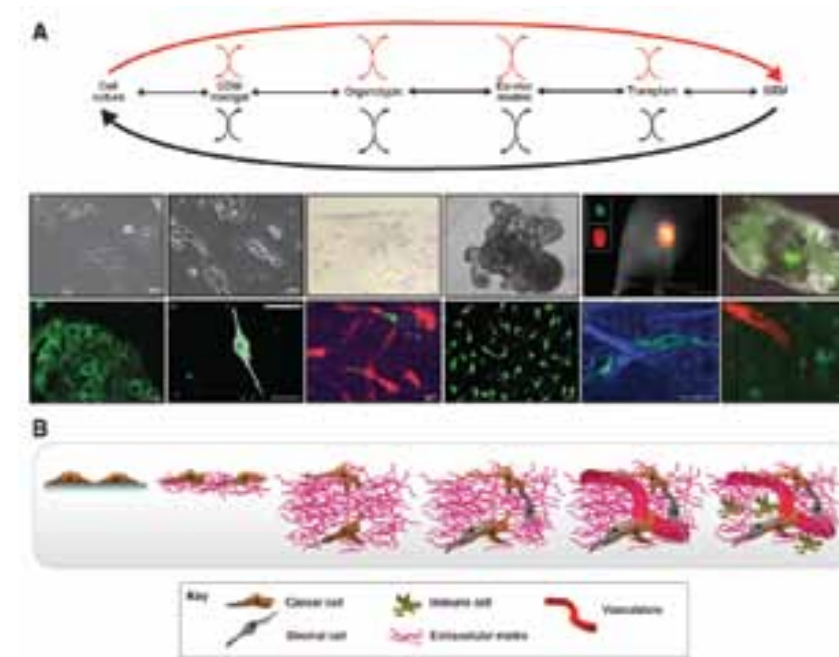
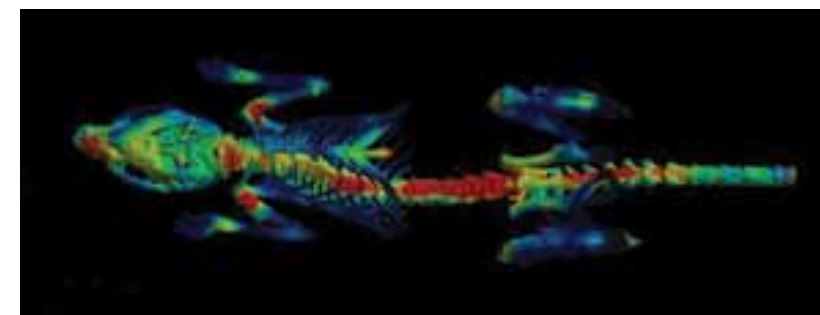


Figure 1
We employ a variety of experimental systems, from cell culture to mouse genetic models. Together, these methods comprise an experimental pipeline in which increasing biological fidelity is exchanged for decreasing experimental control and flexibility.

been adapted to study the activation of GTPases (including RhoA) in cell culture. The use of FRET-based biosensors is technically challenging and has not previously been applied to the study of protein dynamics in mouse tumours. However, there is much evidence to suggest that the behaviour of cells in tumours is critically different that of cells under artificial culture conditions, especially in response to drug treatment. Therefore, we have endeavoured to study the activation of RhoA and response to drug treatment *in situ* using cells derived from the Kras/p53 PDAC model.

We first established cell lines expressing GFP-RFP variants of the Rho-Raichu probe in both mutant p53 and p53 deletion PDAC cell lines. Probe response was characterised in a variety of *in vitro* experimental systems (see 'Imaging development pipeline') using drug treatments and a variety of dominant negative and constitutively active mutants. Mutant p53 cells invaded rapidly into an organotypic collagen matrix, whereas p53 deletion cells remained on the surface of the gel. FLIM of the two cell types indicated that invasion was associated with activation of RhoA, especially in the poles of invading cells, whereas RhoA was not active in non-invasive cells. Finally, the same cells were subcutaneously injected and allowed to form tumours in mice. Again, we found that

Figure 2 - Three-dimensional rendering of fused SPECT and CT data showing Tc-99m-MDP uptake within the skeleton of a healthy mouse.
Display of SPECT signal is limited to regions of overlap with bone determined by CT. Probe incorporation corresponds to regions of active bone remodelling (red and yellow colors, primarily joints and spine), and is used clinically to detect bone metastasis.



higher RhoA activity was present in the poles of mutant p53 cells. Dasatinib has been shown to prevent metastasis of mutant p53 driven cells from the pancreas to the liver, so we examined the effects of dasatinib treatment on RhoA activation. Interestingly, we found that dasatinib treatment selectively inhibited RhoA activation at the cell poles but did not reduce the average activation level within the cell body. This was the first demonstration of sub-cellular drug response *in vivo*. Current work is focused on the spatial and temporal regulation of Src in this model, again using a FRET biosensor.

Imaging development pipeline

Our goal is to study tumour cell migration at the molecular level in mouse cancer models using advanced imaging approaches. We have found that a wide variety of experimental systems are needed to bridge the gap between imaging in cell culture and imaging *in vivo*. Such systems are important for the characterisation of probes and validation of experimental approaches prior to undertaking *in vivo* work. For example, we used organotypic cultures, consisting of a rat-tail collagen gel contracted by primary human epidermal fibroblasts, as an intermediate model system to assess invasion of PDAC cells. This form of three-dimensional collagen matrix is more realistic than cell culture but more amenable to experimental manipulation than the tumour micro-environment. We are currently exploring the use of many intermediate experimental systems (Fig. 1) to build an experimental pipeline ranging from cell culture to transgenic models.

Pre-clinical imaging

This year saw the installation of an Albira (Bruker) tri-modal scanner for PET, SPECT and CT imaging of mice, extending our imaging range from sub-cellular molecular dynamics to whole body molecular imaging. This new work is being undertaken in close collaboration with colleagues from the Greater Glasgow and Clyde NHS Trust, including Gerry Gillen and the clinical team from the Glasgow PET Centre, Jonathan Owen from the Gartnavel Cyclotron Unit and Sally Pimlott from the Radiopharmaceutical Dispensary. Our goal is to develop the use of pre-clinical imaging both as an investigative tool for the study of disease and response to therapy, and as an operational tool for staging disease progression. Initial studies have focused on using CT and FDG-PET to identify both primary tumours and metastasis in pancreatic tumour models, and use of NaF-18 and Tc-99m-MDP to study bone metastasis in a prostate tumour model. Use of pre-clinical imaging and clinical probes will increase the translational utility of our mouse cancer models.

Publications listed on page 82



Group Leader

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Current strategies in the development of new therapies for malignant disease are based on exploiting our increasing understanding of the molecular and cellular basis of cancer development and progression. Work in our group is aimed at developing novel laboratory models that will allow us to understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours, and that will allow us to identify and characterise the signalling pathways that are deregulated at the early stages of pancreatic cancer as well as during the development and progression of the invasive and metastatic phenotype. Using these models, we will determine how potential anti-cancer agents might best be evaluated in subsequent clinical trials.

Infiltrating ductal carcinoma of the pancreas (PDAC) is the fifth commonest cancer and the fourth commonest cause of cancer deaths in the UK. Aggressive invasion and early metastases are characteristic of the disease, such that 90% of patients have surgically unresectable disease at the time of diagnosis. Furthermore, most systemic therapies are largely ineffective in advanced, inoperable disease, and the estimated five-year overall survival is less than 5%. Gemcitabine has modest clinical benefit and a marginal survival advantage in patients with advanced pancreatic cancer. Further small improvements in overall survival may be achieved with the addition of either erlotinib or capecitabine to gemcitabine in combination therapy regimens, and encouraging results have been observed in clinical trials with the FOLRIFINOX regimen. However, the median survival of patients with advanced pancreatic cancer remains poor. In addition, the majority of the selected patients who undergo potentially curative resection for small, localised lesions inevitably develop recurrent or metastatic disease, presumably due to the presence of distant micro-metastases at initial diagnosis. Adjuvant (post-operative) chemotherapy can improve outcome, although overall survival remains disappointing. Consequently, the development of more

effective strategies to treat pre-invasive pancreatic cancer, micro-metastatic disease and advanced disease is of paramount importance.

Our work aims to develop strategies for early detection of pre-invasive disease, to evaluate putative anti-invasive therapies with the aim of improving relapse-free and overall survival following resection of invasive pancreatic cancer, to determine the role of intra- and peri-tumoural inflammation in PDAC development and progression, and to develop a 'personalised medicine' approach to treatment of pancreatic ductal adenocarcinoma models from a range of genetic backgrounds that ultimately might influence the management of advanced disease in the human population.

Evaluation of putative anti-invasive therapies in pancreatic cancer models

One aim of our work is to determine how potentially anti-invasive agents might best be used in the clinical management of epithelial cancers. Specifically, we are defining the mode of action of anti-cancer drugs that are currently in clinical evaluation, and testing the hypothesis that these agents may have anti-migratory and hence anti-invasive and/or anti-metastatic properties. For this work we are using clinically

relevant, pharmacologically active anti-cancer agents as experimental tools.

Previously, we demonstrated that Src kinase may be a relevant target for therapeutic intervention following resection of PDAC and that Src activity is upregulated during progression to invasive PDAC in the *Pdx1-Cre, Z/EGFP, LSL-KrasG12D/+*, *LSL-Trp53R172H/+* model. We also showed that treatment with dasatinib, an inhibitor of Src family kinases, *in vitro* resulted in a dose-dependent inhibition of Src kinase activity, and in inhibition of migration and invasion of PDAC cells. Furthermore, we demonstrated that *in vivo* treatment with dasatinib from 10 weeks of age significantly reduced the number of *Pdx1-Cre, Z/EGFP, LSL-KrasG12D/+*, *LSL-Trp53R172H/+* mice with metastases compared with those treated with vehicle control. However, there was no improvement in survival when compared with vehicle control due to the morbidity of the primary tumour burden. Currently, we are pursuing these observations to determine if the administration of dasatinib, as monotherapy or in combination with gemcitabine, after potentially curative resection of the localised primary tumour can inhibit the development of metastases, and therefore reduce or delay disease recurrence and improve relapse-free and overall survival. We are also exploring whether the inhibition of metastases by dasatinib can be combined with local disease control of the primary tumour by gemcitabine, radiation therapy or both *in vivo* to improve survival in the *Pdx1-Cre, Z/EGFP, LSL-KrasG12D/+*, *LSL-Trp53R172H/+* model with locally advanced, unresectable disease but without evidence of visible metastatic disease. These observations and our ongoing studies have contributed to the development of a clinical trial that aims to improve progression-free and overall survival in patients with locally advanced disease by inhibiting the development of metastases.

Biological function of key tumour suppressor genes and oncogenes in PDAC

We have also developed a number of novel models with a range of genetic backgrounds in collaboration with Owen Sansom's group,

including those with targeted deletion of Pten, APC, c-Myc or p19ARF. These models will help us understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours. They will also allow us to identify and characterise the signalling pathways that are deregulated at the early stages of pancreatic cancer, and during the development and progression of the invasive and metastatic phenotype.

For example, we previously demonstrated that LKB1 haplo-insufficiency cooperates with KrasG12D to cause PDAC. Mechanistically, we showed that LKB1 deficient KrasG12D-induced tumours exhibited reduced levels of the tumour suppressors p53 and p21, and we proposed that this reduction in p53 and p21 allows KrasG12D bearing cells to overcome a senescent barrier to tumour formation. Moreover, haplo-insufficiency for p21 also synergised with KrasG12D to drive PDAC. We have extended these observations to show that activation of the PI3K/Akt/mTOR pathway, which occurs in approximately 20% of patients, is associated with poor prognosis in human PDAC patients. Loss or deficiency of Pten (and consequent activation of Akt) abrogates Ras-induced senescence and leads to acceleration of PDAC progression in laboratory models. Currently, we are using putative inhibitors of this pathway as clinically relevant laboratory tools to explore the activity of these agents in laboratory models with the relevant genetic background. These observations have led to the development of a clinical trial proposal to determine the safety and activity of mTOR inhibition in combination with chemotherapy in selected patients with advanced PDAC. Critical to these approaches will be identifying potential biomarkers in murine models and confirming the potential clinical relevance of these in human tissue microarrays, and developing robust assays for patient selection to enrich the clinical trial population and to demonstrate a biological, as well as a clinical, anti-tumour effect.

Publications listed on page 83



Group Leader

Robert Insall

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Olivia Susanto¹
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Peter Thomason

Clinical Research Fellow

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Graduate Students

José Batista
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David Knecht²

¹ from October

² until August

Crawling movement is fundamental to the behaviour of most eukaryotic cells. During tumour invasion the regulation of cell movement goes wrong - cancer cells invade other tissues, and spread through the blood and lymph systems to form metastases.

We are particularly interested in two related questions. The first is chemotaxis, in which external signals orient and attract cells, and which is increasingly seen as a fundamental cause of metastasis. The second is the regulation of the Arp2/3 complex, an assembly of proteins that promotes movement by driving the formation of actin microfilaments. Our approaches are mostly genetic, so the precise questions within that framework can vary substantially as our results redefine the areas in which we can most efficiently succeed.

Cell movement is a central part of biology, from conception to death. Embryos form their complexity and shape from the movement of layers of cells as well as the migration of individual cells through tissues. Adult wound healing and responses to infection require skin and immune cells to migrate to where they are needed. Metastasis, one of the most feared features of cancer, is caused when cells migrate out from a tumour into the blood, lymph or other tissues. Chemotaxis, the connection between chemical signals outside the cell and its movement, is important in these processes but remains very poorly understood. We are trying to understand cell movement – what drives it and most importantly how it is steered. Most mammalian cells use pseudopods made of polymerised actin to power migration. Our current research focuses on the proteins and pathways that control these pseudopods. We use three approaches. For genetic studies we use *Dictyostelium*, taking advantage of its ease of manipulation, and prominent cell movement and chemotaxis. To apply our knowledge to cancer, we use a range of melanoma cells cultured from tumours with different degrees of metastasis, and actual tumours from mouse models and, when possible, from fresh patient tissue. We also develop computational models

in collaboration with the Mathematics Department at the University of Strathclyde and the School of Engineering at the University of Glasgow. In the long term, we will work on anything that will help us to understand the conserved and fundamental mechanisms that drive cell movement.

Regulators of the Arp2/3 complex in actin dynamics

Actin drives nearly all cell movement, and the principal driver of actin is an assembly called the Arp2/3 complex. When turned on, the Arp2/3 complex causes new actin filaments to form and push against the membranes inside and at the leading edge of cells. We are particularly interested in the family of proteins that turns on the Arp2/3 complex.

One such regulator is SCAR. SCAR proteins - also called WAVES in mammals - are fundamentally important regulators of cell movement. Mutants in a variety of species show that SCAR is required whenever cells need to make large structures such as lamellipods; without SCAR such structures are either small and malformed, or completely absent. SCAR is found as part of a five-membered complex with the Rac-binding protein PIR121, Nap1, Abi and HSPC300. Without the other members of the complex, SCAR is rapidly removed from the cell. The prevailing view in the field is that all these proteins act simultaneously as a huge, homogenous complex that couples Rac signalling to actin polymerisation. However, our genetic studies in *Dictyostelium* show that each complex member may have a different function within the whole – with Nap1 controlling adhesion, and Abi inactivation of movement during processes like cytokinesis. These studies point to the SCAR complex being a nexus integrating multiple inputs - signalling, adhesion

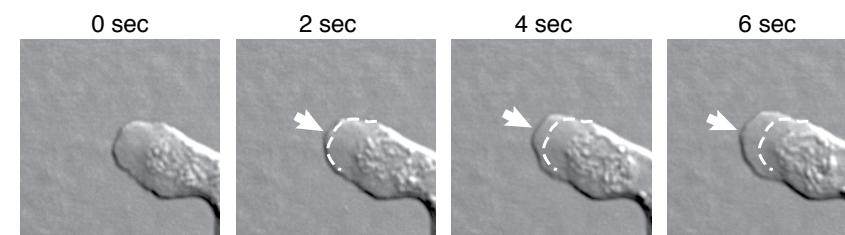


Figure 1. *Dictyostelium* cells in which the basic domain of SCAR/WAVE has been mutated. This causes SCAR/WAVE hyperactivation leading to excessively large, dominant pseudopods (from Ura S *et al.*, Curr Biol 2012; 22: 553).

and a cell's internal state - and coupling the integrated output to cell movement.

Our experiments are currently focused on identifying the activators and other proteins that regulate each component of the complex. SCAR and the other complex members are phosphorylated at multiple sites but the biological significance of these phosphorylations is not understood. We have shown that control of SCAR phosphorylation is centrally important – nearly all cellular SCAR is heavily phosphorylated but a rare dephosphorylated form seems to be particularly important. It is also very active in extending pseudopods, and very unstable, explaining its rarity. We are now seeking the phosphatases. We have also shown – very unexpectedly – that nearly all the same signals regulate the localisation of SCAR and its relative WASP. We are now seeking to understand what those signals are, and how they connect to upstream signalling molecules such as receptors and G-proteins.

We have also investigated the roles of another, poorly understood regulator of the Arp2/3 complex, WASH. WASH is important for cancer cell invasion, and plays a fundamental role in the sorting of vesicle contents. Gene knockouts in *Dictyostelium* have defects in the intracellular transport of vesicles, specifically in the retrograde sorting of proteins such as the vacuolar ATPase from lysosomes. This makes mutant cells retain huge amounts of labelled dextran long after normal cells have expelled it. We are using this observation to drive genetic screens for new WASH interactors. We have also found that one subunit of the WASH regulatory complex – FAM21 – works differently from the others. Instead of making actin on vesicles, it couples the WASH complex to the cell's cytoskeleton and thus allows it to be recycled. We are now focussing on the mechanism that connects FAM21 to the actin cytoskeleton.

Mechanisms underlying chemotaxis

Chemotaxis, migration towards chemical signals, is emerging as a major driver of tumour metastasis. We have shown that chemotaxis in *Dictyostelium* cells works by a different mechanism than that which is usually described. Pseudopods are constantly generated in random directions, then the ones that point in

the best directions are selected and maintained. We are now performing similar studies into the migration of cancer cells. We are using high-resolution, three-dimensional microscopy to ask questions including whether human tumour cells use the same mechanism as *Dictyostelium* for chemotaxis and which properties are most important to the metastatic phenotype. Parameters such as cell speed are widely used but are broadly irrelevant in our new model. Instead, we measure the rate at which pseudopods are made and change shape, the instantaneous velocity at which pseudopods move, and the regulation of pseudopod retraction. We also want to identify which proteins are used to regulate tumour cell chemotaxis and whether they are the same for all metastatic cells, and if not, how broad the range is.

The work involves the examination of a large range of tumour cell lines and primary cultures, using a number of techniques including quantitative DIC (differential interference contrast), immunofluorescence and live-cell confocal microscopy. We have also used chemotaxis chambers of our own design to show that melanoma cells are exquisitely chemotactically sensitive. They can navigate up a gradient of serum with unprecedented accuracy, irrespective of their stage – early melanomas are slower but still highly chemotactic. We are now working on the molecular details of the attractant in serum and the chemotactic receptors that detect it.

We are collaborating with the University of Strathclyde Mathematics Department to make computational models representing moving cells. Our models already faithfully mimic some aspects of the movement of *Dictyostelium* cells. We are now using the model to test our predictions about the underlying mechanisms of chemotaxis, and the proteins that are involved. We are showing that chemotaxis is mostly likely mediated by several dissimilar mechanisms acting in parallel, including regulated pseudopod growth, pseudopod retraction and the control of adhesion. We can also determine which components can safely be ignored, which is increasingly important – hundreds of genes are newly associated with motility and invasion every year so we urgently need a mechanism to determine which are the most important.

Publications listed on page 84



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Yafeng Ma
Xinzi (Amber) Yu²
Tobias Zech

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Hao Ran Tang^{5,6}
Emma Woodham

Visitors
Arthur Charles-Orszag
Amr Hosni
Sina Sender
Mark Sprott

¹ currently a new lecturer at Hull University
² funded by the AICR
³ MRC Fellow
⁴ funded by the British Heart Foundation
⁵ currently a postdoc at the Paterson Institute, Manchester
⁶ until September

Cancer metastasis requires cells to break away from the primary tumour and to survive in a variety of environments before settling into a new site. We aim to gain insights into mechanisms of metastatic spread by determining the roles of key actin cytoskeletal proteins, such as the actin filament nucleation machinery and the bundling protein fascin, in cancer cell invasive and migratory behaviour. The actin cytoskeleton is important not only for cell strength and migratory capacity but also for adhesion-dependent survival, membrane trafficking and establishment of polarity. We aim to understand how various actin regulators control these processes and thus contribute to tumour initiation, growth and spread as well as to fundamental mechanisms of mammalian development.

Role of actin nucleating proteins in cell migration, invasion and membrane trafficking

The Arp2/3 complex is the major inducer of actin filaments in response to extracellular signals. The Wiskott-Aldrich Syndrome Protein (WASP) family proteins transmit signals to the Arp2/3 complex to trigger actin assembly (Fig. 1: Scar/WAVE, N-WASP, WASH, NHS-1A, WHAMM and JMY). Each of these proteins is regulated differently and one of our aims is to understand the mechanisms of regulation and the involvement of these proteins in invasion and metastasis of cancer as well as their normal cellular function. WASP family proteins regulate actin assembly in multiple essential and pathological cellular processes, such as endocytic trafficking, protrusion of lamellipodia and filopodia, assembly of invasive structures such as podosomes and invadopodia, and cell division.

This year, postdocs Amber Yu and Tobias Zech identified the actin nucleation promoting protein N-WASP as an important regulator of invasion and migration of breast cancer cells in a three-dimensional matrix (Yu and Machesky, PLoS1 2012; 7: e30605, Yu, Zech *et al.*, J Cell Biol 2012; 199: 527). We used a human tissue array in collaboration with Karin Oien (University of

Glasgow) to show that N-WASP expression is upregulated in invasive breast cancer. We found that while N-WASP was not important for migration of breast cancer cells across a rigid two-dimensional surface, loss of N-WASP led to severe invasion defects in a three-dimensional matrix. Since N-WASP depleted cells showed severe defects in collagen degradation assays, we investigated a role for N-WASP in delivery of the major transmembrane collagenase MT1-MMP to the plasma membrane. N-WASP depletion did not affect overall levels of MT1-MMP or the total amount of MT1-MMP detectable on the surface of cells but it did reduce the polarised trafficking of late endosomal vesicles containing MT1-MMP into invasive pseudopodia. Furthermore, photobleaching experiments revealed that MT1-MMP appeared to be captured in invasive pseudopods and stabilised there by the actin cytoskeleton. Capture of MT1-MMP was reduced in N-WASP depleted cells, indicating that cytoskeletal assemblies, which we call ‘actin hotspots’, were likely sites of MT1-MMP capture and stabilisation for effective matrix degradation (Fig. 2).

In contrast to the important role of N-WASP in invasive migration, we find that Scar/WAVE

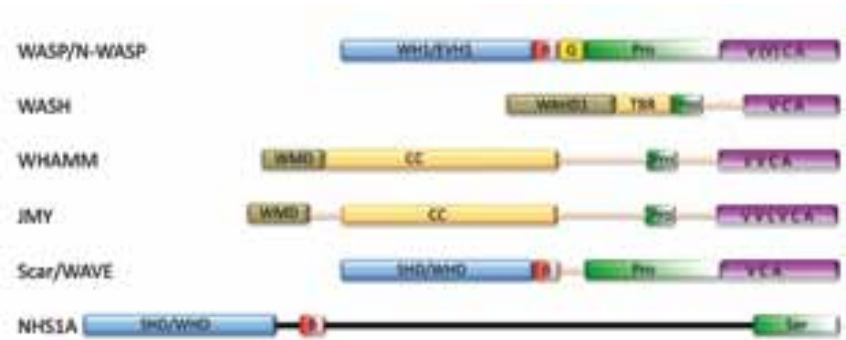


Figure 1

Figure 1 - The WASP-family in mammals
Domain structure of selected WASP and Scar/WAVE related proteins: WH1/EVH1 WASP-homology 1; B basic; G GTPase binding; Pro proline rich; VCA denotes the region of the protein that interacts with and activates the Arp2/3 complex; WAHD1 WASH homology 1; TBR tubulin binding region; WMD WHAMM homology domain; SHD/WHD Scar WAVE homology domain; Ser serine rich.

Figure 2
Matrix metalloprotease is delivered into invasive pseudopods and captured in actin hotspot networks
Drawing by Tobias Zech, from Yu, Zech *et al.*, JCB 2012

Figure 3
Melanoblasts move between keratinocytes with long protrusions
From Li and Machesky, Small GTPases, 2012

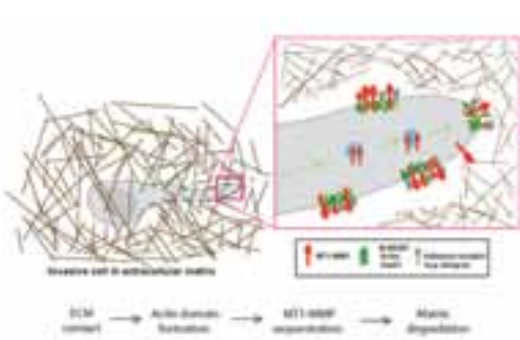
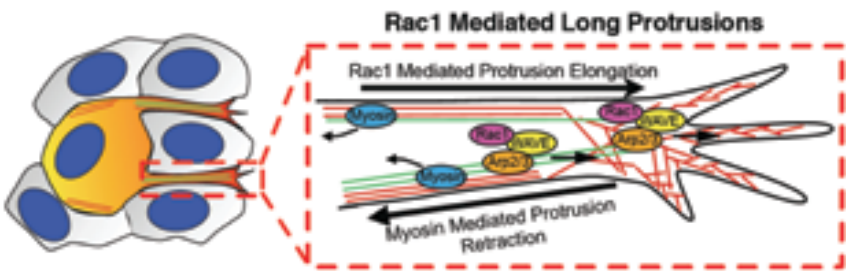


Figure 2

complex depletion can make some cells more invasive. Student Hao Ran Tang found that loss of Scar/WAVE complex could promote increased recruitment of N-WASP and Arp2/3 complex to cell leading edges and thus promote invasion into a three-dimensional extracellular matrix. He further showed that this enhanced three-dimensional migration was dependent on increased activation of focal adhesion kinase and formation of hybrid actin structures containing components of focal adhesions and invadopodia. Clearly, the interplay between the different WASP family actin nucleation promoting proteins needs further exploration with respect to their role in three-dimensional invasive migration.

Fascin, a migration and invasion promoting protein, is a potential therapeutic target
Fascin is an actin-bundling protein implicated in filopodia assembly and migration. Over 50 studies correlate upregulation of fascin expression with worse grade, stage and/or metastatic status of epithelial cancers. We (Yafeng Ma, Ang Li, Richard Stevenson and Heather Spence) are currently collaborating with Martin Drysdale (Drug Discovery) as well as MRC Technology and Cancer Research Technology to identify compounds that target fascin and cancer invasion. Martin’s group has been screening for small molecules that target fascin and we now have several candidates to take forward into further functional studies. Clinical research fellow Richard Stevenson is developing fascin as a potential biomarker in human cancer studies together with Jeff Evans. Student Ang Li has been studying the role of fascin in pancreatic cancer metastasis using a KRasG12D p53R172H model of PDAC (pancreatic ductal adenocarcinoma; Hingorani

et al., Cancer Cell 2005; 7: 469) and postdoc Yafeng Ma is studying the role of fascin in melanoblasts and melanoma. These models will be useful for preclinical testing of the role of fascin in migration and metastasis. Additionally, scientific officer Heather Spence and postdoc Amber Yu have performed siRNA screens together with Lynne McGarry and Emma Shanks (RNAi Screening) to identify additional targets for invasion and metastasis and we now have several candidates to follow up.

Control of actin-based motility in melanoblasts in vivo by Rac1 and implications for melanoma

The small GTPase Rac1 is often activated during tumour progression and correlates with increased cell motility and invasive behaviour. Rac1 is well studied *in vitro* in tissue culture cells but we are only beginning to understand its role *in vivo* in mammals. We (Ang Li, Yafeng Ma and Xinzi Yu, and collaborators; Li *et al.*, Dev Cell 2011; 21: 722) deleted Rac1 in the melanocytic lineage to determine the role of Rac1 and its downstream targets during embryonic development. We further went on to show a role for Rac1 in melanocyte and melanoma proliferation downstream of activated NRas and a role in spread of melanoma to lymph nodes (Li *et al.*, J Invest Dermatol 2012; 132: 2610). Together with students Ang Li and Hannah Schachtner, and collaborators Douglas Strathdee and David Stevenson (Transgenic Technology), we developed a reporter mouse to express Lifeact (Schachtner *et al.*, Eur J Cell Biol 2012; 91: 11) in response to Cre deletion. When we expressed Lifeact in melanocytic cells moving in the epidermis, their long protrusions were actin-driven and Arp2/3 complex-dependent even in the absence of Rac1. Together with new student Emma Woodham, we are continuing to work toward a molecular model of how melanoblasts move in the epidermis (Fig. 3) and will continue to build on this framework. We are further seeking to understand the signals and machinery that control actin-based protrusions and the parallels between melanoblast migration and melanoma metastasis.

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Integrins are cell surface receptors that engage the extracellular matrix and help cells to move. Cancer cells use integrins in order to migrate away from primary tumours to form metastases. Like many other receptors, integrins are internalised (or endocytosed) from the cell surface into endosomes. Once within endosomes, integrins can either be sorted for degradation or can recycle to the cell surface via a number of different routes. We have found that a range of drives to cancer metastasis operate by altering the way in which integrins recycle to the plasma membrane. Moreover, we now know that components of the integrin recycling machinery influence clinical outcomes in patients with pancreatic and breast cancer. We will continue to assemble a detailed molecular picture of integrin recycling and how this contributes to metastasis, and we hope to reveal which are the most promising components of the pathway to target for cancer therapy.

Mutant p53 drives cancer invasion via diacylglycerol kinase-mediated trafficking of α5β1 integrin (collaboration with Vousden lab)

We have previously shown that oncogenic mutants of p53 drive tumour cell invasion by promoting the association of the Rab GTPase effector protein, Rab-coupling protein (RCP) with α5β1 integrin. RCP contains a C2 domain that is known to bind to the acidic lipid metabolite phosphatidic acid (PA). PA can be produced by an enzyme called diacylglycerol kinase alpha (DGKα), which phosphorylates diacylglycerol to PA. We have shown that DGKα and the binding of RCP to PA is required for integrin vesicles to be tethered at the extreme front of invading cells, and for allowing RCP-dependent α5β1 recycling and the resulting invasiveness of tumour cells (Fig. 1a). We are currently using transgenic mice that have targeted disruptions in the genes for RCP and/or DGKα to evaluate the role of the mutant p53/RCP/DGKα axis in controlling invasion and metastasis in mouse models of cancer, and are

intending to combine these transgenic approaches with in vivo imaging to gain a clearer picture of the contribution of integrin recycling to the dissemination and spread of cancer.

Phosphoproteomic approach to identify new kinase substrates that control integrin trafficking

We have previously shown that protein kinase D (PKD) mediates signals from PDGF-R to control αvβ3 integrin recycling. We have now deployed a phosphoproteomic approach to show that Rabaptin-5, a Rab5 effector in endosomal membrane fusion, is a PKD substrate in the PDGF-R response to integrin recycling. PKD phosphorylates Rabaptin-5 at Ser407 and this is both necessary and sufficient for PDGF-dependent short-loop recycling of αvβ3 that in turn inhibits α5β1 integrin recycling. Rab4, but not Rab5, interacts with phosphorylated Rabaptin-5 toward the front of migrating cells to promote delivery of αvβ3 to the leading edge, thereby driving persistent cell motility and

Figure 1 - Kinase control of integrin recycling. Upper panel: Expression of mutant p53²⁷³ᴴ promotes recruitment of RCP to endosomal α5β1. Association of α5β1 with RCP is not a DGK-dependent event and does not require RCP's PA-binding C2 domain. RCP/integrin recycling vesicles can then move up and down the pseudopod shaft, and the role of DGKα is to generate PA species that enable the tethering of RCP at pseudopod tips - an event that requires RCP's C2 domain. Inhibition or silencing of DGKα most significantly affects the interconversion of 38:4 DAG to 38:4 PA. The 38:4 species of PA is therefore depicted as the most likely to be involved in tethering RCP at pseudopod tips. Lower panel: protein kinase D1 (PKD1) associates with the cytodomain of β3 integrin at the early endosomal membrane. PKD1 phosphorylates Rabaptin-5 at Ser⁴⁰⁷, which promotes recycling of αvβ3. These events are required to support persistent cell migration, and the anterior positioning of Rab4-positive early endosomes as cells migrate into wounds.

invasion that is dependent on this integrin. Consistently, disruption of Rabaptin-5 Ser407 phosphorylation reduces persistent cell migration in two-dimensions and αvβ3-dependent invasion. Conversely, invasive migration that is dependent on α5β1 integrin is promoted by disrupting Rabaptin phosphorylation. These findings demonstrate that the PKD pathway functions to interface receptor tyrosine kinase and integrin signalling through Rabaptin-5 phosphorylation (Fig. 1b).

Use of SILAC mass spectrometry to identify new components of the invasive 'recyclome' (collaboration with Zanivan lab)

We have used gene expression arrays to identify rab17 as a gene whose expression must be suppressed in order for MAP kinase signalling pathways to drive invasive migration. To identify which of the receptor cargoes of Rab17 are responsible for its ability to oppose tumour cell invasion, we have developed a novel stable isotope labelling in cell culture (SILAC) approach. By using this quantitative mass spectrometry approach in combination with membrane purification methods, we are able to compare the distribution of proteins between the plasma membrane and recycling endosomes under control conditions and when Rab17 levels are suppressed. By careful analysis of peptides from proteins that are differentially trafficked, we have been able to determine that Rab17 affects α6 integrin trafficking (Fig. 2) in a way that depends on the particular splice variant of the integrin in question. Thus, this novel approach has produced the first description of the role of integrin alternative splicing in their intracellular trafficking. And we anticipate that further characterisation of the invasive 'recyclome' will identify more new receptor cargoes that are key to invasion, and that these may represent new targets at which to aim anti-cancer drugs.

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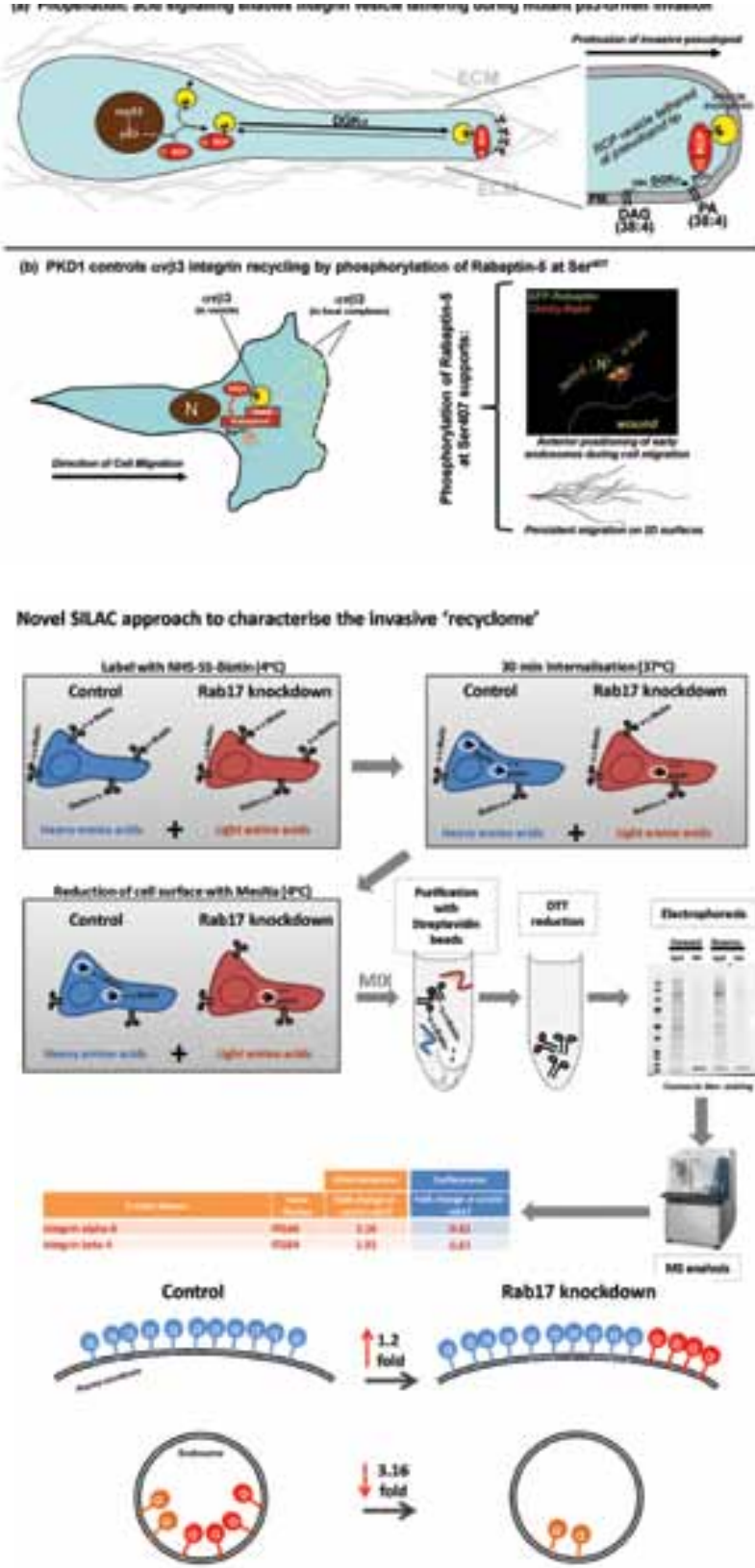


Figure 2. Stable isotope labelling in cell culture (SILAC) approach to identification of novel components of the invasive 'recyclome'. Control and Rab17 knockdown cells are labelled with heavy and light amino acids respectively, and the surface proteins then tagged with HNS-SS-Biotin. Internalisation is allowed to proceed for 30 min at 37°C, then biotin-tags remaining at the cell surface are removed by treatment with the membrane-impermeant reducing agent, MesNa. The biotinylated endosomal proteins are then enriched by streptavidin affinity chromatography and analysed by mass spectrometry. This analysis has shown that a splice variant of the α6 integrin (and its cognate partner β4 integrin) move from endosomes to the plasma membrane following knockdown of Rab17. Given that Rab17 is an anti-invasive protein, these data indicate that certain splice variants of α6β4 are recruited to the cell surface as cancer cells invade.



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A major function of the actin cytoskeleton is to provide the structural underpinning that gives a cell shape and mechanical strength. The actin cytoskeleton is dynamic, undergoing constant rearrangement and reorganisation in response to external factors. Alterations to the cytoskeletal architecture have significant consequences on the entire cell - such as morphology, cytokinesis, adhesion and motility - but also at the subcellular level. As well as these structural functions, the actin cytoskeleton additionally acts as a scaffold, bringing together proteins that not only contribute to cell shape but also proteins that have diverse activities such as signal transduction and gene transcription. The scaffolding function is not limited to the spatial organisation of protein complexes as the actin cytoskeleton may also recruit or stabilise specialised membrane domains.

The Rho family of small molecular weight GTPases consists of 20 members. Regulation of the actin cytoskeleton is a common activity of this group of proteins, although the fine details of how they affect actin structures and the proteins that mediate these responses differ between individuals. Now an intensive area of research, Rho GTPases have been shown to be major players in many diverse biological processes.

Actin-regulatory LIM kinase 2 in colorectal cancer

The LIM kinase family consists of two members; LIM kinase 1 (LIMK1) and LIM kinase 2 (LIMK2), which play central roles in Rho GTPase regulation of the actin cytoskeleton by phosphorylating cofilin proteins on Ser3 and inactivating their filamentous actin (F-actin) severing activity. By virtue of their situation within several important Rho family signal transduction pathways, LIM kinases act as central network hubs in the regulation of cytoskeletal dynamics and have been proposed to be attractive potential anti-cancer drug targets. In contrast to other cancers such as

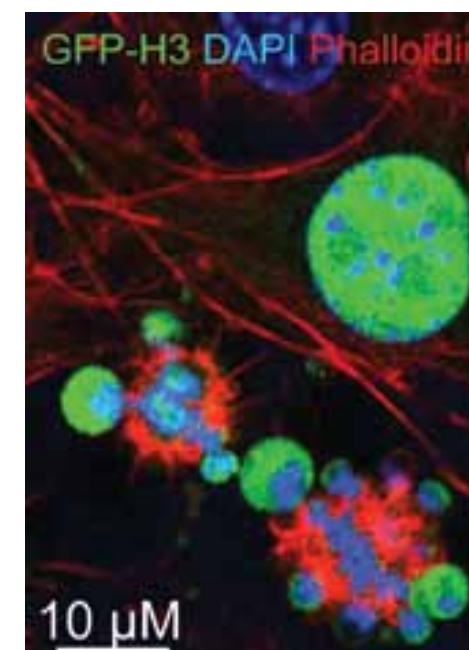
breast and prostate in which LIM kinase expression has been reported to be increased, we found that LIMK2 expression is reduced in intestinal tumours from genetically modified mice, and in human colorectal cancer cell lines and tumours. Protein levels were progressively lower with advancing tumour grade, and reduced LIMK2 expression or substrate protein phosphorylation was each associated with shorter patient survival times. Using genetic methods, we identified a repressive role for LIMK2 in intestinal stem cell proliferation in *Drosophila* and mice. Crossing LIMK2 knockout mice with colorectal cancer prone mice increased intestinal tumour size and colon tumour incidence. These data support the hypothesis that there is selective pressure for reduced LIMK2 expression in colorectal cancer to relieve negative constraints imposed upon gastrointestinal stem cells.

Actomyosin contractility in acute apoptosis

Apoptosis is a fundamental homeostatic mechanism essential for the normal growth, development and maintenance of every tissue in the body. Dying cells have been defined as

Figure 1. Histone H3 translocates from nuclei to blebs during apoptosis.

Fluorescence micrograph of apoptotic NIH3T3 cells expressing GFP-Histone H3, stained for DNA with DAPI and filamentous actin with Texas Red-conjugated phalloidin.



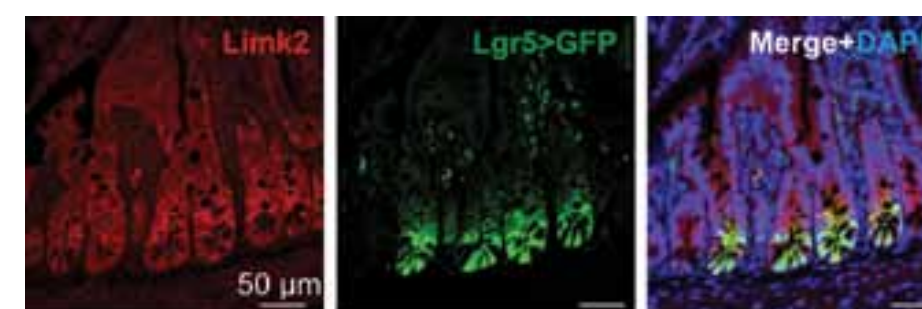
apoptotic by distinguishing features including; membrane blebbing, apoptotic body formation, and maintenance of intact cellular membranes to prevent massive protein release and consequent immunological activation. We found that limited membrane permeabilisation

occurs in blebs and apoptotic bodies, which allows release of proteins that may affect the proximal microenvironment and influence apoptotic cell clearance, prior to the catastrophic loss of membrane integrity during secondary necrosis. Blebbing, apoptotic body formation and protein release during early apoptosis are dependent upon ROCK activity and myosin ATPase activity to drive actomyosin contraction. We identified numerous proteins released from actomyosin-dependent blebs and apoptotic bodies by adapted SILAC (stable isotope labelling with amino acids in cell culture) combined with mass spectrometry analysis. The most enriched proteins released were the nucleosomal histones, which have previously been identified as damage-associated molecular pattern molecules (DAMPs) that can initiate non-infectious inflammatory responses. These results indicate that limited membrane permeabilisation occurs in blebs and apoptotic bodies prior to secondary necrosis, leading to acute and localised release of immunomodulatory proteins.

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Figure 2. Limk2 expression in mouse intestines overlaps with the stem cell marker Lgr5.

Limk2 expression (red) in intestine sections from wild type mice that had been crossed with the stem cell marker Lgr5-GFP (green). Merged image also has DAPI staining of cell nuclei.





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Colorectal cancer is the third most common cancer in the UK and the second most common cause of cancer mortality. The focus of our group is to understand the early changes associated with intestinal neoplasia in order to identify novel markers of the disease as well as new targets for therapy. The key intestinal tumour suppressor is the *Apc* gene that is mutated in approximately 80 percent of sporadic cancers. Central to our work is the use of novel inducible models of intestinal tumorigenesis that allow us to study the functions of specific tumour suppressor genes.

Rac1 integrates ROS and NF-κB signalling to drive hyperproliferation, stemness and transformation following *Apc* loss

We have previously shown that c-Myc is the key effector downstream of *Apc* loss *in vivo*. Since it may be difficult to pharmacologically inhibit transcription factors such as c-Myc, investigating more druggable targets of the Wnt/c-Myc pathway within the intestine may reveal potential therapeutic targets for colorectal cancer. Recent work in the laboratory has shown that Rac GEFs and the active Rac1 splice isoform Rac1b are upregulated following *Apc* loss and Rac1 is required for the hyperproliferative and progenitor/stem cell phenotypes that ensue (Fig. 1). Mechanistically, Rac1 is required for the production of ROS following *Apc* loss, which subsequently activates NF-κB signalling, resulting in p65 binding to the promoters of the intestinal stem cell genes. Physiologically, *Rac1* deletion stops adenoma formation following *Apc* loss and instead results in small lesions that do not form tumours and lack stem cell markers such as Lgr5 and which recapitulate those formed when *Apc* is deleted from non-stem cell compartments. Taken together, our data highlight that Rac1 activation downstream of Wnt signalling and upstream of ROS/NF-κB signalling is a critical event at the early stages of intestinal

carcinogenesis to maintain the progenitor cell phenotype. Given a number of preclinical inhibitors of Rac1 are available, our data suggest that these could be of benefit in colorectal cancer.

Kras mutation expands the cell of origin of colorectal cancer and drives resistance to therapy

In colorectal cancer, *Kras* mutation is often co-mutated with *Apc* (in approximately 40% of cases). These mutations are activating and tumours carrying *Kras* mutations have a poorer prognosis and are less likely to respond to therapy. Therefore, my laboratory has been investigating the cooperation of *Apc* and *Kras* mutations in models of colorectal cancer. The additional mutation of *Kras* exacerbates many of the phenotypes of *Apc* loss, increasing proliferation rates and accelerating tumorigenesis. Interestingly, one of our key findings was that mutation of both *Apc* and *Kras* resulted in a much larger number of cells within the intestinal epithelium having the capacity to form tumours. To prove this, we used the recently developed primary intestinal organoid culture system to show that even differentiated intestinal epithelial cells (villus enterocytes) had the capacity to de-differentiate and form tumours when both *Apc* and *Kras* were

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mutated. Single mutations in either *Apc* or *Kras* were not sufficient to drive tumour formation in these cells (Fig.2). Mechanistically, heightened Rac, NF-kappaB and TGF-beta signalling was observed in cells with mutations in both *Apc* and *Kras*. Therefore, by increasing the levels of the pathways that are required for the progenitor cell phenotype following *Apc* loss, an additional *Kras* mutation can confer this phenotype to many more cell types than *Apc* loss alone.

Kras mutation is also predicted to drive activation of the MAPK/ERK, PI3K/mTOR and RalA/B pathways. Therefore, we have been investigating if inhibition of these pathways can

suppress the phenotype of cells carrying *Apc* and *Kras* mutations. Remarkably, whilst many of these inhibitors can have clear beneficial effects of intestinal cells carrying just *Apc* or *Kras* mutations, when cells carry both mutations they are intrinsically resistant to all of these agents. We are now examining the pathways of resistance in these cells and hope to come up with rationale combination therapies that will overcome this resistance mechanism and specifically target cells carrying both of these mutations.

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Figure 1 - *In vivo* multiphoton imaging of the intestinal stem cell marker Lgr5 shows Rac deficiency suppresses the increased levels following *Apc* loss.

In collaboration with Kurt Anderson, we have quantified the levels of the stem marker Lgr5 through the use of a reporter GFP in murine intestinal whole-mounts. As can be seen here, there is a marked increase in the amount of Lgr5-GFP following *Apc* loss *in vivo* (APC), however following combined loss of *Apc* and *Rac* (APC RAC) this is restored to wild type levels. Importantly, loss of *Rac* alone (RAC) has equivalent levels of GFP to wild types. Red here is second harmonics imaging of collagen.

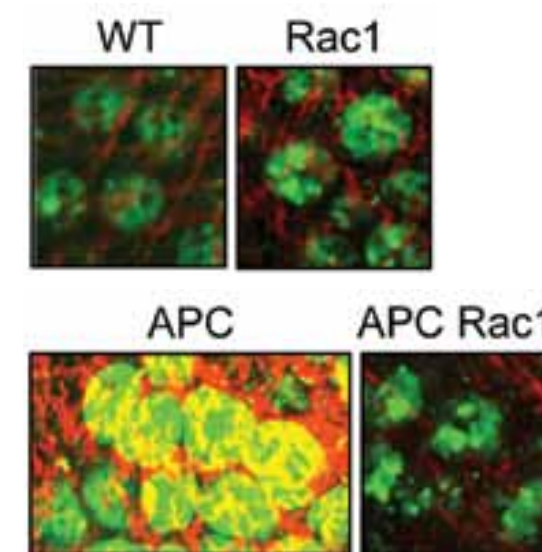
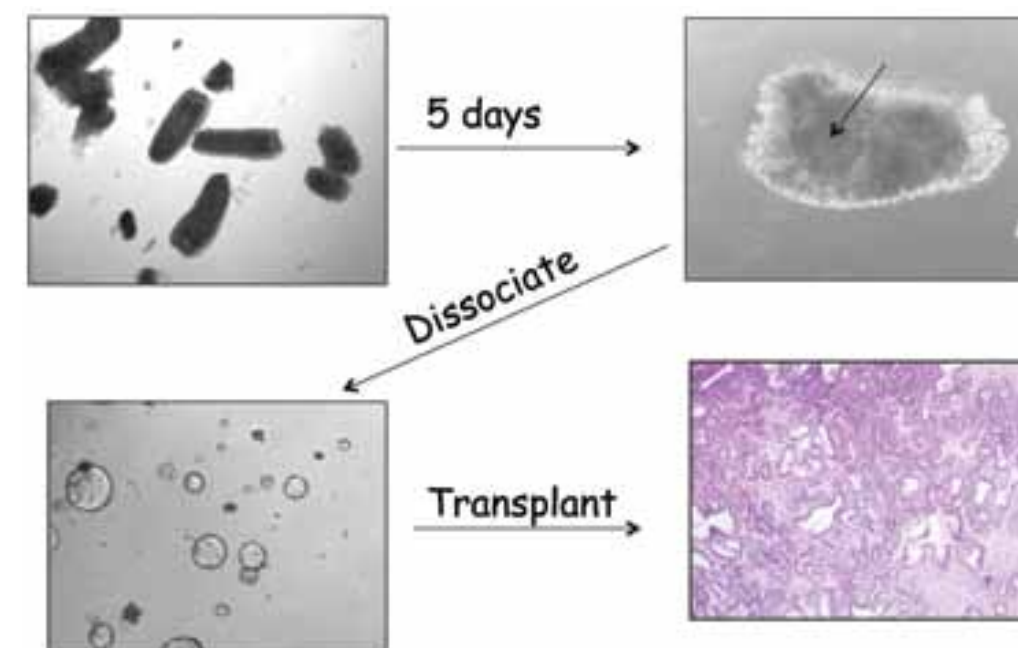


Figure 2

Differentiated cells (villi) were isolated, and *Apc* and *Kras* were mutated leading to tumour sphere formation on the villi (arrowed). The spheres were then dissociated and finally transplanted - these then form tumours. No spheres were formed when *Apc* or *Kras* was mutated alone.





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Figure 1
Confocal microscopy images of wing discs from transgenic flies expressing oncogenic RET, FAK or both in a vertical stripe of cells (labelled in green by GFP). Note that RET expression activated the MAPK pathway (yellow arrow in top right panel). FAK expression did not impact MAPK by itself but suppressed MAPK activation by RET (bottom right).

We utilise the fruit fly *Drosophila* to understand fundamental aspects of cancer biology. We have recently reported an inflammatory reaction from the innate immune system to the rise of tumours, comparable to the one observed in cancer patients. We have also focused on the biological role of the non-receptor tyrosine kinase, focal adhesion kinase (FAK). FAK is over-activated in a wide range of tumours, and is considered a valid therapeutic target. We used a model of tumours driven by the RET oncoprotein and observed FAK activation suppresses RET's oncogenic signal. Thus, in this context FAK may constitute an anti-target. Finally, we have characterised the role of Wnt signalling during regeneration and tumorigenesis in the fly intestine. These results suggest that the JAK/STAT pathway could be a useful therapeutic target in early stage colorectal carcinomas.

Drosophila FAK constitutes an anti-target in the context of oncogenic RET

Drosophila FAK mutant flies are viable and fertile, proving that dFAK is not essential for development. Therefore, we hypothesised that dFAK could be required in conditions of stress, such as oncogenic stress. To test this, we used a *Drosophila* cancer model driven by the receptor tyrosine kinase RET (rearranged during transformation). Flies expressing RET show a tumour-like phenotype. FAK would be expected to participate in the cascade of kinases signalling downstream of ectopic RET. Unexpectedly, when dFAK was deleted, RET's phenotype was enhanced dramatically; and when FAK was co-expressed with RET, it suppressed RET's phenotype. Thus, FAK acts as an inhibitor of RET's signalling. Our studies further indicate that FAK impairs RET signalling via the MAPK pathway (Fig. 1, Macagno *et al.*, submitted). Therefore, in the context of RET-driven tumours, rather than a target, FAK may constitute an anti-target.

Modelling colorectal cancer in *Drosophila*

The adult fly intestine is a tissue of highly regenerative capacity that is constantly replenished from a population of adult intestinal

stem cells (ISCs). This discovery suggested that the biology of intestinal homeostasis is highly evolutionary conserved across metazoa. The highly regenerative capabilities of the

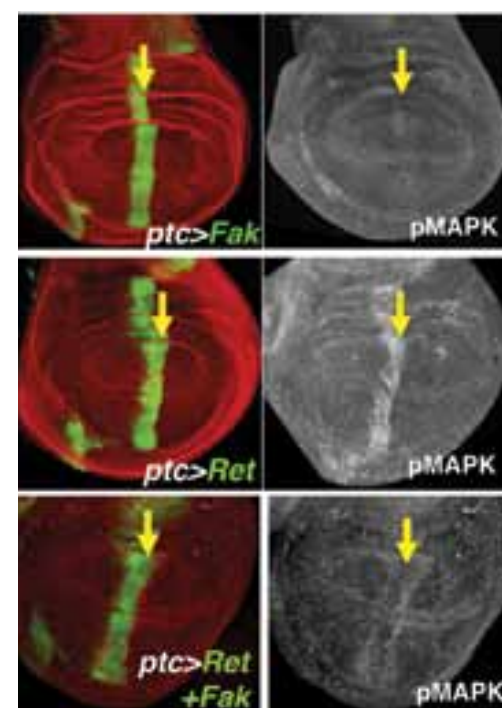
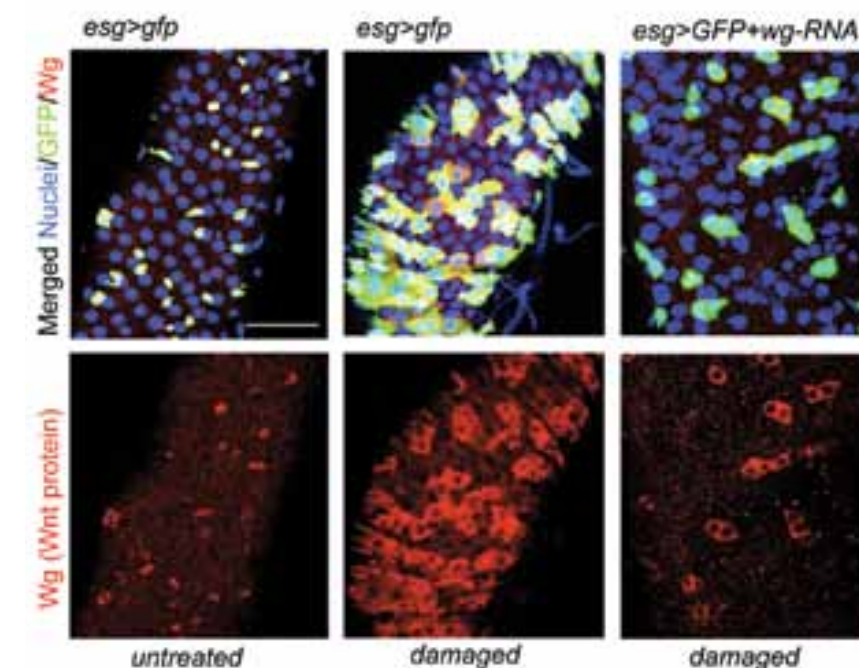


Figure 2
Drosophila intestines from transgenic flies expressing GFP in the stem/progenitor cell compartment (experimental controls: left and middle panels) or GFP and RNAi for the Wnt ligand Wg (right panels). Top panels show merged images stained for nuclei, Wg protein and GFP. Bottom panels show Wg protein in red. Note that damage to the control tissue (middle) resulted in an expansion of the stem/progenitor compartment and Wg levels. RNAi for Wg prevented this regenerative response.



mammalian intestine are suspected to mediate the high propensity of this tissue for neoplastic transformation. We therefore speculated that the fly intestine could model important aspects of intestinal cancer. We have characterised a novel source of the Wnt ligand Wg in enteroblast cells that is absolutely required for intestinal regeneration upon damage (Fig. 2, Cordero *et al.*, EMBO J 2012; 31: 3901). Wg is also produced from the visceral muscle but this source is not required for regeneration. Importantly, various sources of Wnt ligand have recently been reported in the mammalian intestine. Therefore, the mechanism uncovered in *Drosophila* could be conserved and impact our understanding on intestinal regeneration.

2009; 8: 2926). We have now characterised a non-cell autonomous signalling network acting upon APC1 loss in stem cells. The results demonstrate that APC1 mutant ISCs secrete epidermal growth factor, which acts in part by activating its receptor in differentiated epithelial cells. This results in the production of the interleukin-6 homologue Unpaired, which acts on ISCs to promote their proliferation via the activation of the STAT signal transducer (Cordero, Stefanatos *et al.*, Development 2012; 139: 4524). These results suggest that STAT signalling is a potential target in APC-driven colorectal carcinomas at early stages and without an obvious inflammatory component.

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Finally, we previously showed that depletion of the *dApc1* gene in *Drosophila* ISCs results in intestinal hyperplasia (Cordero *et al.*, Cell Cycle



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Tumour cells have continuous need of oxygen and nutrients to support their uncontrolled proliferation. To meet this demand, tumour cells secrete pro-angiogenic factors and promote sustained vessel growth. This process is referred to as tumour angiogenesis and drives tumour growth, tumour progression to malignancy, and metastasis. The angiogenic process is defined by a multitude of cellular mechanisms, such as sprouting, cell-cell adhesion, cell-extracellular matrix adhesion, migration, proliferation, lumen formation and mural cells recruitment. Our group is working with state-of-the-art mass spectrometry in combination with stable isotope labelling with amino acids in cell culture (SILAC) to perform quantitative proteomic and post-translational modification - such as phosphorylation - analyses. Our aim is to develop a better understanding of the complexity of the signalling driving vessel formation with a particular focus on how the environment surrounding newly forming vessels impacts on this process.

Endothelial cell signalling in angiogenesis

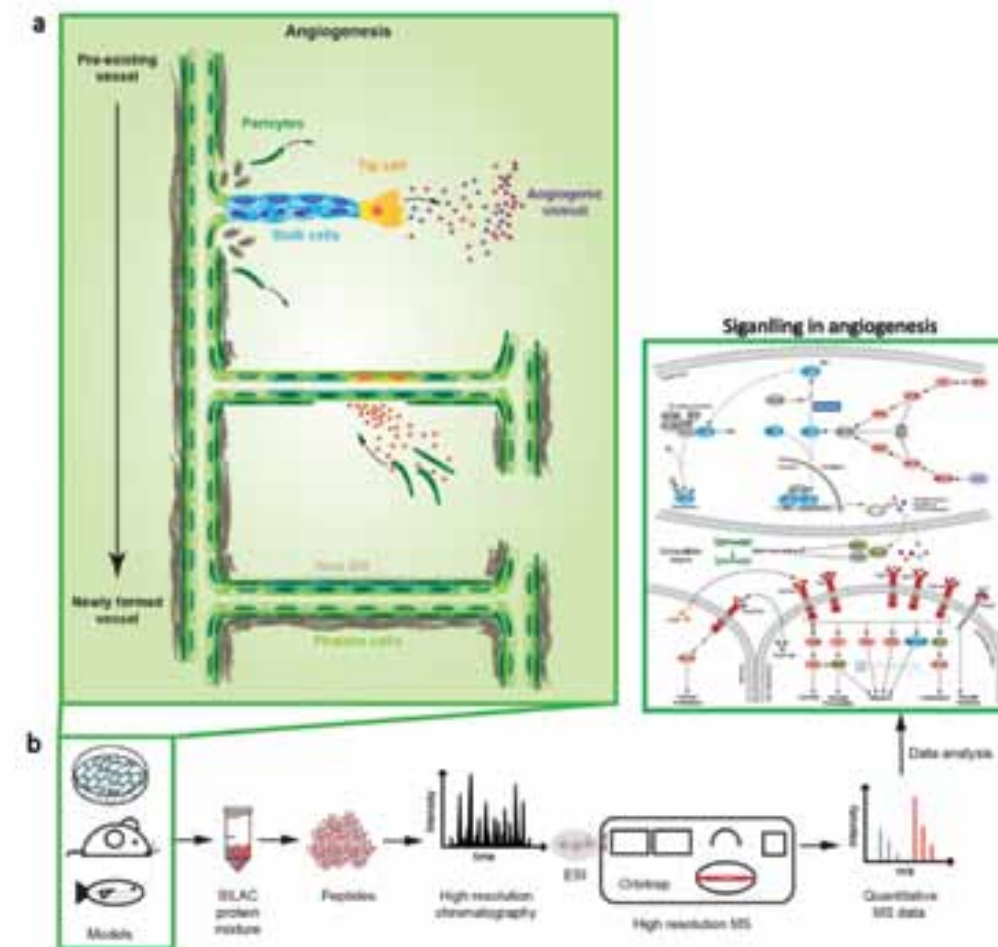
Endothelial cells constitute the first layer of the vessel wall and are the major cell players in the angiogenic process. Indeed, endothelial cells orchestrate a multitude of cellular mechanisms that trigger the formation of new and mature vessels – for example they sprout, digest and migrate through the extracellular matrix (ECM), proliferate, change morphology, and recruit mural cells (see Fig. 1 for details). A better understanding of the molecular mechanisms underpinning angiogenesis will be required to interfere more efficiently with this mechanism in pathological conditions such as cancer.

When endothelial cells are plated on an appropriate matrix, such as matrigel (that is the basement membrane soluble extract from the Engelbreth-Holm-Swarm tumour), they assemble into tubular structures complete with a lumen, and thereby morphologically recapitulate the *in vivo* process. We previously used this *in vitro* model in combination with SILAC-based quantitative mass spectrometry to investigate proteomic changes occurring in

primary human endothelial cells while assembling into tubular structures. The detailed temporal choreography of the cellular processes regulated during this process showed that the interplay between cells and the ECM plays a prominent role. Additionally, we found that the morphogenetic process is associated with metabolic changes in endothelial cells, and that this alteration is functional. We are currently investigating this aspect further. Additionally, the proteomic profile of endothelial cells forming tubular structures in matrigel provided a source of novel molecules potentially involved in (tumour) angiogenesis. We focused on the glycoproteins CLEC14A and MMRN2. By studying two distinct genetic mouse models of multistep carcinogenesis we validated these proteins as blood vessel markers, modulated during tumour progression.

Our future work will be devoted to better characterisation of the complexity of the signalling network during vessel formation with a particular focus on the remodelling of the metabolic machinery, and the signalling

Figure 1 - Quantitative proteomics in angiogenesis. (a) Schematic overview of the major steps involved in the formation of a new mature blood vessel from a pre-existing one. Colour of the text reflects the specific cell type or extracellular matrix (ECM) as follows: dark brown, ECM; light brown, newly synthesised ECM; yellow, tip cell; blue, stalk cells; light green, phalanx cells (quiescent ECs); dark green, pericytes. The arrows indicate the direction of cell movement. (b) Schematic overview of a MS shotgun proteomic approach, where models representing different aspects of the angiogenic process (a) are used in combination with SILAC for quantitative proteomic analysis. In a typical workflow, peptides from digested SILAC-containing protein mixture are resolved using liquid chromatography and electrosprayed into LTQ-Orbitrap. ESI = electrospray ionisation.



triggered by the extracellular environment (Fig.1). We aim to integrate these data with those previously generated to draw a more comprehensive picture of the molecular mechanisms regulating the formation of new vessels, and to identify and further characterise new key proteins as possible targets to interfere with tumour vascularisation in the context of anti-cancer therapies.

The extracellular environment is important to drive tumour progression and angiogenesis

The environment surrounding cells strongly affects their behaviour. Interestingly, the extracellular environment is diverse in physiological and pathological conditions. In cancer, the tumour stroma, composed of extracellular matrix (ECM) and non-cancer cells such as fibroblasts and immune cells, has a different composition and structure compared to normal tissue. This contributes to tumour progression to malignancy. Making use of the SILAC mouse technology, we recently performed an *in vivo* quantitative proteomic study of a mouse model of skin carcinogenesis, where we observed that ECM composition differs between benign and malignant tumours, and that specific cell adhesion proteins are highly expressed in tumour cells associated with malignancy. In the context of angiogenesis, the ECM composition and its mechanical properties can affect endothelial cell functions, such as adhesion, migration and proliferation. Therefore, there is a clear link between the

tumour stroma and the angiogenic process in cancer and we aim to investigate this further.

Our group applied in depth SILAC-based quantitative proteomics to characterise the composition of the proteome, ECM and secreted proteins produced by fibroblasts of different origins, normal and tumour, to provide a detailed picture of proteins specifically expressed or with an altered level of expression in the tumour environment. We are currently investigating the function of some of the identified proteins in the context of angiogenesis by using an endothelial cell-fibroblast co-culture *in vitro* model.

We are additionally exploring an improved strategy to SILAC-label human primary endothelial cells. This will allow further dissection of proteomic and post-translational modification changes occurring in endothelial cells. Particularly, we aim to characterise in depth the signalling networks activated in endothelial cells by the mechanical properties and composition of the ECM, typical of the tumour environment. This will hopefully reveal signalling and identify specific proteins involved in tumour angiogenesis. Candidate proteins will be further characterised as potential suitable targets to interfere with the process of angiogenesis in cancer.

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DRUG DISCOVERY PROGRAMME

**THE BEATSON INSTITUTE FOR
CANCER RESEARCH**

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Metastasis is responsible for approximately 90% of cancer deaths, however there are currently no therapeutics specifically designed to combat this process. Exploiting the basic research themes of Beatson Institute scientists, we have made significant advances in our portfolio targeting novel approaches to manage invasion and metastasis. These are the kinase MRCK (myotonic dystrophy kinase-related Cdc42-binding kinase), a key effector in the Rho signalling pathway, and fascin, an actin bundling protein. We have continued to utilise our fragment-based hit identification expertise to target other protein-protein interactions that, though challenging biological targets, have a high degree of validation as cancer targets. In particular, we are targeting RAS, one of the most highly validated cancer targets that is mutated in 30% of all human cancers.

Myotonic dystrophy kinase-Related Cdc42-binding Kinase (MRCK)

Increasing evidence implicates MRCK as an attractive anti-metastatic target. MRCK is a key effector in the Rho GTPase signalling pathway that acts to power cell movement by initiating actomyosin contractility and cytoskeletal reorganisation. Multiple tumour types show increased Rho GTPase expression, loss of negative regulators and/or upregulation of downstream effectors such as MRCK, leading to enhanced signalling through the Rho GTPase pathway (Sahai & Marshall, Nat Rev Cancer 2002; 2: 133).

In collaboration with Mike Olson, we are aiming to develop small molecule inhibitors that target the α and β isoforms of MRCK, which alone or in combination may be used in the management of metastatic disease. Initial screens identified several chemical series that show activity against both MRCK isoforms as well as selectivity over other key kinases such as Rho kinases (ROCKs). By adopting a focused medicinal chemistry approach, the activity against both isoforms of MRCK has been optimised to provide a number of chemical

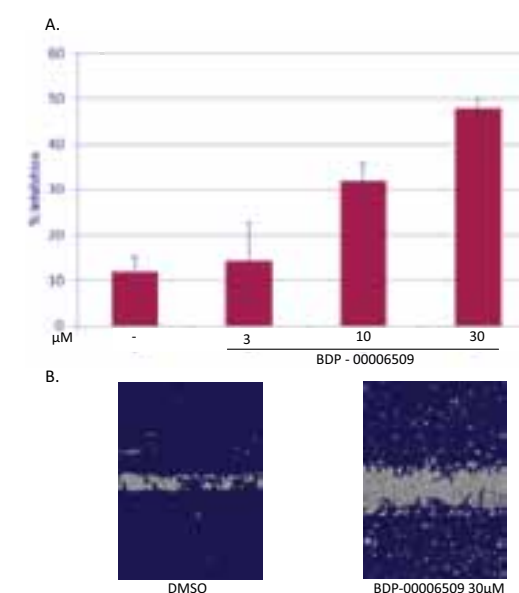
series that exhibit good MRCK biochemical activity (MRCK α/β Ki < 10 nM) and excellent biochemical selectivity (routinely >100-fold selective and often >400-fold selective) over ROCKs. Furthermore, this encouraging biochemical selectivity is confirmed in cellular assays with a key compound, BDP-00006509 showing good activity in an MRCK β selective cellular assay (IC₅₀ ~ 1.5 μ M), while also demonstrating selectivity over ROCK2 (IC₅₀ >10 μ M) (Fig.1A). In line with these data, compounds show robust decreases in cellular invasion *in vitro* (Fig. 1B and C). Optimisation of the ADMET (absorption distribution metabolism excretion toxicity) profile of each series is ongoing in preparation for profiling analogues in *in vivo* pharmacodynamic (PD) and efficacy models.

Fascin

Elevated expression of the actin-bundling protein fascin is strongly correlated with invasiveness and poor clinical outcome in a variety of tumour types. Fascin is implicated in cell migration, formation of filopodia and degradative invasion into extracellular matrix, and evidence is mounting that fascin represents a target for anti-invasive therapeutics. Last year,

Figure 1

(A) MDA-MB-231 D3H2LN cell invasion through matrigel was measured using an Incucyte kinetic imaging system. The % inhibition of invasion was calculated 30 hours post-treatment with BDP-00006509 at the indicated concentrations. (B). Representative images of invading cells treated with DMSO or BDP-00006509, 30 hours post-treatment, are shown. The grey area indicates the wound width at the end of the experiment.



we carried out a fragment screen to identify small molecules binding to fascin with the potential to disrupt the actin-fascin interaction. As a protein-protein interaction mechanism, this represents a challenging target but one for which fragment-based approaches are becoming increasingly used. The SPR and NMR fragment screen identified hits with ligand efficiencies as high as 0.39 and we now have

crystal structures for six of these fragments binding at two different sites. There are currently no published crystal structures of fascin in complex with drug-like molecules. We have used a combination of commercially available analogues and in-house synthesis to identify compounds with potency increases of 10-100 fold. Site 1 is adjacent to a series of amino acids that upon site-directed mutagenesis block actin bundling, suggesting this site has physiological relevance. The crystal structures of more potent compounds have been solved to inform further design and one is shown alongside the original site 1 fragment hit in Figure 2. A crucial aspect of the project is collaboration with Laura Machesky's lab. There, cell-based assays are being developed to help identify fascin-actin disrupting compounds and current work is aimed at increasing the throughput of this assay (Fig. 3). In addition to these mechanistic assays, high throughput phenotypic assays have been developed to identify inhibitors of tumour cell invasion and these are being deployed within the Drug Discovery Programme. Our current focus is to increase the potency of the hit series to a point where they have the potential to demonstrate activity in biochemical and cell-based assays.

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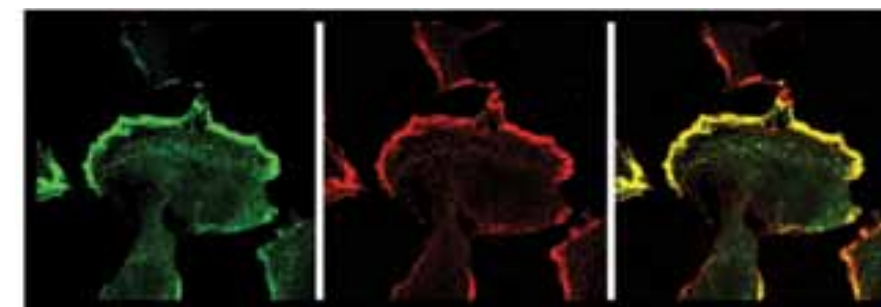
Figure 2

A fascin-binding fragment (BDP-000013, left) and a lead-like compound (BDP-007029, right) binding at the same pocket in fascin.



Figure 3

CHL-1 melanoma cells showing colocalisation (yellow) of fascin (green) and actin (red) at the leading edge of the cell and at localised points of invasion (Ang Li and Laura Machesky).





ADVANCED TECHNOLOGIES

THE BEATSON INSTITUTE FOR CANCER RESEARCH

Kurt Anderson - Beatson Advanced Imaging Resource (BAIR)

Gabriela Kalna - Bioinformatics

Nick Morrice - Proteomics and Mass Spectrometry

Emma Shanks - RNAi Screening

Karen Blyth - Transgenic Models of Cancer

Douglas Strathdee - Transgenic Technology

BEATSON ADVANCED IMAGING RESOURCE (BAIR)

www.beatson.gla.ac.uk/advanced_technologies



Head
Kurt Anderson

Scientific Officers

Tom Gilbey
Ewan McGhee
Agata Mrowinska
Margaret O'Prey
David Strachan

Light microscopy is a fundamental technique in cell biology and cancer research. The development of genetically encoded fluorophores (fluorescent proteins) has revolutionised research by enabling direct visualisation of any gene product.

Concomitant with the development of new genetic tools, there have been tremendous advances in fluorescence imaging technology to visualise molecular dynamics in living cells, tissues and organisms. These powerful techniques are increasingly sought after by researchers, who require assistance in both the evaluation and application of imaging technology in order to address fundamental questions in cancer biology. Our mission is to support basic imaging and the development of advanced applications. Basic imaging support primarily consists of training new users in simple acquisition and analysis techniques, such as FACS analysis, immunofluorescence microscopy and the export of raw data into presentation software. Development of advanced applications requires close work with our users to understand their scientific questions and help them develop appropriate imaging strategies. The following advanced techniques have been identified through consultation with Beatson group leaders as important: medium throughput long term time lapse imaging; high-resolution live cell imaging; confocal microscopy, especially for the use of photo-activation, -bleaching, and -switching; total internal reflection fluorescence (TIRF) microscopy; *in vivo* mouse imaging; and fluorescence lifetime imaging for the determination of fluorescence resonance energy transfer (FLIM-FRET). Collectively we provide our users with a powerful technology toolbox for cellular and molecular level investigations of disease and response to therapy *in vitro* and *in vivo*.

The BAIR staff serve as a repository of expert knowledge who train users and assist with advanced applications. We install, maintain, troubleshoot and manage the repair of equipment, serving as an important link between commercial partners and users. We also provide vision for future trends and help to

identify new technology of potential benefit to our researchers. The BAIR occupies a purpose-built space in the basement of the Institute. Central features of the floor plan include: space flexibility, achieved through the use of large rooms divided by curtains; a central laser room, which provides a more stable operating environment for delicate equipment and removes a source of heat, noise and hazard from the user environment; a computer room, situated near the imaging systems to facilitate the flow of data from acquisition to analysis; and a staff office with natural lighting overlooking the hallway, which promotes rapid user assistance.

Changes this year

2012 saw the installation of an Albira (Bruker) tri-modal scanner for PET, SPECT and CT imaging of mice. We also employed a new member of staff (Agata Mrowinska) to support the use of this new equipment. Agata brings extensive mouse experience to the BAIR, and will assist with aspects of experimental use including injection of probes, instrument operation and analysis of data. Our adaptive optics project on the multi-photon system, led by Ewan McGhee, has continued in collaboration with Caroline Mullenbroich, who was awarded a six-month SUPA Inspire Fellowship (supported by Coherent) to design and implement an adaptive pathway for our OPO laser. Preliminary data suggest 1.5-3 times improvement in signal intensity at sample depths from 10-70 μm . Finally, our easy-STED project, led by David Strachan and based on incorporation of a 1.5 W 592 nm STED laser into a Leica TCS SP2 confocal microscope, has progressed to completion of the hardware modifications. Preliminary images have shown a resolution improvement of approximately 20% over conventional confocal microscopy, although system alignment and image acquisition parameters remain to be optimised.

BIOINFORMATICS

www.beatson.gla.ac.uk/advanced_technologies



Head
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Informatician

Ann Hedley

The Bioinformatics Unit provides support for a range of research projects that require a computational approach, advanced statistical analysis and/or modelling. Currently, the key drivers are the increased demand for complex analysis of datasets from microarrays-based technologies, proteomics, metabolomics and siRNA screening and a move towards next generation sequencing.

Our team focuses on exploratory data analysis with an aim to provide essential insights for better understanding of cancer biology. We offer routine processing of data, differential expression, supervised and unsupervised machine learning as well as graph and network theory-based analyses. R-based BioConductor packages, commercial Partek Genomics Suite, and Matlab Bioinformatics and Statistics Toolboxes are used for many of the data analysis tasks. In addition to the available software packages, we offer analytical routines that have been developed in-house and with our collaborators from the areas of mathematics, statistics, computer science and biology.

Lists of genes and metabolites are submitted for functional annotation, clustering, enrichment, ontology and pathway analysis. To deal with these tasks, licenses for Ingenuity Pathway Analysis and GeneGo Meta Core are available to researchers at the Beatson. In addition to these tools, the Oncomine Research Premium Edition database has been in use to deal with an increasing interest in mining publicly available datasets. Many tasks (e.g. motif incidence and isoform identification) involve the use of online databases either by searching them directly or by writing customised scripts to download the relevant data and mine the download for the required information. The CLC Genomics Workbench has been selected as a result of an open tendering process for a site-wide DNA software analysis package. The Beatson shares this license with the University of Glasgow.

Our aim is to ensure that appropriate statistical methods are used and presented in publications. We offer advice on experimental designs, statistical techniques and data presentation, and have rich experience with multivariate analysis of high-throughput data with clinical and survival information from patient cohorts. We are also involved in testing and developing of new computational approaches.

In 2012, we made significant changes in terms of new equipment and staff. Our priority was to accommodate next generation sequencing at the Beatson. A new Linux server was set up with help from Information Services, and the installation and trial of software is in progress. These large datasets bring new challenges and our future plans will particularly focus on the implementation of novel algorithms for their analysis.

We are happy to see the constant interest in our service and try our best to deal with all incoming projects effectively and within a reasonable timeframe. However, we have had a number of new kinds of data and modelling projects that require study, trials of different methodologies and development of new workflows. To meet these requirements, we have appointed a new member of the team who will be starting in January 2013.

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PROTEOMICS AND MASS SPECTROMETRY

www.beatson.gla.ac.uk/advanced_technologies



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The facility has five mass spectrometers used for both proteomics and metabolomics: two Orbitrap Velos mass spectrometers (one of which is used by Sara Zanivan's group) coupled to Easy-LC II nano HPLCs; two Exactive mass spectrometer systems for metabolomics; and an AB-Sciex 5600 triple TOF system.

The Orbitrap Velos systems are primarily used for protein and peptide identification, post-translational modification analysis and quantitative proteomics using SILAC. The 5600 Triple TOF is used for quantitative proteomics using SILAC, iTRAQ or label free analyses. We have recently adapted this LC-MS system with a capillary HPLC coupled to an optimised ESI source which has reduced sample analysis times 3-5 fold with little loss in sensitivity or number of proteins identified. We have also introduced data independent LC-MS using SWATHMS technology, which creates a LC-MS peptide map that can be interrogated with spectral data, rather than using a database search. This technology is thought of as a mass spectral Western blot that can be interrogated with spectral library data from proteins of interest long after the raw data has been acquired.

The experienced staff members provide advanced knowledge and expertise for initiation of proteomics projects and state-of-the-art proteomics analyses to the Institute's scientific community. It is advisable to contact one of the staff members prior to initiating a project so that the best experimental design can be drawn up. Although most of the activity of the service is related to the analyses of samples for protein identification and characterisation by MS, the analysis of post-translational modifications, especially protein phosphorylation, is a speciality. Along with the investment in MS instrumentation, we are expanding the informatics capability of the facility. There is now a dedicated MaxQuant server for the analysis of SILAC data, a new Mascot server, Proteome Discoverer 1.3, Protein Pilot 4 for the analysis of 5600 data as well as Scaffold Q + S for integrating Mascot, Proteome Discoverer

and MaxQuant data into one user friendly viewer. Training is always available from the facility staff on how to use any of these software packages. The metabolomic analyses are mainly performed by members of Eyal Gottlieb's and Karen Vousden's groups but Gillian Mackay has been appointed as the facility manager to oversee the metabolomics service. Metabolite profiling can be done on the two Exactive LC-MS systems and if metabolite identification is required, this can be performed on the Orbitrap Velos or 5600 Triple TOF systems. The groups are mainly focused on the analysis of polar metabolites, such as amino acids, organic acids, nucleotides, sugars and sugar phosphates, but work is ongoing to expand the repertoire of metabolites to include fatty acids, phospholipids and triglycerides. The metabolomics service uses software packages such as SIEVE 2, LC Quan2.6 that are used for the identification and quantification of previously characterised metabolites. As for the proteomics service, it is recommended that you contact Gillian ahead of any project, so that the design of the experiment is optimised.

The facility focuses on four major areas:

1. Quantification of protein complexes, proteomes and secretomes using label and label-free methods.
2. Post-translational modification analysis of individual proteins, in particular phosphorylation, acetylation, methylation and ubiquitination.
3. Metabolomic profiling of known, naturally occurring metabolites.
4. Method development for proteomic and small molecule analysis.

Publications listed on page 87

RNAi SCREENING

www.beatson.gla.ac.uk/advanced_technologies



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Since its establishment in early 2011, the RNAi Screening Facility has developed an extensive collaborative portfolio with group leaders at both the Beatson Institute and the University of Glasgow. Our facility couples high throughput RNA interference (RNAi) screening with high content imaging to translate fundamental cancer research towards new therapies. Using RNAi screening, we can sequentially knockdown every gene in the genome and quantify its effect on a biological system. With appropriate supporting validation, this approach is a powerful tool for elucidating new interactions, and has the potential to identify novel drug targets and/or drug interacting partners to improve existing cancer therapeutic approaches.

Developments in 2012

The duration of 2011 saw creation of the facility infrastructure and working practises, and completion of two siRNA screens; one targeting over 9000 murine genes in two genetically distinct pancreatic tumour-derived cell lines, the other targeting 720 kinase genes in a docetaxel resistant prostate cancer cell line. We entered into 2012 with a further six projects in the pipeline, all of which have progressed through assay development and screening. This includes our first whole genome viability screen (18175 genes) investigating genes that promote cell death selectively under hypoxic conditions, with minimal effect under normoxic conditions. We have also completed our first combination screens looking for LIMK inhibitor synergist partners, using a dual drug versus drug and a drug versus siRNA approach. These projects are currently at various stages of hit validation and deconvolution; the screening of our sixth project (an investigation into genes regulating cell invasion) will be completed before the end of the year.

The overall focus of the portfolio continues to be a balance between synthetic lethality and identification of novel players within a system of interest. The former aims to identify key lethality-causing genes within specific genetic cancer backgrounds, potential targets that will

re-sensitise drug resistant cancer cell lines, and synergistic partners for putative novel cancer drugs to increase efficacy. We remain committed to supporting the downstream deconvolution work with collaborating groups and have expanded certain projects to include focused small molecule screens and also to investigate a use for existing drugs for non-cancer related indications (drug repurposing), which is adding a breadth of knowledge to our portfolio. We have obtained a pilot grant from Breast Cancer Campaign to support a drug repurposing investigation in a panel of breast cancer cell lines with different genetic backgrounds. We look forward to entering 2013 with four exciting projects ahead of us.

Accessing the facility

We aim to be as flexible as possible in supporting requests from researchers, and welcome informal screening enquiries throughout the year. To engage in a screening campaign with the facility, we have an application process and invite proposal submissions every six months.

TRANSGENIC MODELS OF CANCER

www.beatson.gla.ac.uk/advanced_technologies



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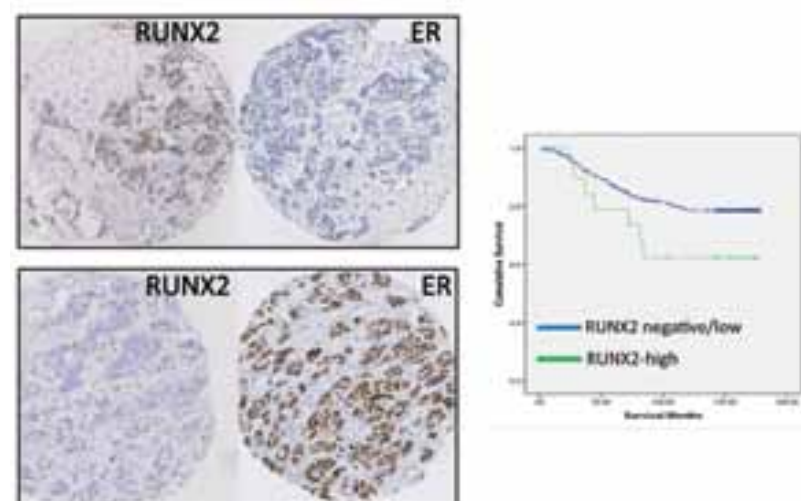
³ University of Glasgow

Preclinical genetic models significantly contribute to our understanding of how cancers develop and metastasise. Our lab maximises the potential of these models to accurately recapitulate all stages of the human disease in a physiologically relevant manner. One family of genes that the lab has a particular interest in is the RUNX genes and their role in breast and prostate cancer.

Using *in vivo* models of human cancer we are assessing the genetic mutations and signalling pathways that are associated with cancers such as colorectal, pancreatic, breast and prostate cancer, and melanoma. In collaboration with many of the Beatson research groups, and utilising sophisticated genetic models generated by the Transgenic Technology Laboratory, we can dissect the processes that drive cancer initiation, progression and metastasis. Furthermore, these preclinical models, which faithfully recapitulate the human disease, allow us to monitor responses and modes of action of novel therapeutic agents (e.g. Eg5 kinesin inhibitors) in an attempt to

Figure 1 - Expression of RUNX2 correlates with ER/PR/HER2-negative human breast cancer.

Invasive breast carcinomas stained for RUNX2 and oestrogen receptor (ER) depicting the reciprocal expression pattern. Survival is plotted for patients showing that high RUNX2 expression in tumours is associated with poorer overall survival.



develop better-targeted treatments in a physiologically relevant system. We also work with Kurt Anderson and the BAIR to maximise the potential of *in vivo* imaging, which is part of our 3Rs (reduction, replacement and refinement) initiative.

The RUNX genes in epithelial cancer

The *RUNX* genes, which are essential for normal mammalian development, are context dependent oncogenes and tumour suppressor genes. In collaboration with researchers at the University of Glasgow, we have been dissecting the role of *Runx1* and *Runx2* in epithelial cancer, and in particular in breast and prostate cancer. Differential and temporal expression of these genes is observed during mammary epithelial differentiation *in vivo*, strongly indicative of an important functional role. Indeed, we believe that *Runx2* must be tightly regulated in order for normal mammary gland development to occur, and preliminary evidence suggests a putative role in the regulation of mammary stem and/or progenitor cells. As with many genes important in cell fate decisions, *RUNX2* has also been linked to metastatic cancer where in some established breast cell lines, retention of expression is associated with a more invasive phenotype. Recent work in the lab on a human tumour tissue microarray has shown that *RUNX2* is overexpressed in a particular subset of breast cancers that are ER/PR/HER2 negative, a subtype of breast cancer for which targeted therapy is not currently available. Furthermore high expression of *RUNX2* is associated with poorer overall patient survival (Fig. 1).

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TRANSGENIC TECHNOLOGY

www.beatson.gla.ac.uk/advanced_technologies



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The Transgenic Technology Laboratory generates models of human cancers in collaboration with other scientists at the Beatson. Using gene targeting in stem cells we can accurately introduce defined genetic alterations into the germline, allowing us to generate increasingly accurate and sophisticated cancer models.

Taking advantage of the high rates of DNA recombination in embryonic stem cells allows us to modify genes in a highly controlled manner. This type of approach enables the analysis of gene functions and how these processes are disrupted during the development of cancer. The lab is especially interested in developing and implementing new technologies to allow the refinement and improve the accuracy of cancer models. We collaborate on a wide variety of different projects and use a number of strategies, such as point mutations or conditional knockouts, to produce finely controlled alterations in gene activity. As examples, these include projects such as altering the activity of two genes simultaneously or tagging a protein of interest with a fluorescent marker (Fig. 1). More recently

we have started a number of project designed to allow us to control the level of gene expression in tumours directly. This should allow for example the validation of candidate genes as potential targets for drug development.

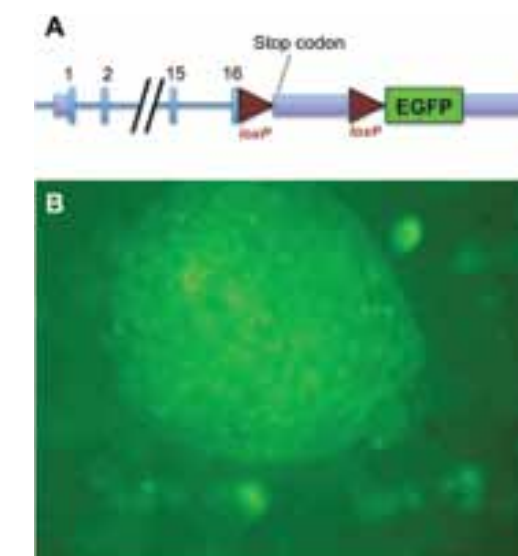
Using stem cells to model cancer

Once the desired genetic alteration has been introduced into stem cells, the cells can be phenotyped to assess the consequence of the desired mutation on the biology and behaviour of the cells. One advantage of using stem cells is that they can be differentiated in culture to produce a wide variety of cell types. So, the consequence of the mutation can be analysed not only by the stem cells themselves but in a variety of specialised cell types derived from them. In addition, it is possible to reverse the differentiation process and reprogramme a variety of somatic cells to induced pluripotent stem (iPS) cells. This process is reminiscent of anaplasia, the loss of differentiation frequently seen in cancer. Genes crucial for this type of reprogramming are often involved in cancer development. We are studying some of the genes involved in this process and some of the genes involved in maintenance of the stem cell phenotype.

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Figure 1 - Targeted locus incorporating inducible protein tag

(A) Diagram of the modified locus. Following deletion of the stop codon, by Cre recombinase activity at the loxP sites, EGFP is expressed as a fusion to the endogenous protein. (B) Expression of the EGFP fusion protein in a colony of embryonic stem cells.





BEATSON ASSOCIATES

UNIVERSITY OF GLASGOW

Peter D. Adams - Epigenetics of Cancer and Ageing
Daniel J. Murphy - Oncogene-Induced Vulnerabilities
Stephen Tait - Mitochondria and Cell Death

EPIGENETICS OF CANCER AND AGEING

www.gla.ac.uk/researchinstitutes/cancersciences/staff/peteradams/



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¹ joint with David Gillespie

The Adams lab is investigating the impact of chromatin structure on cell proliferation and, ultimately, tissue ageing and cancer. To investigate this hypothesis we are using cell and molecular approaches, animal models and whole-genome technologies. We are collaborating with clinicians to test the relevance and utility of our findings to better understand and combat common diseases of human ageing.

A specific focus of our research is the epigenetic basis of cellular senescence. Cell senescence impacts a variety of physiological processes, such as tumour suppression and wound healing, and also cell, tissue and organismal ageing. Using massively parallel DNA sequencing technologies (ChIP-seq, RNA-seq and methyl-seq) we are building a comprehensive map of the epigenetic landscape of senescent cells. From this map, we are generating and testing remarkable and exciting hypotheses regarding the interwoven epigenetic basis of ageing and cancer.

Cell senescence is an irreversible proliferation arrest instigated by a variety of molecular triggers including acquisition of activated oncogenes, and shortened telomeres caused by excess rounds of cell division. In addition, senescent cells secrete a cocktail of inflammatory cytokines, chemokines and matrix proteases (the 'inflammatory secretome' or senescence-associated secretory phenotype, SASP) that is capable of influencing behaviour of neighbouring cells, including immune cells. Compelling evidence now indicates that cell senescence is a potent tumour suppression mechanism, notably in cells harbouring activated oncogenes. Senescence-associated proliferation arrest and the SASP act in concert to achieve tumour suppression: proliferation arrest directly curtails tumour growth and the SASP calls on innate immune cells to eliminate the offending damaged cells. Because of senescence, most primary human cells have a finite proliferative lifespan, and evidence has been presented that senescence contributes to tissue ageing *in vivo*, in part by limiting the proper self-renewal of stem cells and tissues. In sum, cell senescence has both beneficial and

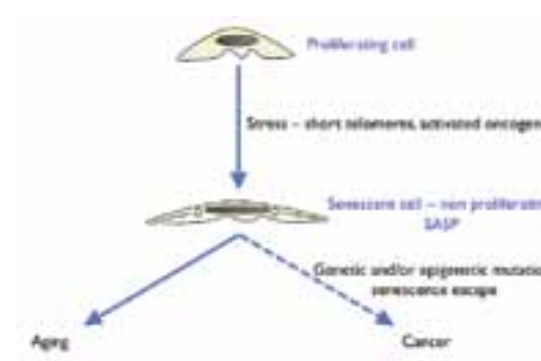
detrimental effects, and bypass of senescence can lead to tumour formation through uncontrolled proliferation of damaged cells (Fig. 1).

Cellular senescence, ageing and cancer are all accompanied by marked changes in chromatin structure. We are interested in the epigenetic changes associated with senescence, and their contribution to the senescent phenotype. In addition, since senescent cells promote ageing, we are testing the hypothesis that senescence-associated changes in chromatin structure contribute to age-associated changes in chromatin structure, and onset of diseases of ageing, including cancer.

The origin of epigenetic changes in cancer

To better understand the structure and function of chromatin in senescent cells, we are performing genome-wide analyses of histone modifications and DNA methylation to compare chromatin structure and gene expression profiles in proliferating and senescent cells. To do this, we are using massively parallel DNA sequencing technologies, ChIP-seq, RNA-seq and methyl-seq. To complement this analysis of epigenetic marks and the transcriptome in senescence, we are also exploring the genome-wide distribution of histone chaperones in senescent cells, again using state-of-the-art approaches. These genome-wide analyses are generating many exciting insights. For example, we have obtained compelling data to indicate that a shared hallmark of cancer cells and aged tissues (global genome hypomethylation and CpG island hypermethylation) originates in the pre-neoplastic senescent cell, underscoring the dual role of senescence in both ageing and cancer.

Figure 1 - Senescence as a tumour suppressor mechanism. Acquisition of an activated oncogene or inactivation of a tumour suppressor initially causes a proliferative burst. Ultimately, senescence kicks in to arrest proliferation of the cells harbouring the oncogenic event. Proliferation arrest is reinforced through the senescence-associated secretory phenotype (SASP). Senescence-associated proliferation is likely to arrest tumour progression by preventing proliferation of neoplastic cells and suppressing accumulation of additional genetic alterations. In addition, senescence recruits the innate immune system to clear the genetically altered cells that threaten the host with malignant disease.



Linking histone chaperones to tumour suppressors through large-scale epigenomics

DNA replication-independent nucleosome assembly pathways are likely critical for chromatin function and cell phenotype in non-proliferating cells, including senescent cells. The human HIRA/UBN1/CABIN1/ASF1a (HUCA) complex is thought to deposit the histone variant H3.3 into chromatin in a DNA replication-independent manner. Integrated ChIP-seq analysis of three subunits of HUCA revealed that the complex co-localises with histone H3.3 at gene transcription start sites and enhancers, and is required for H3.3 deposition at these sites. Our results also pointed a novel role for the SWI/SNF ATP-dependent chromatin remodelling complex, containing tumour suppressors BRG1 and INI1, in placement of histone variant H3.3. This has been confirmed by *in vitro* studies with purified recombinant proteins, showing that HUCA stimulates chromatin remodelling by SWI/SNF.

Barrett's oesophagus and oesophageal cancer

Barrett's oesophagus is a precursor of oesophageal adenocarcinoma, via intestinal metaplasia and dysplasia. Risk of cancer increases substantially with dysplasia, particularly high-grade dysplasia. Thus, there is a clinical need to identify and treat patients with early stage disease (metaplasia and low grade dysplasia) that are at high risk of cancer. Activated Wnt signalling is critical for normal intestinal development and homeostasis, but less so for oesophageal development. Therefore, in an active and ongoing collaboration with clinicians at the Glasgow Royal Infirmary (Lisa Moyes, Hamish McEwan,

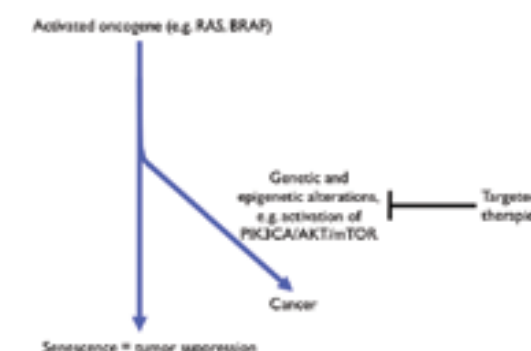


Figure 2 - Senescence, ageing and cancer.

At the cellular level, senescence is triggered by various molecular stresses. Cellular senescence contributes to a tissue-level response to these stresses, by arresting proliferation and promoting immune clearance of incipient cancer cells via the SASP. However, in the long term, senescence-mediated proliferation arrest and production of inflammatory cytokines in the SASP can damage the tissue, leading to tissue decline and ageing. Bypass or escape of cells from senescence can promote development of cancer.

Douglas MacKenzie, James Going and Grant Fullarton) we asked whether abnormally increased Wnt signalling contributes to the development of Barrett's oesophagus (intestinal metaplasia) and/or dysplasia. In human tissues, expression of nuclear activated β -catenin was found in dysplasia, particularly high grade. Barrett's metaplasia did not show high levels of activated β -catenin. Upregulation of Ki67 and Wnt target genes was also mostly associated with high-grade dysplasia. Aberrant activation of Wnt signalling in mouse oesophagus caused marked tissue disorganisation with features of dysplasia, but only selected molecular indicators of metaplasia. Based on these results in human tissues and a mouse model, we conclude that abnormal activation of Wnt signalling likely plays only a minor role in initiation of Barrett's metaplasia but a more critical role in progression to dysplasia. Ongoing studies are developing a comprehensive oesophageal cancer research programme, incorporating tissue microarrays, whole-exome sequencing, *in vitro* studies and mouse models.

Not all oncogenes are equal - a basis for oncogene cooperation

Given the significant role of senescence in tumour suppression, it is important to understand how the genetic alterations commonly found in human cancers interact to overcome the senescence barrier to tumorigenesis. Mutations in both RAS and the PTEN/PIK3CA/AKT signalling module are found in the same human tumours. PIK3CA and AKT are downstream effectors of RAS, and the selective advantage conferred by mutation of two genes in the same pathway is unclear. Based on a comparative molecular analysis, we have shown that activated PIK3CA/AKT is a weaker inducer of senescence than is activated RAS. Moreover, concurrent activation of RAS and PIK3CA/AKT impairs RAS-induced senescence. *In vivo*, bypass of RAS-induced senescence by activated PIK3CA/AKT correlates with accelerated tumorigenesis. Thus, not all oncogenes are equally potent inducers of senescence and, paradoxically, a weak inducer of senescence (PIK3CA/AKT) can be dominant over a strong inducer of senescence (RAS). For tumour growth, one selective advantage of concurrent mutation of RAS and PTEN/PIK3CA/AKT is suppression of RAS-induced senescence. In tumours harbouring activated RAS and inactivation of PTEN, inhibition of a downstream effector of the PIK3CA/AKT pathway, mTOR, restores cell senescence. Thus, our new understanding of interaction between the RAS and PIK3CA/AKT pathways might be exploited in rational development and targeted application of pro-senescence anti-cancer therapies (Fig. 2).

Publications listed on page 92



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Oncogenic signalling profoundly alters how cells respond to their environment, typically putting tumour cells under tremendous pressure to reconcile conflicting cues. For example, tumour cells must re-organise their metabolic pathways to balance competing needs for biosynthetic precursors with energetic homeostasis, commonly while surviving in a milieu of limiting oxygen and nutrients. Our overarching hypothesis is that such oncogene-induced biological perturbations can be exploited for cancer therapy, even in the absence of direct suppression of driver oncogenes. We use deregulated Myc as our paradigm oncogene coupled with a mixture of candidate and RNAi-based screening approaches to identify induced vulnerabilities *in vivo* and *in vitro* and are actively exploring several strategies for selective elimination of cells that overexpress Myc.

Myc in cancer

Overexpression of the transcription factor Myc occurs in a huge number of human cancers arising from almost every tissue type. Myc overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability or indeed increased signalling through upstream regulatory factors such as Ras, Notch or β -catenin. In a number of *in vivo* settings, Myc overexpression is sufficient to initiate or exacerbate tumorigenesis and moreover is typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits Myc overexpression would likely have a tremendous impact on human health.

Intrinsic tumour suppression

Some 20 years ago, work on Myc exposed the paradigm of intrinsic tumour suppression when it was shown that Myc overexpression in otherwise normal cells potently induces cell death. It has since been recognised that the interweaving of apoptosis and/or senescent signalling with the machinery of proliferation serves as a primary and general defence

mechanism to protect multicellular organisms from the detrimental effects of overexpansion of individual cell types. How then can oncogene expression initiate tumorigenesis? One mechanism through which emerging tumour cells evade such intrinsic restraints is by the acquisition of secondary mutations that either directly impair or indirectly circumnavigate specific tumour suppressive responses. Alternatively, we have previously shown that threshold levels of oncogenic signalling are required to trigger intrinsic tumour suppression: Myc expressed at near-physiological levels can drive ectopic proliferation of most cell types *in vivo* without provoking apoptosis. Importantly we also showed that this level of Myc could nonetheless sensitise cells to a second pro-apoptotic signal such as DNA damage. Thus, modest Myc overexpression induces a vulnerability to apoptotic stimuli that can potentially be exploited to eliminate such cells. One ongoing project aims to identify the mediators of Myc-induced sensitisation in different tissues to better establish the contexts in which apoptosis can be exploited for therapy.

Figure 1

Acute deregulation of Myc is sufficient to drive ectopic proliferation in terminally differentiated adult bronchiolar epithelium. Proliferating cells are labelled with BrdU (green), while CK19 (red) identifies mature bronchiolar epithelium. Note that the level of Myc required for this effect does not trigger apoptosis or induce expression of p19Arf, thereby effectively flying below the radar of intrinsic tumour suppression.

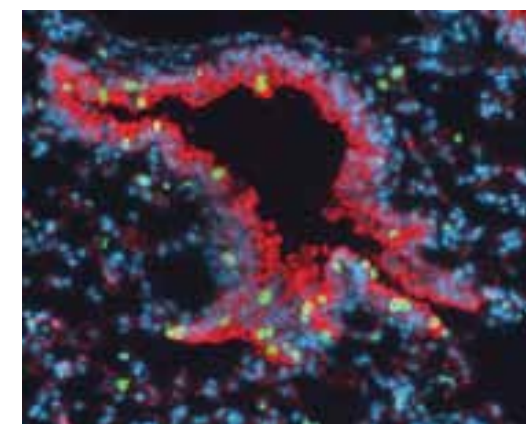


Figure 2

Overexpression of Ark5 in human pancreatic ductal adenocarcinoma, detected by immunohistochemistry (brown stain). All PDAC cases examined to date exhibit elevated expression of Ark5 when compared with adjacent normal tissue.

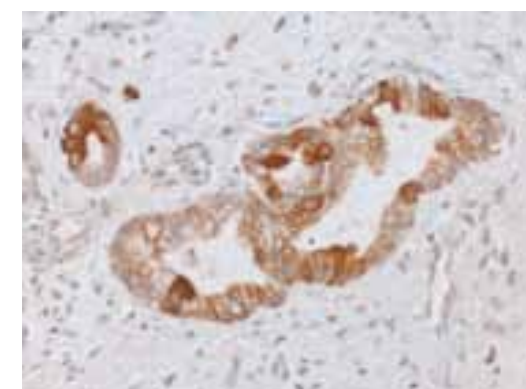
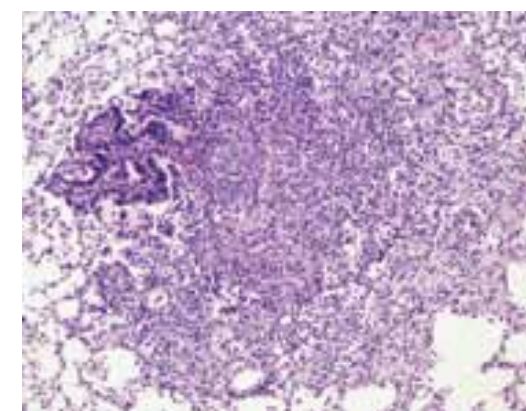


Figure 3

Progression of KRas-initiated lung tumours to aggressive disease in the presence of modestly elevated Myc expression. The darkly staining tumour region at 10 o'clock is relatively benign, well organised and well circumscribed; the paler region growing radially outwards contains clusters of adenocarcinoma embedded in a meshwork of tumour stroma.



Myc induced metabolic vulnerability: Liu et al., Nature 2012; 483: 608

Rather than attempting to target Myc directly, a number of groups including our own have turned to 'synthetic lethal' screening approaches in order to identify induced vulnerabilities, i.e. proteins and pathways to which cells overexpressing Myc – but, crucially,

not normal cells – are addicted for survival. Our siRNA screen of the human kinome surprisingly failed to return predicted cooperating oncogenes such as Ras or PI3K. Instead, we identified a number of metabolic regulators, including the AMP-activated protein kinase AMPK and the closely related Ark5 (aka Nuak1), as required for viability specifically when Myc is overexpressed. Why these kinases? Myc-driven biosynthetic processes cause cells to consume ATP at a much higher rate than normal and, despite elaborate reorganisation of carbon metabolism to balance ATP production with biosynthetic precursor provision, this engenders a state of energetic stress that leads to AMPK activation. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with production. For reasons that are presently unclear, Ark5 is required for this homeostatic feedback mechanism. Depletion of either AMPK or Ark5 thus leads to ATP collapse and consequently loss of viability, selectively in cells overexpressing Myc, strongly suggesting that targeting these kinases may be therapeutically effective against Myc overexpressing cancers.

Myc induced lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide and incidence of lung cancer is on the rise especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have developed a model for early tumour progression using tractable combinations of conditional alleles, including KRas and Myc. We are using laser-capture microdissection combined with gene expression analysis and functional screening to identify proteins with enzymatic activities that are required for progression from benign to malignant disease. The dual goal of this endeavour is to identify both new therapeutic targets and surrogates for early detection.

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MITOCHONDRIA AND CELL DEATH

www.gla.ac.uk/researchinstitutes/cancersciences
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Cell death is a key tumour suppressor mechanism that must be inhibited in order for cancer to develop. Importantly, cell death sensitivity also governs therapeutic efficacy because anti-cancer therapies often act by killing cells. The major form of programmed cell death is apoptosis, a process in which mitochondria play an essential role. Our research focuses upon understanding how mitochondria regulate cell death and addressing how this process is deregulated in cancer. Clinical translation of our findings will lead to improvements in existing anti-cancer therapies and the development of new approaches that permit tumour selective killing.

Mitochondria, cell death and cancer

Apoptosis requires caspase protease activation leading to widespread substrate cleavage and rapid cell death. During apoptosis, mitochondrial outer membrane permeabilisation (MOMP) occurs, a crucial event that is required for caspase activation. Following MOMP, mitochondrial intermembrane space proteins, such as cytochrome c, are released into the cytoplasm where they drive caspase activation and apoptosis. Given its key role in controlling cell survival, mitochondrial outer membrane integrity is highly regulated, largely through interactions between pro- and anti-apoptotic Bcl-2 proteins. Cancer cells commonly inhibit apoptosis by preventing MOMP, often through upregulation of anti-apoptotic Bcl-2 proteins or by inhibiting caspase activity downstream of MOMP. Newly developed anti-cancer therapies target these apoptotic blocks. For example, BH3-mimetic compounds exploit the Bcl-2 addiction of certain cancer cells leading to tumour specific killing.

Regulating the mitochondrial gateway to death

The mechanism of MOMP inhibition by Bcl-2 proteins is controversial. Two prominent models have been proposed: Bcl-2 proteins inhibit MOMP either through binding BH3-only proteins (a subfamily of Bcl-2 proteins that relay

the apoptotic signal to the mitochondria) or by binding Bax and Bak (the effector proteins that cause MOMP). Our recent data supports a unified model; under conditions of low apoptotic stress, Bcl-2 proteins inhibit MOMP by binding BH3-only proteins and under high levels of stress they block MOMP by binding activated Bax and Bak. Cells in which anti-apoptotic Bcl-2 proteins suppress MOMP by binding active Bax or Bak are much more resistant to the addition of BH3-mimetic compounds, a result that may have important clinical implications. We are currently investigating the relevance of these findings in a therapeutic context.

Cell survival following mitochondrial permeabilisation

Following MOMP, cells often die irrespective of caspase activation, suggesting that MOMP represents a 'point-of-no-return'. However, we have found that cells can sometimes survive and proliferate following MOMP. This may be important in cancer development and regulating therapeutic sensitivity as cancer cells often display defective caspase activity. We determined that survival following mitochondrial permeabilisation was dependent, at least in part, upon glycolysis and autophagy. More recently, using novel live cell imaging techniques, we have discovered that MOMP can be incomplete such that some mitochondria remain intact. Intact mitochondria are required

for cell survival following MOMP probably by serving as a source of healthy mitochondria that allows mitochondrial repopulation. We found that the ability of certain mitochondria to evade MOMP is due to increased anti-apoptotic Bcl-2 protein expression on their outer membrane. Following from this, treatment with BH3-mimetic compounds effectively induced complete MOMP and prevented clonogenic survival, a result that may have therapeutic relevance. Current work is aimed at addressing the importance of post-MOMP cell survival in tumorigenesis and treatment response using a variety of approaches including developing a means to report cell survival following MOMP *in vivo*. Secondly, we are investigating the mechanisms that enable cell survival, focusing upon the protective role of autophagy in this process.

independent cell death, necroptosis and mitotic cell death. These alternate forms of cell death function as either back up pathways in the event of failed apoptosis or are distinct cell death programmes in themselves. Although these cell death modalities are likely important in cancer (particularly in response to therapy) little is known about their underlying molecular mechanisms. We are currently addressing the hypothesis that mitochondria play central roles in non-apoptotic cell death by disrupting mitochondrial functions or generating mitochondria-free cells and asking if non-apoptotic cell death is affected (Fig. 1). These approaches will form a starting point for understanding the molecular mechanisms regulating non-apoptotic cell death and their role in cancer.

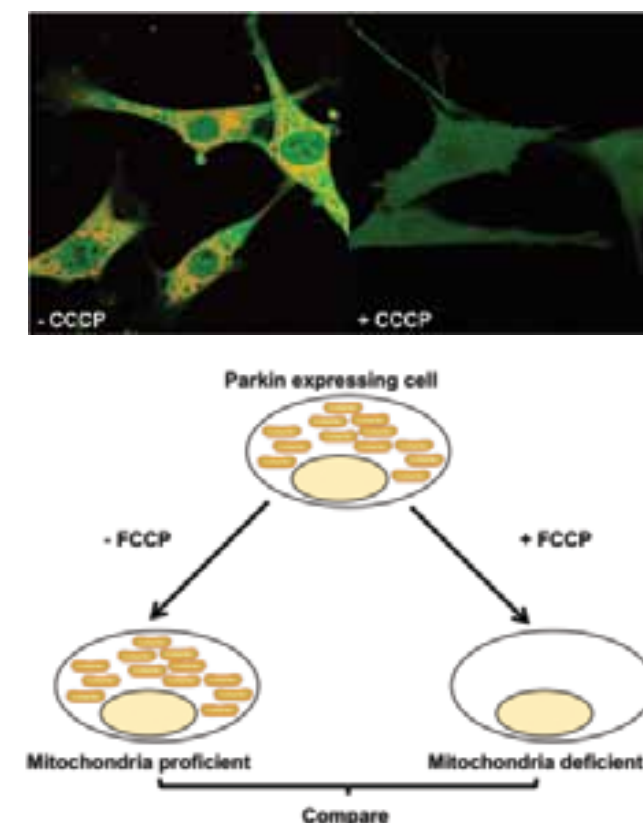
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Mitochondria and non-apoptotic cell death

A variety of other forms of programmed cell death exist besides apoptosis, including caspase

Figure 1 - A new approach to defining mitochondrial importance in cell death and beyond.

Top: Following mitochondrial uncoupling (CCCP treatment) the ubiquitin E3 ligase Parkin (green) can effectively and specifically deplete mitochondria (red) through a process termed mitophagy. Bottom: Using this approach, we can address the importance of mitochondria in cell death and other processes by comparing mitochondria proficient and deficient cells.





RESEARCH GROUPS

UNIVERSITY OF GLASGOW

Tessa Holyoake - Paul O’Gorman Leukaemia Research Centre
Jeff Evans - Institute of Cancer Sciences



Head
Tessa
Holyoake FRSE

The haemopoietic system provides a unique tractable model for investigating how normal cell functions are altered by oncogenes to cause cancers. Leukaemias are devastating diseases of the haemopoietic system that arise from normal stem and progenitor cells that have acquired corrupted cell fate decisions. These aberrant cells, called leukaemic stem cells (LSCs), initiate and drive the process of leukaemogenesis.

Current therapies fail to efficiently eradicate LSCs and therefore new curative treatments need to be identified. The research programmes within the Paul O'Gorman Leukaemia Research Centre (POG-LRC) collectively focus on (i) understanding the fundamental mechanisms governing normal haemopoietic stem cell (HSC) and LSC functions, (ii) identification of novel therapeutic targets against LSCs and (iii) exploiting these targets in clinical trials.

Normal HSCs reside in niches within the bone marrow and have a unique capacity to sustain life-long multilineage haemopoiesis (Fig. 1). HSCs face tightly orchestrated cell fate decisions between quiescence, self-renewal, apoptosis and differentiation. Strict regulation of these fates is essential to maintain the adult HSC pool and dysregulation of the balance between these fates is a common feature of blood malignancies. LSCs have gained the capacity for enhanced self-renewal, increased cell survival and defective differentiation, and instead of generating normal blood lineages they give rise to leukaemias. Since current therapies often fail to eradicate LSCs, it is of paramount importance to uncover the mechanisms governing LSC functions. We hope that our investigations will assist in the identification of curative therapies aimed at eradicating LSCs.

The POG-LRC provides a multidisciplinary and collaborative setting that brings together basic and clinical scientists. Our research covers multiple aspects of haematology including normal HSC biology, and myeloid and lymphoid malignancies.

Mhairi Copland

Chronic myeloid leukaemia (CML) is an HSC disorder, characterised by the Philadelphia chromosome and its unique fusion gene product BCR-ABL, a constitutively active tyrosine kinase. Standard therapy for CML is the BCR-ABL tyrosine kinase inhibitor (TKI) imatinib (Glivec™). However, despite producing long term disease control in the majority of patients in chronic phase, imatinib does not cure CML. Aberrant LSC self-renewal and altered bone marrow microenvironment or 'niche' allows the LSCs to survive the most effective chemotherapeutic strategies (including TKIs) available today, resulting in refractory leukaemia or relapses. We investigate differences between normal HSC and CML stem cell functions. With this information, we will be in a position to target CML stem cells in a selective manner and identify agents that will work alongside TKIs to eradicate CML stem cells. We employ a range of techniques including fluorescence activated cell sorting, gene and protein expression analysis, and cell biology approaches to assess the differences between normal and leukaemic cells. Small molecule inhibitors are increasingly becoming available to target essential stem cell pathways. We aim to target the identified pathways in order to eradicate CML stem cells.

Projects include:

- Investigation of the control of cell division and quiescence in leukaemic versus normal haemopoietic stem and progenitor cells;
- Hedgehog signalling as a LSC self-renewal pathway in CML;
- Development of a biomarker assay for the assessment of the Hedgehog pathway in haematological malignancies;

- Investigation of TKI-induced quiescence in CML stem cells;
- Antagonising the CXCR4/SDF-1 interaction in CML for sensitisation to TKI.

Tessa Holyoake

Small molecule TKIs such as imatinib form the standard therapy for CML, however we have shown that CML stem and progenitor cells are insensitive to these drugs, which cause cell cycle arrest rather than programmed cell death resulting in disease persistence in patients. CML is therefore very unlikely to be cured using TKI monotherapy. Our research focuses on novel approaches including combination therapies to eradicate the LSC population in CML. Uniquely, we have a large biobank of normal and leukaemic cells and are able to purify the stem cells of interest from clinical samples for study.

Our recent work has shown that the rare, primitive and transplantable CML LSCs are insensitive to the action of TKI as the cells are not dependent on BCR-ABL for their survival unlike mature (differentiated) white blood cells. This means that alternative targets need to be found in order to efficiently eradicate persistent LSCs without damaging normal HSCs. Quiescence, a property of normal stem cells, is a key factor in LSC drug resistance. Mechanisms that maintain CML LSCs out of the cell cycle, for example the dephosphorylation and subsequent activation of a protein called FOXO in the presence of imatinib through its inactivation of BCR-ABL, may represent a novel target pathway for designing new therapies.

In our previous gene expression studies, chemokine expression was highlighted to be central to the quiescence of normal HSCs and LSCs. We are examining which chemokines are highly expressed in quiescent HSCs and what effect modulating their expression has on cell cycle. Analysis for significant differences in gene regulation of potential survival factors for CML LSCs versus normal HSCs identified HLA class II family to be downregulated in CML. We are interested to know how the latter is regulated in LSCs and if modulation of MHC-II expression can sensitise CML LSCs to be more immunogenic. Further mRNA and microRNA profiling is under investigation to identify a tractable set of genes regulating proteins/ pathways important for TKI resistance in CML stem cells, which could then be targeted with a second agent. We are also looking by proteomic methodologies at the signatures of TKI treated CML LSCs. There is growing evidence of a role for JAK2/STAT5 in survival of CML LSCs, indeed our data suggest the combination of a JAK2 inhibitor with TKI is more effective than single agent in terms of cytotoxicity and inhibition of signalling in CML LSCs. A number of candidate proteins shown to change in response to the

combination of JAK inhibitor plus TKI with respect to TKI alone are currently being validated as targets.

We have shown that TKIs induce autophagy (the normal physiological process of breakdown of intracellular components to maintain balance of cellular products) as a survival pathway in BCR-ABL+ cells, including primary CML LSCs. Suppression of autophagy by pharmacological inhibitors or knockdown of essential autophagy genes enhances TKI-induced cell death in primary CML cells. Critically, the combination of TKI with autophagy inhibitors results in near complete elimination of CML LSCs *in vitro*. Together, these findings suggest that autophagy inhibitors will enhance the therapeutic effects of TKI in the treatment of CML patients. This preclinical work has now progressed to an MRC sponsored phase 2 clinical trial investigating the addition of hydroxychloroquine to imatinib to reduce minimal residual disease levels in patients. In the laboratory, we are extending our work to investigate if autophagy serves as a survival mechanism following inhibition of BCR-ABL/PI3K/AKT/mTOR pathway in TKI-sensitive and resistant (BCR-ABL mutant) CML LSCs and if autophagy can be targeted with specific inhibitors in preclinical development.

Karen Keeshan

Acute lymphoblastic leukaemia (ALL) is the leading cause of childhood leukaemia while acute myeloid leukaemia (AML) accounts for 20% of childhood leukaemia and is the number one form of adult leukaemia worldwide. Despite this, AML still has the lowest survival rate of all forms of leukaemia (23.4% compared to 66.3% for ALL). AML, B- and T-ALL have differing clinical courses and distinct responses to therapy. The heterogeneity of the disease underlies the variable response of individual patients to therapy. Paediatric AML has an overall survival of ~60-70% but the risk of relapse is ~40%. Given the poor prognosis of paediatric AML and the significant cardiotoxicity of standard therapy, novel therapies are needed. Current therapies are quite effective at killing the bulk leukaemic populations but are not as effective at killing the leukaemia stem cell, which may differ between adults and children and is yet to be specified.

Understanding the molecular aberrations that occur during cellular leukaemic transformation is key to the advance of new, targeted therapies. Central to our understanding of the pathogenesis of leukaemias has been genetically modified mouse models and pre-clinical mouse models that recapitulate cellular transformation. Trib proteins are emerging as central modulators of survival and differentiation pathways, involving MAPK signalling and C/EBP transcription factors. In the

human AML disease, elevated Trib2 is associated with a leukaemic subset with a mixed myeloid and lymphoid phenotype. We have shown previously that Trib2 is a Notch1 gene target, and this has important implications as activating Notch1 mutations are found in 50% of T cell acute leukaemia. Additionally, our data demonstrates that E2F1, 3 and 4 are potent inducers of Trib2, and E2F1 cooperates with C/EBP α -p30 (dominant negative truncated C/EBP) to further activate Trib2 expression. Overexpression of Trib2 in haemopoietic progenitors drives robust murine AML and can cooperate with HOXA9 to accelerate AML. Trib2 (and Trib1) -induced AML occurs through inactivation of C/EBP α , a transcription factor responsible for granulocytic differentiation and blocking of cellular proliferation. Trib2 inactivates C/EBP α by inducing proteasomal degradation. Recently, it was shown that terminal differentiation of FLT3ITD leukaemic cells by FLT3ITD inhibitors required functional C/EBP α . ATRA induced differentiation in AML also requires functional C/EBP α . These data highlight the important role of C/EBP α in AML therapy and place Trib proteins in a central role in AML signalling pathways. Therefore, Trib2-induced murine AML is a very useful model to dissect 1) the role of Trib2 in the induction of AML 2) the role of Trib2 signalling pathways in chemoresistance and 3) the signalling pathways dysregulated in AML that can be therapeutically targeted.

Projects include:

- Establishment of *ex vivo* culture and *in vivo* transplant models of paediatric and adult primary AML cells to assess novel therapeutics and their efficacy in blast and AML 'stem' cells, and differences in adult and paediatric stem cell phenotypes;
- Investigation of the prognostic implications and chemotherapeutic profile of elevated Trib in adult and paediatric AML and ALL. Investigation of how to therapeutically target the adult and paediatric AML chemoresistant cell;
- Investigation of Trib2-mediated effects on downstream signalling pathways such as C/EBP α , cell cycle regulators p53 and CDC25, and cooperative effects with e.g. C/EBP α p30, EVI1, HoxA9, PML-RAR and Notch1, and the resultant effects on the leukaemic phenotype and the chemotherapeutic responses.
- Investigation of the role of Trib2 in HSC function and ageing;
- Establishment of the role for E2F1 in AML cells. Investigation of the role of E2F1-Trib2 network in AML;
- Investigation of myeloid, B and T cell transcription factors in lineage choice and

development and their role in leukaemic transformation.

Kamil Kranc

We investigate mechanisms orchestrating HSC fate decisions and study how these decisions are corrupted to generate LSCs. While HSCs reside in the hypoxic bone marrow microenvironment, the impact of hypoxia and hypoxia-dependent pathways on normal HSC/progenitor fate decisions and leukaemogenesis remains largely unexplored. We found that Cited2, a direct target gene of HIF-1 and HIF-2, functions as a selective and essential regulator of adult HSC maintenance (Kranc *et al.*, Cell Stem Cell 2009; 5: 659). We subsequently found that Cited2 is induced during leukaemogenesis and is required for the generation of LSCs. We are currently investigating the molecular mechanisms through which this happens.

In another collaborative study, we investigated the role of a hypoxia-inducible process of autophagy in HSC functions (Mortensen *et al.*, J. Exp. Med. 2011; 208: 455). Autophagy is a process by which cells degrade their own components and is critical for their metabolic homeostasis. We demonstrated that conditional deletion of the essential autophagy gene Atg7 results in loss of HSCs and in a multilineage bone marrow failure. HSCs lacking autophagy accumulate damaged mitochondria, have increased production of reactive oxygen species and undergo apoptosis. We propose that the hypoxic stem cell niche microenvironment promotes autophagy in HSCs to sustain their life-long integrity.

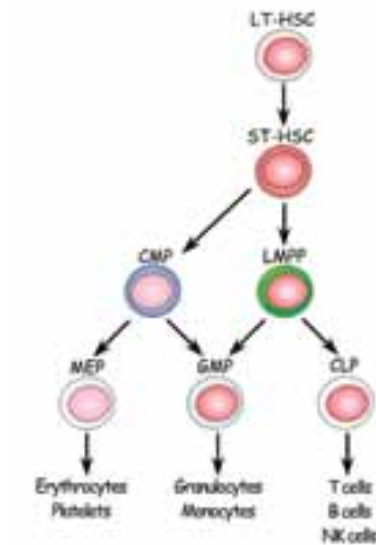
Taken together, these studies suggest that the strict regulation of HSC responses to hypoxia is essential for their normal functions. Ongoing investigations in our laboratory address how hypoxia signalling pathways are subverted to cause leukaemias. Finally, we hope that by exploiting the strengths of LSCs as a model system our studies will reveal important common mechanisms operating in other cancer stem cells.

Alison Michie

Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in the Western world, for which there is currently no chemotherapeutic cure. Despite the significant progress made recently in the understanding of the biology of CLL, the cause of CLL remains elusive and no single genetic event has been identified. While investigating the role of protein kinase C- α (PKC α) in early B lymphocyte development, we established that subversion of PKC α , by expression of a dominant-negative PKC α construct (PKC α -KR), acts as an oncogenic trigger for B cells. Indeed, culturing

Figure 1 - Haemopoietic differentiation hierarchy.

Normal HSCs (long term HSCs, LT-HSCs) are positioned at the apex of the hierarchy and differentiate to primitive and committed progenitors, which eventually give rise to all blood lineages. LSCs arise from HSCs and/or progenitors that have acquired uncontrolled self-renewal capacity. Abbreviations: ST-HSCs, short-term HSCs; CMP, common myeloid progenitor; LMPP, lymphoid-primed multipotent progenitors; CLP, common lymphoid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythroid progenitor.



transduced HSCs in B cell generation systems *in vitro* and *in vivo* results in the formation of a population of cells that bear hallmark characteristics of human CLL cells at the level of phenotype, cell cycle phase (G_0/G_1 *ex vivo*), and resistance to apoptosis. Moreover, PKC α -KR-expressing cells possess an enhanced proliferative capacity both *in vivo* and *in vitro*, reflecting the dynamic cellular kinetics that exist during progressive human CLL. Mouse CLL cells generated *in vivo* accumulate in the lymphoreticular system similar to that noted in CLL patients, demonstrating the applicability of this mouse model to the human disease. These findings have prompted us to develop this mouse model to test potential novel therapeutic agents, which show promise upon treatment of patient-derived CLL samples in *in vitro* systems that replicate pro-survival and/or proliferative conditions *in vivo*. Taken together, we have developed translational models systems to carry out preclinical studies with novel drugs, with the aim of providing improved treatments for CLL that can elicit a cure.

Helen Wheadon

Our research focuses on regulation of haemopoiesis, in particular the role of the Wnt signalling pathway and how active tyrosine kinases alter the signalling and transcriptional networks to deregulate myelopoiesis. Myeloproliferative disorders often arise due to a constitutively active tyrosine kinase, with TEL-PDGFR β in chronic myelomonocytic leukaemia (CMML), BCR-ABL in CML and V617F Jak2 in polycythaemia vera (PV) being the best characterised. Although these tyrosine kinases activate similar downstream signalling pathways, the aetiology of the diseases are distinct with TEL-PDGFR β causing a myeloproliferative disorder involving monocytic cell accumulation often associated with eosinophilia, BCR-ABL resulting in the accumulation of neutrophilic granulocytes and

V617F causing an erythroid disorder. Previously, we performed a high throughput proteomic screen to identify the initiating events involved in leukaemic transformation by TEL-PDGFR β . This revealed an upregulation of several IFN γ response proteins, including interferon consensus binding protein (ICSBP). This aberrant increase in IFN γ signalling is in sharp contrast to patients with BCR-ABL induced CML, who have reduced levels of ICSBP and corresponding low levels of PML and high levels of the anti-apoptotic protein BCL-2, suggesting that ICSBP has tumour suppressor activity. In order to investigate whether ICSBP actually has tumour suppressor or tumour enhancer properties, we are using human embryonic stem (ES) cells and patient derived induced pluripotent stem cells to model CMML, CML and PV to analyse how important IFN signalling is in these disease phenotypes.

Wnt signalling plays a crucial role during developmental haemopoiesis and is implicated in adult haemopoiesis, regulating both the proliferation and self-renewal capacity of HSCs and multipotent myeloid progenitors (MMPs). Using mouse models and ES cells we are investigating the role of canonical Wnt signalling in haemopoiesis and lineage commitment during haemopoietic ontogeny. By overexpressing a constitutively active form of β -catenin, a downstream mediator of canonical Wnt signalling, or inhibiting GSK3 β to activate the pathway we are monitoring how this alters early differentiation decisions. In particular we are focusing on how activating the pathway changes expression of mesodermal, haemangioblast, HSC and haemopoietic lineage commitment by assessing individual cell populations isolated during key stages of haemopoietic differentiation for surface markers, proteomic and transcriptome alterations. Deregulation of Wnt signalling is an emerging event in both myeloid and lymphoid leukaemias especially CLL. In CLL we have identified an important role for non-canonical Wnt signalling that regulates a multitude of genes including cytoskeletal filaments, involved in cell migration and intracellular signalling. We have shown a striking correlation between high levels of the intermediate filament vimentin and poor prognosis in CLL. Stimulation of CLL cells with non-canonical Wnts upregulates vimentin, which associates with several signalling molecules involved in cell survival. Using peptide aptamer technology, a new generation of 'biologic therapeutics', we aim to block the essential sites for vimentin assembly and its interaction sites with these signalling molecules to determine what role vimentin plays in conferring apoptosis resistance in poor prognosis CLL patients.



Head
Jeff Evans

The Institute of Cancer Sciences (ICS) spans basic cancer biology, drug discovery, translational, and clinical cancer research as well as three campuses, at the Gartcube, Gartnavel and Southern General. Its primary goal is to deliver world-class research that can be translated to patient benefit, contribute to developing research leaders of the future and provide a leading-edge environment for research and training.

The ICS consists of a number of research units, which are described here and include the Paul O’Gorman Leukaemia Research Centre (see page 66). Notable achievements in 2012 included the renewal of the CR-UK Clinical Trials Unit’s programme and the completion of the Translational Cancer Research Centre (TCRC) building. We welcomed Iain McNeish to a Chair of Gynaecological Oncology, greatly strengthening our long-standing interests in ovarian cancer and experimental cancer therapeutics. Karen Keeshan, Daniel Murphy and Stephen Tait joined as Senior Lecturers, Nicola Valeri as a Clinical Lecturer in Medical Oncology and Chiara Braconi as a Lord Kelvin Adam Smith University Fellow. Kamil Kranc was awarded a CR-UK Senior Cancer Research Fellowship and both he and Mhairi Copland were promoted to Readerships. Prabs Rajan was awarded a CR-UK Clinician Scientist Fellowship and Chiara Braconi a Scottish Senior Clinical Research Fellowship from the Academy of Medical Sciences, to commence in 2013.

We were delighted to recruit Andrew Biankin to the Regius Chair of Surgery and Directorship of the TCRC. Both he and David Chang (Senior Clinical Lecturer in Surgery) will take up post in 2013. Andrew is currently the Head of the Pancreatic Cancer Research Programme at the Garvan Institute of Medical Research in Sydney, and his appointment will greatly strengthen our research interests in pancreatic cancer and further develop our interests in medical genomics. The ICS has long-standing links with our research-active NHS colleagues, and Brenda Gibson and Nick Reed were appointed Honorary Professors at the University in 2012. These links were further consolidated with the

allocation of NHS Research Scotland awards from the Chief Scientist Office to NHS consultants Andrew Clarke, Ros Glasspool, Hilary Glen and Stephen Harrow.

Epigenetics Unit (Head: Peter Adams)

The malignant properties of cancer cells have their origins in DNA mutations and other forms of genetic damage but also in epigenetic changes. These are heritable through cell division but are not directly coded in the DNA. Instead, they are coded within chromatin, through DNA methylation, non-canonical histone variants, histone modifications, histone binding proteins and higher order patterns of chromatin folding. We are interested in basic mechanisms of chromatin regulation, epigenetic inheritance and how epigenetic dysfunction contributes to cancer and response to cancer therapies.

Peter Adams

See page 58.

Rob Mairs

Our goal is to improve the poor survival rate of childhood neuroblastoma patients with advanced disease. Targeted radiotherapy is the selective irradiation of tumour cells by radionuclides conjugated to tumour-seeking molecules. To date, the most promising agent is [¹³¹I]MIBG, an analogue of adrenergic neurone blockers, that is selectively concentrated in neuroblastoma cells via the noradrenaline transporter. Our experimental observations of synergy between [¹³¹I]MIBG and topotecan without marrow toxicity provided the rationale for current combination approaches applied

clinically. In our current research (supported by the Neuroblastoma Society, Molecular Insight Pharmaceuticals, Children with Cancer UK and Great Ormond Street Hospital Children’s Charity and Sport Aiding Medical Research for Kids), we have observed that inhibitors of poly(ADP-ribose) polymerase and the 26S proteasome interact with [¹³¹I]MIBG and topotecan treatment to give supra-additive kill of neuroblastoma clonogenic cells and enhanced survival of athymic mice bearing human neuroblastoma xenografts. This indicates the possibility of tripartite treatment to achieve the greatest benefit from targeted radiotherapy.

Paul Shiels

Glasgow has an extremely steep socioeconomic gradient with associated mortality and morbidity, unexplained by conventional risk factors for disease. The biological pathways underlying this ‘Glasgow effect’ are not well defined. We previously demonstrated that accelerated biological age is a feature of many diseases in Glasgow including cancer, and that lower socioeconomic status and poor diet can accelerate biological ageing and predispose to early onset of disease. Notably, we showed that significant associations exist between epigenetic status and emerging markers of disease risk. This association appears to be established *in utero* and recent data from this lab indicates that such epigenetic status has significant impact on cognitive ability, physical fitness and the expression of biomarkers of ageing in old age. We have also undertaken translational studies in a number of interdisciplinary collaborations to investigate mechanistic links between biomarkers of ageing and disease progression and outcome. These have identified a bio-ageing gene as a key mediator of the Warburg effect in cancer and cellular senescence as a mediator of rejection phenomena in renal transplantation. This is now being evaluated clinically.

David Vetrie

In chronic myeloid leukaemia (CML), a subpopulation of BCR-ABL⁺ cells in the bone marrow niche exhibit properties of haematopoietic stem cells (HSCs) (quiescence and self-renewal), and are resistant to current therapies with tyrosine-kinase inhibitors (TKIs). Our aim, in collaboration with Tessa Holyoake, has been to obtain a global view of the epigenetic processes involved in maintaining the stem cell population in CML (leukaemic stem cells, LSCs). We demonstrated that expression levels of the pathways involved in stem cell identity are not significantly different in LSCs to those found in HSCs. These pathways include Wnt and TGF-beta signalling, and several neurotransmitter signalling pathways that we have shown are capable of promoting

the maintenance and quiescence of LSCs *in vitro*. By examining genome-wide histone modification patterns using ChIP-sequencing, we showed that these stem cell pathways are heavily dependent on polycomb proteins and enriched for both the activating H3K4me3 and repressive H3K27me3 marks. Whilst repression of stem cell identity is mediated through the same pathways in both normal development and CML, intriguingly, epigenetic reprogramming in CML mediates this repression via a different polycomb mediated mechanism. Our analysis has defined the chromatin landscape of a cancer stem cell for the first time and provides new therapeutic targets for the eradication of TKI resistant CML stem cells.

Adam West

Whole genome profiling of histone modifications, chromatin factors and nuclear proximity has revealed prevalent organisation of genes into domains, or neighbourhoods, of common chromatin state. This domain organisation is radically altered in cancer cells. The maintenance of chromatin domain integrity requires the setting of boundaries that can be established by DNA sequence elements called insulators. We studied how the USF transcription factor establishes chromatin boundaries by manipulating the language of histone modifications, and found that USF recruits the candidate tumour suppressor RNF20 to direct histone H2B mono-ubiquitination at the beta-globin HS4 insulator element. Disruption of RNF20 recruitment leads to the loss of a panel of histone modifications at HS4 and a collapse of chromatin boundary integrity. We showed, in collaboration with Marie-Noëlle Prioleau (Jacques Monod Institute, Paris), that the activities recruited by USF also act to control early replication timing, providing further evidence for the coordination of chromosomal domains by insulator elements. We are currently investigating the role of H2B ubiquitination in chromatin boundary integrity across the human genome (funded by BBSRC).

Katherine West

Epigenetic mechanisms are fundamental to the maintenance of stem cell pluripotency and regulation of differentiation. The chromatin structure in embryonic stem (ES) cells is different to that of somatic cells, appearing to be less condensed, and permissive for low-level transcription of many, if not all, somatic genes. Key developmental genes also have a distinct epigenetic signature in ES cells that is thought to regulate their expression during various differentiation pathways. We focus on a family of small chromatin-binding proteins called the Hmgn proteins that influence both chromatin structure and epigenetic marks. Hmgn2 is essential for early embryonic development, and

our current data shows that Hmgn proteins are crucial for maintaining the expression of key pluripotency genes (Oct4, Nanog and Sox2) in embryonal carcinoma cells that are very similar to ES cells. Loss of expression of these genes is known to cause ES cells to lose their properties of self-renewal and pluripotency. We also show that Hmgn proteins are very highly expressed in the dividing neural progenitor cells in the developing brain, and that they are important for maintaining the correct pattern of gene expression in differentiating neurons *in vitro*. We are using genome-wide and gene-specific approaches to investigate how Hmgn proteins regulate chromatin structure, gene expression and cell identity during ES cell differentiation.

Experimental Therapeutics (Head: Jeff Evans)

Current strategies in the development of new therapies for malignant disease are based on exploiting our increasing understanding of the molecular and cellular basis of cancer development and progression. We aim to identify novel therapeutic targets from molecular pathology studies of pre-malignant and malignant tissue specimens, validating these targets through *in vitro* and *in vivo* studies, identifying and characterising the signalling pathways that are deregulated at the various stages of cancer development and progression, exploiting these pathways as potential therapeutic targets, and identifying biomarkers of anti-tumour activity to specific agents that can be applied to biological samples in clinical trials. The main areas of focus are in cellular senescence and cancer cell invasion and metastasis, with a particular emphasis on gastro-intestinal and endocrine tumours.

Analytical Services Unit (Jeff Evans)

The ASU is the centrepiece of the Glasgow ECMC and supports our work in translating ideas from the laboratory to the clinic. Sample acquisition, handling, processing and reporting are all performed to standards that allow us to make important decisions on the drug development pathway based on the results of these pharmacodynamic studies. In particular, this function has been applied to the CR-UK supported clinical trials of epigenetic therapies, mainly in ovarian cancer, and we continue to have a strong collaboration with the Scottish Gynaecological Cancer Trials Group. In addition, the ASU has a crucial role in support of the early phase clinical trials programme allowing us to apply our understanding of the scientific basis of cancer to answer clinically relevant questions. This includes support for a CR-UK study in patients with carcinoma of unknown primary origin in collaboration with the NCRN Upper GI Group and Karin Oien. It

also performs pharmacokinetic (PK) assays in support of an MRC multicentre phase 2 study in patients with chronic myeloid leukaemia, led by Tessa Holyoake, and PK assays of docetaxel in patients with prostate cancer. We continue to investigate the role of blood biomarkers in the management of patients with gastro-intestinal cancers. Specifically, we are investigating the role of markers associated with cellular senescence in blood samples from patients with gastro-oesophageal cancer during chemotherapy with clinical colleagues in Scotland and Belfast, and in collaboration with Nicol Keith, and are supporting the first clinical trial under the umbrella of the Astra Zeneca-ECMC Combinations Alliance, investigating the addition of FGFR inhibitors to standard chemotherapy in patients with advanced gastro-oesophageal cancer. Exploratory studies of novel biomarkers have been initiated in collaboration with Laura Machesky (pancreatic and colon cancer), Gerry Graham (University of Glasgow; melanoma) and Chiara Braconi (HCC).

Nicol Keith

Senescence is an irreversible arrest of cell proliferation and provides a barrier to excessive cellular growth. It is triggered by four main mechanisms; telomere-dependent replicative senescence, cell stress, oncogene induced senescence and drug induced senescence-like phenotype. As senescent cells do not proliferate, it has been proposed that cellular senescence is a major barrier to cancerous transformation, and premature induction of cellular senescence can therefore be targeted for anti-cancer therapy in tumorigenic cells and/or as a cytostatic mechanism to prevent further proliferation. As our understanding of the genes and pathways regulating senescence grows, so will the pool of potential targets for cancer therapies. Cancer cell senescence is currently an underserved area in drug development. Over recent years interest in the application of a pro-senescence therapy for oncology has gained momentum and this has been fuelled by increasing evidence of the importance of senescence as a block to tumour progression. Our research involves a senescence screening programme consisting of both target and drug-hunting activities. This approach is uncovering new biological understanding of cancer cell senescence and immortality and a series of targets for drug development.

Iain McNeish

We focus on two areas in ovarian cancer. The first is the development of novel biological therapies, especially oncolytic virus therapies. Specific areas include identifying the role of genomic DNA damage responses in the

response to adenovirus infection, the mode of cell death following virus infection in malignant cells and the role of innate immune responses in modifying virus efficacy in ovarian cancer. With funding from the Technology Strategy Board, and in collaboration with PsiOxus Therapeutics, a phase 1 trial of ColoAd1, an oncolytic Ad11 vector, in recurrent ovarian cancer will commence in Glasgow in 2013. Secondly, we aim to identify biomarkers of response in high-grade serous ovarian cancer, the commonest subtype of the disease. In collaboration with James Brenton (CR-UK Cambridge Research Institute), we lead BriTROC, the UK Ovarian Cancer Translational Collaborative, funded by Ovarian Cancer Action, and we have recently been awarded a CR-UK programme grant to explore defective homologous recombination in recurrent high-grade serous disease and the use of circulating tumour DNA as a biomarker of response.

Joanne Edwards

We aim to ensure that patients receive maximum benefit by identifying novel therapeutic targets and establishing clinical biomarkers that are able to best predict and monitor response to targeted therapies, using an *in vitro* approach but also utilising clinical cancer specimens linked to full clinical follow-up data. We focus on the mechanisms underlying the development and progression of breast and prostate cancer, using a twinned human tissue and cell line based approach. Our emphasis is on the role of Src kinase family members, phosphorylation of oestrogen and androgen receptors, and the IKKs/NF- κ B signalling cascade. Having established a clinically relevant biomarker, we investigate its functional effect and regulation. We then establish a unique set of genes under control of the biomarker and translate these back to our clinical cohort at the protein level to confirm which have prognostic value. We aim to identify novel therapeutic targets but also develop predictive tests to ensure that patients receive maximum benefit. We have established tissue microarrays for prostate, breast, renal and colorectal cancer that are linked with patient information to provide unique follow-up data. In a cohort of prostate cancer patients, this approach identified that phosphorylation of the androgen receptor at serine 515 is associated with decreased disease-specific survival and is independent of known clinical parameters. This site is phosphorylated in response to CDK1 and could potentially provide a novel biomarker and a novel therapeutic target for treatment of prostate cancer. We are currently collaborating with the Pathology department to extend and improve our clinical cohorts.

Iain MacPherson, a Senior Clinical Lecturer in Medical Oncology, is developing translational research in breast cancer as part of Jim Norman's group and in collaboration with Karen Blyth. The way in which proteins are trafficked to and from the cell membrane and between intracellular compartments is emerging as an important contributor to the malignant phenotype in breast cancer. He is deploying a variety of techniques to characterise the role of recently identified regulators of endocytic trafficking such as Rab-coupling protein and CLIC3 and identify cargoes of these proteins that are of particular relevance to breast cancer. He has also initiated a clinical trials research programme in patients with advanced breast cancer, particularly in the development of novel agents for patients with advanced disease.

Nicola Valeri

Our research focuses on the role of microRNAs (miRs) in colorectal cancers. Initially, our efforts focused on understanding the role of miRs in causing microsatellite instability (MSI). About 10-15% of MSI tumours lack a genetic or epigenetic event able to explain MMR (mis-match repair) deficiency. We showed that two miRs frequently upregulated in colon cancer, miR-21 and miR-155, could cause downregulation of core MMR proteins causing MSI and tolerance to fluorouracil *in vitro* and *in vivo*. A prospective case-control study by Albert de la Chapelle is currently ongoing to validate these findings in human colon cancer. We also studied the mechanisms by which chromosomal instability can cause miR deregulation, showing that miR-135b is frequently deregulated in colon cancer because of mutations occurring in key oncogenes or onco-suppressor genes such as APC and PI3K. Targeting miR-135b *in vivo* reduces tumour size and number, and might represent a target for therapy in colon cancer. We are now investigating mutations in miRNA genes and miR processing genes, and their role in colon cancer progression. While mutations in protein coding genes have a clear role in colon cancer development and progression, very little is known about mutations in non-coding genes. Initially, we will use massive parallel sequencing to study mutations in a limited screening cohort. Driver mutations detected in the screening will be confirmed in a large case control study run by the NIH in the USA. Finally, mutations will be correlated to clinical-pathological variables.

Chiara Braconi

Our interest focuses on primary liver cancers. Hepatocellular carcinoma and cholangiocarcinoma represent a major health issue in Scotland, as the mortality for liver cancer in Scottish men has increased by 47% in

the last decade. We have shown that non-coding RNAs have a significant impact on the promotion and progression of these diseases. We showed that microRNAs are modulators of hepatocyte chemo-sensitivity and key effectors of biliary transformation. Long non-coding RNAs are involved in the progression of liver cancer cells by taking part in several biological processes of cancer cells, such as apoptosis and cell cycle regulation. Our activity is now focused on studying the interaction between different classes of non-coding RNAs in liver cancer cells, and on exploring non-coding RNAs as clinical tools for the management of liver cancer patients, specifically as targets for the development of novel therapeutics as well as novel prognostic tissue and serological markers to better personalise cancer treatments.

Clinical Pathology (Head: Massimo Pignatelli)

Stratified Medicine Programme

Translational research often requires high quality human tissue samples, which are collected with full patient consent and accompanying clinical, pathological and follow-up information. In Glasgow, such tissue samples are collected, stored and distributed to researchers by the Bio-Repository, within Clinical Pathology. This resource is funded by the NHS, Chief Scientist's Office and CR-UK (via the Glasgow ECMC), and its importance to research has been underscored by our successful application to join CR-UK's Stratified Medicine Programme as one of the seven designated 'collection hubs' in the UK. This is led by Karin Oien, Massimo Pignatelli and Jane Hair (Clinical Pathology) in collaboration with NHS consultant pathologists, Glasgow ECMC and NHS R&D (Roma Armstrong). The ultimate vision of the Stratified Medicine Programme is to develop a molecular diagnostics service within the NHS that delivers high quality, cost effective tests for patients, with routine consent for the collection and research use of population-scale genetic and outcome data. This will increase our understanding of the genetic basis of cancer development and progression, and facilitate the development of a stratified medicine/personalised medicine approach to patient selection for treatment.

James Going

We are investigating pre-malignant field change and early adenocarcinogenesis in human breast and upper gastro-intestinal tract cancers, especially Barrett's oesophagus and cancers of the proximal stomach. This includes the investigation of inflammatory responses in breast cancers (in collaboration with Donny McMillan). In Barrett's oesophagus a pro-

inflammatory, unstable luminal environment promotes malignancy via a complex metaplasia-dysplasia-carcinoma sequence that remains poorly understood. Current studies are focused on identifying pre-malignant biomarkers of risk in the development of Barrett's adenocarcinoma in collaboration with Peter Adams, and on piloting oesophageal cytology in cancer risk stratification in Barrett's oesophagus with Grant Fullarton (Glasgow Royal Infirmary). Other research interests are in cancer of the upper stomach (collaboration with Kenneth McColl, University of Glasgow) and advanced imaging and cancer diagnosis in screening-detected colorectal neoplasia (collaboration with Frank Carey, Dundee).

Karin Oien

We focus on the identification, validation and optimal clinical application of cancer biomarkers in tissue samples that can then be applied for the purposes of diagnosis, prognosis or prediction of treatment response. This is in collaboration with colleagues at the Beatson Institute (Owen Sansom, Jeff Evans) and Department of Surgery, Glasgow Royal Infirmary (Colin McKay, Ross Carter). This work has yielded new insights into pancreatic cancer development and biology, and thus potential therapeutic targets, and into novel prognostic factors enabling improved patient stratification and tailored treatment. The concept of molecular profiling to optimise patient management is currently being explored in the CUPONE study, an NCRN study in patients with carcinoma of unknown primary origin. One of its main objectives is to evaluate a number of molecular classifiers that might be applicable to refine treatment regimens based on the molecular classification of the tumour and its likely primary origin.

Torsten Stein

Our interest is in mammary gland morphogenesis and breast cancer. Using transcriptome analysis, we have identified gene expression changes during key stages of mammary gland development. One particular gene that was identified was annexin A8 (AnxA8). AnxA8 was strongly expressed when, after lactation, the mammary tissue is re-modelled, preparing it for a new round of differentiation and lactation. This protein is mainly found in non-dividing cells of not fully differentiated tissues with low proliferation. However, its expression is also associated with a subgroup of breast cancers with poor prognosis, including hereditary breast cancers that carry a dysfunctional BRCA1 gene. Recently, we have shown that AnxA8 expressing cells represent a subgroup of luminal epithelial progenitor cells in the mouse mammary gland, and that these cells express several progenitor

and stem cell markers in the human breast, including the stem cell factor receptor c-kit. This group of cells is understood to be the origin of BRCA1-associated breast cancers. Our current work aims to understand the role that AnxA8 plays within these progenitor cells as well as its contribution to the malignancy of BRCA1-associated breast cancers.

Clinical Cancer Research (Head: Jeff Evans)

We are focused on the development, application and conduct of clinical studies. Jim Paul is senior statistician in the CTU and has a pivotal role in the management and conduct of our (mainly) large-scale randomised controlled trials. Rob Jones is Director of the CTU, and both he and Hing Leung are lead figures in the field of urological oncology. Hing also heads a group at the Beatson Institute (see page 18). The Radiotherapy Research Group, led by Stefano Schipani, has been launched with the aim to initiate, facilitate, execute and publish high quality, innovative and clinically relevant research that will establish Glasgow as a leading international centre for academic radiotherapy research and will lead to better treatments for cancer patients. Early phase clinical trials and translational clinical studies are developed and led through the Glasgow ECMC (Lead: Jeff Evans). Chloe Cowan, CR-UK Senior Research and Information Nurse, leads on public engagement and education, which remain important themes for our clinical research programmes.

Rob Jones, CR-UK Clinical Trials Unit

The CTU develops, coordinates and delivers national and international multicentre studies. With the CTU in Edinburgh, it forms CaCTUS (Cancer Clinical Trials Unit Scotland), which has both NCRI (National Cancer Research Institute) accreditation and UKCRC (UK Clinical Research Collaboration) registration. Several new trials have been designed and funded from a variety of sources over the past year that will ensure our central place in the organisation of large-scale trials in Scotland and further afield (for details of all trials see www.crukctuglasgow.org). New trials funded in 2012 include a number in rare cancers that play to our core strengths in gynaecological cancer (trials of BIBF1120 in clear cell cancer, and in uterine leiomyosarcoma) and gastrointestinal cancer (the BALLAD trial in small bowel adenocarcinoma), and reflect our growing expertise in international collaborations. In addition, we gained funding for a large national phase 3 trial comparing different intravenous infusion devices (the CAVA trial). We are also developing a portfolio of studies in lung cancer, with the SYSTEMS trial gaining funding and starting recruitment this

year, and other lung cancer trials planned. Collaborations with industry via NCRN/CR-UK alliances continue to flourish: the PLUTO trial being one of the first projects to commence in collaboration with GSK. A phase 1/2 study exploring the combination of AZD4547, an FGFR inhibitor, with a chemotherapy backbone (cisplatin and capecitabine) in patients with locally advanced or metastatic gastro-oesophageal adenocarcinoma and with FGFR2 polysomy or amplification, recruited its first patient. We also developed new industry collaborations: for example, we have been awarded a grant to develop and coordinate MAdCaP, a randomised phase 2 trial exploring MDM2-targeted therapy in prostate cancer in collaboration with Roche. This study should commence recruitment early next year. Our trial portfolio in urological cancers continues to grow: PLUTO, which compares cytotoxic chemotherapy with the VEGFR TKI, pazopanib, in second line treatment of bladder cancer, enrolled its first patients and the SAPROCAN phase 1/2 trial will complete its phase 1 component (in collaboration with Southampton) early in 2013.

Anthony Chalmers, Translational Radiation Biology Group

Our aim is to evaluate the clinical potential of novel targeted agents when delivered in combination with radiotherapy and/or chemotherapy, and to test promising combinations in early phase clinical trials. In 2012, stage 1 of the OPARATIC study was completed and recruitment to stage 2 commenced. OPARATIC is a phase 1 clinical trial of the PARP inhibitor olaparib in combination with temozolomide in patients with recurrent glioblastoma, funded by CR-UK. We have demonstrated that olaparib penetrates glioblastoma at therapeutic levels. Next, we will test whether it can be safely combined with continuous low dose temozolomide. Associated translational work is examining markers of DNA damage and repair in glioblastoma with the aim of identifying predictive biomarkers. We have developed an intracranial mouse model of glioblastoma that recapitulates the key features of the disease in patients, and are using this to test the effects of DNA repair inhibitors in combination with radiotherapy and chemotherapy. We are investigating the mechanisms underlying *in vivo* effects by working with two- and three-dimensional *in vitro* models of glioblastoma that incorporate glioma stem cells, hypoxia and other features of the tumour microenvironment



RESEARCH SUPPORT AND MANAGEMENT

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RESEARCH FACILITIES



Head of Research Facilities
Sue Fowler

Research Facilities supports research groups at the Beatson Institute and University of Glasgow on the Beatson site. This year, stage 2 of the new services building has been completed allowing a number of groups to relocate into a dedicated space. There has been continued investment in new equipment for Information Services and Histology. Information Services have invested in new equipment to increase the available network storage and Histology has purchased a new autostainer. Building Facilities have further increased their remit with the completion of stage 2 of the new services building and have also been active with a number of projects to adapt laboratory space to accommodate specialist equipment.

Building Facilities

Alistair Wilson, Alex Kernahan, Michael Daly, Don MacBean

Building Facilities manage the outsourced services provision for catering, cleaning and janitorial services. We provide maintenance support for the Beatson Institute buildings and manage alterations and refurbishments. This year has seen the completion of stage 2 of a new services building further increasing the scope of our responsibilities across the site.

We continue to provide a workshop repair and modification service for three days a week. This covers a diverse array of requests from the design and fabrication of laboratory devices and accessories through to laboratory equipment repairs. The facility is shortly due to be relocated to a new area within the Beatson as the building where it is currently housed is due for demolition.

The new services laboratories are now fully operational and plant is supported using the same third party provision as in the Beatson building, this ensures a consistent level of support across Institute occupied areas. It has been necessary to carry out additional works to the plant within the original building to bring this equipment up to the required standard.

Uptake of the online helpdesk facility continues to be an effective means of logging reactive calls for maintenance and repair as well as requests for workshop services. Minor project work continues at a fairly high level, at present we are upgrading our access control system and our lecture theatre main screen is due to be refurbished.

Central Services

Margaret Laing (Supervisor), Elizabeth Cheetham, Barbara Donnelly, Barbara Lambie, Fiona McKay, Kirstie McPherson, Tracy Shields, Rose Steel, Robert Storey

Central Services perform a wide range of duties that are essential for the support of the research groups across the site. This includes cleaning and sterilisation of reusable laboratory glassware, sterilisation of consumables, and preparation of tissue culture solutions, bacterial culture media and *Drosophila* food. The team is also responsible for the cleaning and checking of items such as centrifuge rotors, X-ray processors, water baths and pH meters. The stocking of the tissue culture suites, and laboratory waste collections and autoclave processing to make waste safe are performed daily.

Histology Service

Colin Nixon, Saira Ghafoor, Mark Hughes, Wendy Lambie, Fiona McGregor, Brenda McGuire, Vivienne Morrison

The Histology Service performs essential processing of tissue samples and cellular material from the wide range of cancer models developed within the Institute allowing the material to be evaluated at a cellular level in order to understand the disease mechanics. The service has recently moved into a new purpose built laboratory that has been designed ergonomically to help improve workflow leading to a reduced sample turnaround time. The Service offers processing for tissue samples fixed in an array of different types of fixative dependent on required subsequent analysis. Once received the tissue samples will be trimmed, appropriately processed and then orientated into paraffin wax blocks to facilitate tissue sectioning and staining. The tissue samples are processed according to type and necessity using previously designated specific, specialised processing cycles. We have three large capacity automated tissue processors allowing large scale consistent processing but when required specialised processing cycles can be designed. Other material such as organotypic assays, cell pellets, spheroids and agar plugs can also be processed to provide a wax block to allow sectioning and further investigation. All paraffin wax blocks sectioned are stained with haematoxylin and eosin in order to allow general analysis of cell morphology and structure. After initial analysis more specialised histology stains can be performed if required to investigate specific tissue structures.

The Institute has a Leica LMD6500 laser microdissection system that allows

subpopulations of tissue cells to be procured from histological prepared slides under microscopic visualisation. We are able to cut sections from both cryostat and paraffin blocks onto specialised slides, which can be stained appropriately allowing cellular material to be identified and separated to permit subsequent downstream analysis to be performed. Consultation regarding the downstream analysis is imperative prior to work beginning as this allows the correct protocols and procedures to be used to maximise the results obtained from the specific analysis required.

Where fixation is not required or disadvantageous to tissue structure and analysis, the facility offers a frozen section resource. Frozen tissue, embryos or cells can be sectioned and when required stained for examination using routine immunohistochemical or immunofluorescence staining methods. Material for PCR analysis and immunofluorescence investigation can also be sectioned from both paraffin-embedded material and frozen tissue.

We also offer a comprehensive immunohistochemistry service using our two large capacity autostainers. We are continually expanding the number of optimised antibodies that, if required, can be batch-stained using the facility's immunohistochemistry autostainers to provide high quality, consistent staining. New antibodies can also be optimised to produce a working protocol that allows the antibody to be used either on the autostainer or for hand staining by the researcher. Training can be provided in order that an individual scientist can understand the rationale and techniques available to allow them to perform the staining to an acceptable standard.



Information Services

Peter McHardy, Iain White

Information Services provide a wide range of support services, including server support, hardware cover, an on-site helpdesk providing both repair and software support as well as help in hardware selection and user training. There are over 350 users with nearly four hundred PCs on site comprising a mixture of Windows computers, Apple Macs and Linux machines, with central authentication, central file store and print sharing.

The servers provide in excess of 300 TB of online storage with nightly backups and tapes stored off-site, to provide support for microscopy, DNA sequencing and mass spectrometry data. Data backup facilities are now available for laptop users when they are off-site, aimed at reducing data loss.

All PCs are built with a common desktop environment, around Windows or Mac OS X and Microsoft Office and are actively managed and upgraded to ensure the best possible working environment. Mac OS X Mountain Lion is being rolled out across the site and we are actively upgrading Windows computers to Windows 7. All e-mail services are now running on Microsoft Exchange giving the benefit of being able to provide local client-based access and web access to email as well as delivering email, diaries and address books to mobile devices including iPhones, iPads and other smart phones.

We are currently migrating over as many physical servers as possible to virtual servers using VMware. We currently provide access to virtualised servers for research groups allowing them greater flexibility for test and production applications. This also allows us to provide virtual workstations for researchers with both high core counts and large amounts of RAM, making them ideal for mass spectroscopy analysis or other computationally intense applications.

Our intranet uses a content management system (CMS) framework, allowing service managers and support departments the ability to easily upload forms and information for users. Bespoke hardware systems have been designed and configured for users allowing them to achieve significant speed gains when they are running large data analyses, in some cases reducing data analysis runs from days to minutes.

A range of replacement hardware is stored on site to allow fast repairs. A good selection of

loan IT hardware, from USB drives to digital projectors, is held centrally. We provide video conferencing facilities, enabling conference calls between the Beatson and other Cancer Research UK sites as well as many other locations. Audio-visual support services for large conferences have been provided at a number of international venues, as well as overseeing the in-house 178-seat state-of-the-art lecture theatre.

Laboratory Management

Robert McFarlane, Richard Selkirk, Michael McTaggart, Joe McFadden, George Monteith

Laboratory Management is responsible for providing advice and information to scientists on health and safety, particularly on how to carry out risk assessments and on appropriate control measures. Safety plays an important part of everyday life in the laboratory and in running the building services. We administer the monitoring and training elements on a day-to-day basis, identifying training needs and ensuring adequate provision is made to fulfil the Institute's legal obligations to staff. This year we have successfully made the transition to using online systems for fire warden training. The completion of stage 2 of the services building and the imminent demolition of the neighbouring laboratory building has necessitated another programme of laboratory decommissioning, monitoring and liaison to ensure safe arrangements and documentation has been completed.

A major function of Laboratory Management is the overseeing of shared equipment servicing, replacement and the purchase of new equipment to facilitate the needs of researchers. Service contracts for core equipment are procured centrally and maintenance or repairs are coordinated. This year, we are reviewing our freezer alarm system with a view to replacement and unifying two distinct systems allowing more remote accessibility and interaction. This has required a significant amount of supplier negotiation to ensure best value for money. We have effective procurement processes and liaise with Cancer Research UK purchasing to take advantage of any centralised agreements. A further essential role is the monitoring of all outgoing orders to ensure compliance with Institute safety procedures, particularly relating to COSHH.

The stores facility stocks a wide range of consumables with rapid re-stocking to ensure high use materials are always available. Items can be withdrawn on a self-service basis with automatic cost centre allocation via swipe card.

A porter service is run to deliver external orders to the researchers, while any outgoing samples or materials are processed by stores for courier collection. We continue to review the services provided by stores to try to improve what is on offer to the scientific staff. This has required negotiating preferential pricing with our suppliers at a local level. As a result of these negotiations and better turnaround times from suppliers, we have been able to reduce the overall value of stock held without compromising supply lines to the laboratories.

Molecular Technology and Reagent Services

Billy Clark, Deborah Gardner, Andrew Keith

This year Molecular Technology and Reagent Services moved into the new services building finally enabling all elements of the service to be in one location.

The Molecular Technology Service provides routine plasmid sequencing and DNA purification on a small and large-scale. Sequencing is performed on an Applied Biosystems 3130xl (16 capillary) Sequencer that provides good sample throughput, long read lengths and a sample turnaround time of 24 hours. In recent years, DNA sequencing has been revolutionised by the introduction of next generation technologies offering large-scale sequencing in a matter of days. This year, we have sequenced a number of Genomic libraries on our Illumina GAIIx sequencer applying ChIP-seq and RNA-seq protocols. Multiplexing has enabled us to sequence more than one library per lane, increasing throughput while reducing time and costs. The researchers are preparing the libraries and the facility is sequencing them. Protocols currently used are ChIP-seq and RNA-seq.

Small-scale DNA purification is performed on a Qiagen 8000 Biorobot. Researchers provide overnight bacterial cultures that are processed by the facility. Sample numbers are consistently in the region of 15,000-17,000 per year. We continue to provide a very popular large-scale DNA purification (maxiprep) service from bacterial cultures.

Human cell line authentication using the Applied Biosystems AmpF/STR Identifier PCR Amplification Kit is available as an internal service. The samples are run on the Applied Biosystems 3130xl Sequencer (Gene Fragment Analysis) and analysed using Genemapper v4.0 software (Applied Biosystems). Regular cell line authentication is important both to confirm integrity of data and is increasingly requested by journals as a requirement prior to publication



Reagent Services provide a diverse range of support to the research groups. The mycoplasma screening service offers testing of each research group's cells every four to five months. Researchers are also encouraged to have newly imported cell lines tested as soon as possible after arrival as we have found that a significant number of newly imported cell lines are infected with mycoplasma. Cell lines are mainly tested using a luciferase assay that detects mycoplasmal enzymes. They may also be tested by: Hoechst staining to detect the presence of mycoplasma DNA; enzyme immunoassay against the four most common species of mycoplasma; or a colorimetric microplate assay to detect 16S ribosomal mycoplasma RNA.

Cell-derived matrices from Tiff 5 cells are prepared to order for the research groups and have proved very popular. Stocks of commonly used tissue culture medium are ordered and the batch testing of serum is coordinated. The facility provides a range of commonly used buffers, for example 10X TBST and bacterial growth reagents. Each product is tested for suitability of use and sterility where possible before being released for general stock. The preparation of antibiotic bacterial culture plates has been automated using a Mediacleve (Integra Biosciences AG) to sterilise and dispense into the plates.

RESEARCH PUBLICATIONS

Kurt Anderson (page 26)
Tumour Cell Migration

Primary Research Papers

Ioannidou K, Anderson KI, Strachan D, Edgar JM, Barnett SC.
Time-lapse imaging of the dynamics of CNS glial-axonal interactions *in vitro* and *ex vivo*. ***PLoS One*** 2012; 7: e30775

Macintosh RL, Timpson P, Thorburn J, Anderson KI, Thorburn A, Ryan KM.
Inhibition of autophagy impairs tumor cell invasion in an organotypic model. ***Cell Cycle*** 2012; 11: 2022-9

Mack NA, Porter AP, Whalley HJ, Schwarz JP, Jones RC, Khaja AS, Bjartell A, Anderson KI, Malliri A.
Beta2-syntrophin and Par-3 promote an apicobasal Rac activity gradient at cell-cell junctions by differentially regulating Tiam1 activity. ***Nat Cell Biol*** 2012; 14: 1169-80

Preisinger C, Schwarz JP, Bleijerveld OB, Corradini E, Muller PJ, Anderson KI, Kolch W, Scholten A, Heck AJ.
Imatinib-dependent tyrosine phosphorylation profiling of Bcr-Abl-positive chronic myeloid leukemia cells. ***Leukemia*** doi: 10.1038/leu.2012.243, published online 27 Aug 2012

Radulescu S, Brookes MJ, Salgueiro P, Ridgway RA, McGhee E, Anderson K, Ford SJ, Stones DH, Iqbal TH, Tselepis C, Sansom OJ.
Luminal iron levels govern intestinal tumorigenesis after Apc loss *in vivo*. ***Cell Rep*** 2012; 2: 270-82

Roth S, Franken P, Sacchetti A, Kremer A, Anderson K, Sansom O, Fodde R.
Paneth cells in intestinal homeostasis and tissue injury. ***PLoS One*** 2012; 7: e38965

Karen Blyth (page 54)
Transgenic Models of Cancer

Primary Research Papers

Li A, Ma Y, Jin M, Mason S, Mort RL, Blyth K, Larue L, Sansom OJ, Machesky LM.

Activated Mutant NRas(Q61K) Drives Aberrant Melanocyte Signaling, Survival, and Invasiveness via a Rac1-Dependent Mechanism. ***J Invest Dermatol*** 2012; 132: 21610-21

Maddocks ODK, Berkers CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH.
Serine starvation induces stress and p53 dependent metabolic remodeling in cancer cells. ***Nature*** doi: 10.1038/nature11743, published online 16 Dec 2012

Ridgway RA, Serrels B, Mason S, Kinnaird A, Muir M, Patel H, Muller WJ, Sansom OJ, Brunton VG.
Focal adhesion kinase is required for beta-catenin-induced mobilization of epidermal stem cells. ***Carcinogenesis*** 2012; 33: 2369-76

Radulescu S, Ridgway RA, Cordero J, Athineos D, Salgueiro P, Poulsom R, Neumann J, Jung A, Patel S, Woodgett J, Barker N, Pritchard DM, Oien K, Sansom OJ.
Acute WNT signalling activation perturbs differentiation within the adult stomach and rapidly leads to tumour formation. ***Oncogene*** doi: 10.1038/onc.2012.224, published online 4 Jun 2012

Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futo K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrady B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM.
N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. ***J Cell Biol*** 2012; 199: 527-44

Zebisch A, Wolfler A, Fried I, Wolf O, Lind K, Bodner C, Haller M, Drasche A, Pirkebner D, Matallanas D, Rath O, Blyth K, Delwel R, Taskesen E, Quehenberger F, Kolch W, Troppmair J, Sill H.
Frequent loss of RAF kinase inhibitor protein expression in acute myeloid leukemia. ***Leukemia*** 2012; 26: 1842-9

Other Publications

Blyth K, Morton JP, Sansom OJ.
The right time, the right place: will targeting human cancer-associated mutations to the mouse provide the perfect preclinical model? ***Curr Opin Genet Dev*** 2012; 22: 28-35

Ferrari N, McDonald L, Morris JS, Cameron ER, Blyth K.
RUNX2 in mammary gland development and breast cancer. ***J Cell Physiol*** doi: 10.1002/jcp.24285, published online 20 Nov 2012

Martin Drysdale (page 46)
Drug Discovery Programme

Primary Research Papers

Davies NG, Browne H, Davis B, Drysdale MJ, Foloppe N, Geoffrey S, Gibbons B, Hart T, Hubbard R, Jensen MR, Mansell H, Massey A, Matassova N, Moore JD, Murray J, Pratt R, Ray S, Robertson A, Roughley SD, Schoepfer J, Scriven K, Simmonite H, Stokes S, Surgenor A, Webb P, Wood M, Wright L, Brough P.
Targeting conserved water molecules: Design of 4-aryl-5-cyanopyrrolo[2,3-d]pyrimidine Hsp90 inhibitors using fragment-based screening and structure-based optimization. ***Bioorg Med Chem*** 2012; 20: 6770-89

Jeff Evans (page 28)
Translational Cancer Therapeutics

Primary Research Papers

Anthoney DA, Naik J, MacPherson IRJ, Crawford D, Hartley JM, Hartley JA, Saito T, Abe M, Jones K, Miwa M, Twelves CJ, Evans TRJ.
Phase I study of TP300 in patients with advanced solid tumors with pharmacokinetic, pharmacogenetic and pharmacodynamic analyses. ***BMC Cancer*** 2012; 12: 536-46

Boss DS, Glen H, Beijnen JH, Keesen M, Morrison R, Tait B, Copalu W, Mazur A, Wanders J, O'Brien JP, Schellens JH, Evans TR.
A phase I study of E7080, a multitargeted tyrosine kinase inhibitor, in patients with advanced solid tumours. ***Br J Cancer*** 2012; 106: 1598-604

Jamieson NB, Morran DC, Morton JP, Ali A, Dickson EJ, Carter CR, Sansom OJ, Evans TR, McKay CJ, Oien KA.
MicroRNA molecular profiles associated with diagnosis, clinicopathologic criteria, and overall survival in patients with resectable pancreatic ductal adenocarcinoma. ***Clin Cancer Res*** 2012; 18: 534-45

Macpherson IR, Poondru S, Simon GR, Gedrich R, Brock K, Hopkins CA, Stewart K, Stephens A, Evans TR.
A phase 1 study of OSI-930 in combination with erlotinib in patients with advanced solid tumours. ***Eur J Cancer*** doi: 10.1016/j.ejca.2012.09.036, published online 22 Oct 2012

Olmos D, A'Hern R P, Marsoni S, Morales R, Gomez-Roca C, Verweij J, Voest EE, Schoffski P, Ang JE, Penel N, Schellens JH, Del Conte G, Brunetto AT, Evans TR, Wilson R, Gallerani E, Plummer R, Tabernero J, Soria JC, Kaye SB.
Patient selection for oncology phase I trials: a multi-institutional study of prognostic factors. ***J Clin Oncol*** 2012; 30: 996-1004

Salazar R, Jones RJ, Oaknin A, Crawford D, Cuadra C, Hopkins C, Gil M, Coronado C, Soto-Matos A, Cullell-Young M, Iglesias Dios JL, Evans TR.
A phase I and pharmacokinetic study of elisidepsin (PM02734) in patients with advanced solid tumors. ***Cancer Chemother Pharmacol*** 2012; 70: 673-81

Other Publications

Cairney CJ, Bilsland AE, Evans TR, Roffey J, Bennett DC, Narita M, Torrance CJ, Keith WN.
Cancer cell senescence: a new frontier in drug development. ***Drug Discov Today*** 2012; 17: 269-76

Venugopal B, Evans TR.
Late recurrences of renal cell carcinoma at unusual sites: implications for patient management. ***Clin Adv Hematol Oncol*** 2012; 10: 126-8

David Gillespie (page 12)
Checkpoints and Cell Cycle Control

Other Publications

Gillespie DA.
Short-circuiting the cell cycle for cancer therapy. ***Cell Cycle*** 2012; 11: 2777-8

Eyal Gottlieb (page 14)
Apoptosis and Tumour Metabolism

Primary Research Papers

Chaneton B, Hillmann P, Zheng L, Martin AC, Maddocks OD, Chokkathukalam A, Coyle JE, Jankevics A, Holding FP, Vousden KH, Frezza C, O'Reilly M, Gottlieb E.
Serine is a natural ligand and allosteric activator of pyruvate kinase M2. ***Nature*** 2012; 491: 458-62

Duran RV, Mackenzie ED, Boulahbel H, Frezza C, Heiserich L, Tardito S, Bussolati O, Rocha S, Hall MN, Gottlieb E.
HIF-independent role of prolyl hydroxylases in the cellular response to amino acids. ***Oncogene*** doi: 10.1038/onc.2012.465, published online 22 Oct 2012

Duran RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E, Hall MN.
Glutaminolysis Activates Rag-mTORC1 Signaling. ***Mol Cell*** 2012; 47: 349-58

Maddocks ODK, Berkers CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH. Serine starvation induces stress and p53 dependent metabolic remodeling in cancer cells. *Nature* doi: 10.1038/nature11743, published online 16 Dec 2012

Other Publications

Chaneton B, Gottlieb E. PGAMgnam style: A glycolytic switch controls biosynthesis. *Cancer Cell* 2012; 22: 565-6

Chaneton B, Gottlieb E. Rocking cell metabolism: revised functions of the key glycolytic regulator PKM2 in cancer. *Trends Biochem Sci* 2012; 37: 309-16

Schug ZT, Frezza C, Galbraith LC, Gottlieb E. The music of lipids: how lipid composition orchestrates cellular behaviour. *Acta Oncol* 2012; 51: 301-10

Danny Huang (page 16)
Ubiquitin Signalling

Primary Research Papers

Dou H, Buetow L, Hock A, Sibbet GJ, Vousden KH, Huang DT. Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. *Nat Struct Mol Biol* 2012; 19: 184-92

Dou H, Buetow L, Sibbet GJ, Cameron K, Huang DT. BIRC7-E2 ubiquitin conjugate structure reveals the mechanism of ubiquitin transfer by a RING dimer. *Nat Struct Mol Biol* 2012; 19: 876-83

Robert Insall (page 30)
Cell Migration and Chemotaxis

Primary Research Papers

Choi CH, Thomason PA, Zaki M, Insall RH, Barber DL. Phosphorylation of Arp2 is required for normal development and cAMP chemotaxis in Dictyostelium. *J Biol Chem* doi: 10.1074/jbc.M112.435313, published online 5 Dec 2012

Pakes NK, Veltman DM, Rivero F, Nasir J, Insall R, Williams RS. The Rac GEF ZizB regulates development, cell motility and cytokinesis in Dictyostelium. *J Cell Sci* 2012; 125: 2457-65

Spence HJ, Timpson P, Tang HR, Insall RH, Machesky LM. Scar/WAVE3 contributes to motility and plasticity of lamellipodial dynamics but not invasion in three dimensions. *Biochem J* 2012; 448: 35-42

Tang H, Li A, Bi J, Veltman DM, Zech T, Spence HJ, Yu X, Timpson P, Insall RH, Frame MC, Machesky LM. Loss of Scar/WAVE complex promotes N-WASP and FAK dependent invasion. *Curr Biol* doi: 10.1016/j.cub.2012.11.059, published online 22 Dec 2012

Ura S, Pollitt AY, Veltman DM, Morrice NA, Machesky LM, Insall RH. Pseudopod growth and evolution during cell movement is controlled through SCAR/WAVE dephosphorylation. *Curr Biol* 2012; 22: 553-61

Veltman DM, King JS, Machesky LM, Insall RH. SCAR knockouts in Dictyostelium: WASP assumes SCAR's position and upstream regulators in pseudopods. *J Cell Biol* 2012; 198: 501-8

Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futo K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrady B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM. N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. *J Cell Biol* 2012; 199: 527-44

Other Publications

Insall R. How a paper on RAC set the standard. *Nat Rev Mol Cell Biol* 2012; 13: 66

King JS. Autophagy across the eukaryotes: Is S. cerevisiae the odd one out? *Autophagy* 2012; 8: 1159-62

King JS. Mechanical stress meets autophagy: potential implications for physiology and pathology. *Trends Mol Med* 2012; 18: 583-8

Gabriela Kalna (page 51)
Bioinformatics

Primary Research Papers

Galban-Horcajo F, Fitzpatrick AM, Hutton AJ, Dunn SM, Kalna G, Brennan KM, Rinaldi S, Yu RK, Goodyear CS, Willison HJ. Antibodies to heteromeric glycolipid complexes in multifocal motor neuropathy. *Eur J Neurol* doi: 10.1111/j.1468-1331.2012.03767.x, published online 22 Jun 2012

von Thun A, Birtwistle M, Kalna G, Grindlay J, Strachan D, Kolch W, von Kriegsheim A, Norman JC. ERK2 drives tumour cell migration in three-dimensional microenvironments by suppressing expression of Rab17 and liprin-beta2. *J Cell Sci* 2012; 125: 1465-77



Hing Leung (page 18)
Urology Research

Primary Research Papers

Ahmad I, Small DR, Krishna NS, Akhtar MN, Leung HY. Prostate cancer incidence in patients on 5 α -reductase inhibitors for lower urinary tract symptoms: A 14-year retrospective study. *British Journal of Medical and Surgical Urology*. 2012; 5: 179-83

Bennett HL, Stockley J, Fleming JT, Mandal R, O'Prey J, Ryan KM, Robson CN, Leung HY. Does androgen-ablation therapy (AAT) associated autophagy have a pro-survival effect in LNCaP human prostate cancer cells? *BJU Int* doi: 10.1111/j.1464-410X.2012.11409.x, published online 16 Aug 2012

Forbat L, Place M, Kelly D, Hubbard G, Boyd K, Howie K, Leung HY. A cohort study reporting clinical risk factors and individual risk perceptions of prostate cancer: implications for PSA testing. *BJU Int* doi: 10.1111/j.1464-410X.2012.11316.x, published online 21 Jun 2012

Gao M, Patel R, Ahmad I, Fleming J, Edwards J, McCracken S, Sahadevan K, Seywright M, Norman J, Sansom O, Leung HY. SPRY2 loss enhances ErbB trafficking and PI3K/AKT signalling to drive human and mouse prostate carcinogenesis. *EMBO Mol Med* 2012; 4: 776-90

Kelly JD, Dudderidge TJ, Wollenschlaeger A, Okoturo O, Burling K, Tulloch F, Halsall I, Prevost T, Prevost AT, Vasconcelos JC, Robson W, Leung HY, Vasdev N, Pickard RS, Williams GH, Stoeber K.

Bladder cancer diagnosis and identification of clinically significant disease by combined urinary detection of mcm5 and nuclear matrix protein 22. *PLoS One* 2012; 7: e40305

McCall P, Bennett L, Ahmad I, Mackenzie LM, Forbes IW, Leung HY, Sansom OJ, Orange C, Seywright M, Underwood MA, Edwards J. NFkappaB signalling is upregulated in a subset of castrate-resistant prostate cancer patients and correlates with disease progression. *Br J Cancer* 2012; 107: 1554-63

Shafique K, McLoone P, Qureshi K, Leung H, Hart C, Morrison DS. Cholesterol and the risk of grade-specific prostate cancer incidence: evidence from two large prospective cohort studies with up to 37 years' follow up. *BMC Cancer* 2012; 12: 25

Shafique K, McLoone P, Qureshi K, Leung H, Hart C, Morrison DS. Tea Consumption and the Risk of Overall and Grade Specific Prostate Cancer: A Large Prospective Cohort Study of Scottish Men. *Nutr Cancer* 2012; 64: 790-7

Shafique K, McLoone P, Qureshi K, Leung H, Hart C, Morrison D. Coffee consumption and prostate cancer risk: further evidence for inverse relationship. *Nutr J* 2012; 11: 42

Williamson SC, Hepburn AC, Wilson L, Coffey K, Ryan-Munden CA, Pal D, Leung HY, Robson CN, Heer R. Human $\alpha(2)\beta(1)$ (H1) CD133(+VE) Epithelial Prostate Stem Cells Express Low Levels of Active Androgen Receptor. *PLoS One* 2012; 7: e48944



Yang Z, Laird A, Monaghan A, Seywright M, Leung HY.
Incidental seminal vesicle amyloidosis at the same time of diagnostic prostatic biopsies – Is routine investigations for systemic amyloidosis warranted? *Asian Journal of Andrology* doi:10.1038/aja.2012.125, published online 10 Dec 2012

Other Publications

Ahmad I, Iwata T, Leung HY.
Mechanisms of FGFR-mediated carcinogenesis. *Biochim Biophys Acta* 2012; 1823: 850-60

Ahmad I, Sansom OJ, Leung HY.
Exploring molecular genetics of bladder cancer: lessons learned from mouse models. *Dis Model Mech* 2012; 5: 323-32

Krishnan A, Wilson J, Leung HY.
Approaches for developing novel microtubule targeting agents (MTAs) for therapeutic exploitation. *Curr Pharm Des* 2012; 18: 2804-10

Mischak H, Ioannidis JP, Argiles A, Attwood TK, Bongcam-Rudloff E, Broenstrup M, Charonis A, Chrousos GP, Delles C, Dominiczak A, Dylag T, Ehrich J, Egido J, Findeisen P, Jankowski J, Johnson RW, Julien BA, Lankisch T, Leung HY, Maahs D, Magni F, Manns MP, Manolis E, Mayer G, Navis G, Novak J, Ortiz A, Persson F, Peter K, Riese HH, Rossing P, Sattar N, Spasovski G, Thongboonkerd V, Vanholder R, Schanstra JP, Vlahou A.
Implementation of proteomic biomarkers: making it work. *Eur J Clin Invest* 2012; 42: 1027-36

Patel R, Leung HY.
Targeting the EGFR-family for therapy: biological challenges and clinical perspective. *Curr Pharm Des* 2012; 18: 2672-9

Laura Machesky (page 32)

Migration, Invasion and Metastasis

Primary Research Papers

Dawson JC, Bruche S, Spence HJ, Braga VM, Machesky LM.
Mtss1 promotes cell-cell junction assembly and stability through the small GTPase Rac1. *PLoS One* 2012; 7: e31141

Li A, Ma Y, Jin M, Mason S, Mort RL, Blyth K, Larue L, Sansom OJ, Machesky LM.
Activated Mutant NRas(Q61K) Drives Aberrant Melanocyte Signaling, Survival, and Invasiveness via a Rac1-Dependent Mechanism. *J Invest Dermatol* 2012; 132: 2610-21

Maritzen T, Zech T, Schmidt MR, Krause E, Machesky LM, Haucke V.
Gadkin negatively regulates cell spreading and motility via sequestration of the actin-nucleating ARP2/3 complex. *Proc Natl Acad Sci U S A* 2012; 109: 10382-7

Schachtner H, Li A, Stevenson D, Calaminus SD, Thomas SG, Watson SP, Sixt M, Wedlich-Soldner R, Strathdee D, Machesky LM.
Tissue inducible Lifeact expression allows visualization of actin dynamics *in vivo* and *ex vivo*. *Eur J Cell Biol* 2012; 91: 923-9

Spence HJ, Timpson P, Tang HR, Insall RH, Machesky LM.
Scar/WAVE3 contributes to motility and plasticity of lamellipodial dynamics but not invasion in three dimensions. *Biochem J* 2012; 448: 35-42

Tang H, Li A, Bi J, Veltman DM, Zech T, Spence HJ, Yu X, Timpson P, Insall RH, Frame MC, Machesky LM.
Loss of Scar/WAVE complex promotes N-WASP and FAK dependent invasion. *Curr Biol* doi: 10.1016/j.cub.2012.11.059, published online 22 Dec 2012

Ura S, Pollitt AY, Veltman DM, Morrice NA, Machesky LM, Insall RH.
Pseudopod growth and evolution during cell movement is controlled through SCAR/WAVE dephosphorylation. *Curr Biol* 2012; 22: 553-61

Veltman DM, King JS, Machesky LM, Insall RH.
SCAR knockouts in Dictyostelium: WASP assumes SCAR's position and upstream regulators in pseudopods. *J Cell Biol* 2012; 198: 501-8

Yu X, Machesky LM.
Cells assemble invadopodia-like structures and invade into matrigel in a matrix metalloprotease dependent manner in the circular invasion assay. *PLoS One* 2012; 7: e30605

Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futo K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrady B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM.
N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. *J Cell Biol* 2012; 199: 527-44

Other Publications

Garcia E, Jones GE, Machesky LM, Anton IM.
WIP: WASP-interacting proteins at invadopodia and podosomes. *Eur J Cell Biol* 2012; 91: 869-77

Li A, Machesky LM.
Melanoblasts on the move: Rac1 sets the pace. *Small GTPases* 2012; 3: 115-9

Machesky LM, Sansom OJ.
Rac1 in the driver's seat for melanoma. *Pigment Cell Melanoma Res* 2012; 25: 762-4

Stevenson RP, Veltman D, Machesky LM.
Actin-bundling proteins in cancer progression at a glance. *J Cell Sci* 2012; 125: 1073-9

Zech T, Calaminus SD, Machesky LM.
Actin on trafficking: Could actin guide directed receptor transport? *Cell Adh Migr* 2012; 6: 476-81

Nick Morrice (page 52)

Proteomics and Mass Spectrometry

Primary Research Papers

Berggreen C, Henriksson E, Jones HA, Morrice N, Goransson O.
cAMP-elevation mediated by beta-adrenergic stimulation inhibits salt-inducible kinase (SIK) 3 activity in adipocytes. *Cell Signal* 2012; 24: 1863-71

Brook M, McCracken L, Reddington JP, Lu ZL, Morrice NA, Gray NK.
The multifunctional poly(A)-binding protein (PABP) 1 is subject to extensive dynamic post-translational modification, which molecular modelling suggests plays an important role in co-ordinating its activities. *Biochem J* 2012; 441: 803-12

De Wever V, Lloyd DC, Nasa I, Nimick M, Trinkle-Mulcahy L, Gourlay R, Morrice N, Moorhead GB.
Isolation of Human Mitotic Protein Phosphatase Complexes: Identification of a Complex between Protein Phosphatase 1 and the RNA Helicase Ddx21. *PLoS One* 2012; 7: e39510

Henriksson E, Jones HA, Patel K, Peggie M, Morrice N, Sakamoto K, Goransson O.
The AMPK-related kinase SIK2 is regulated by cAMP via phosphorylation at Ser358 in adipocytes. *Biochem J* 2012; 444: 503-14

Neukamm SS, Toth R, Morrice N, Campbell DG, Mackintosh C, Lehmann R, Haering HU, Schleicher ED, Weigert C.
Identification of the amino acids 300-600 of IRS-2 as 14-3-3 binding region with the importance of IGF-1/insulin-regulated phosphorylation of Ser-573. *PLoS One* 2012; 7: e43296

Roget K, Ben-Addi A, Mambole-Dema A, Gantke T, Yang HT, Janzen J, Morrice N, Abbott D, Ley SC.
IkappaB Kinase 2 Regulates TPL-2 Activation of Extracellular Signal-Regulated Kinases 1 and 2 by Direct Phosphorylation of TPL-2 Serine 400. *Mol Cell Biol* 2012; 32: 4684-90

Tran HT, Nimick M, Uhrig RG, Templeton G, Morrice N, Gourlay R, Delong A, Moorhead GB.
Arabidopsis thaliana histone deacetylase 14 (HDA14) is an alpha-tubulin deacetylase that associates with PP2A and enriches in the microtubule fraction with the putative histone acetyltransferase ELP3. *Plant J* 2012; 71: 263-72

Ura S, Pollitt AY, Veltman DM, Morrice NA, Machesky LM, Insall RH.
Pseudopod growth and evolution during cell movement is controlled through SCAR/WAVE dephosphorylation. *Curr Biol* 2012; 22: 553-61

Jim Norman (page 34)

Integrin Cell Biology

Primary Research Papers

Chen Y, Rice W, Gu Z, Li J, Huang J, Brenner MB, Van Hoek A, Xiong J, Gundersen GG, Norman JC, Hsu VW, Fenton RA, Brown D, Lu HA.
Aquaporin 2 Promotes Cell Migration and Epithelial Morphogenesis. *J Am Soc Nephrol* 2012; 23: 1506-17

Christoforides C*, Rainero E*, Brown KK, Norman JC**, Tokar A**.
PKD Controls alphavbeta3 Integrin Recycling and Tumor Cell Invasive Migration through Its Substrate Rabaptin-5. *Dev Cell* 2012; 23: 560-72

*these authors contributed equally to this work
**corresponding authors

Dozynkiewicz MA, Jamieson NB, Macpherson I, Grindlay J, van den Berghe PV, von Thun A, Morton JP, Gourley C, Timpson P, Nixon C, McKay CJ, Carter R, Strachan D, Anderson K, Sansom OJ, Caswell PT, Norman JC.
Rab25 and CLIC3 collaborate to promote integrin recycling from late endosomes/lysosomes and drive cancer progression. *Dev Cell* 2012; 22: 131-45

Eva R, Crisp S, Marland JR, Norman JC, Kanamarlapudi V, Ffrench-Constant C, Fawcett JW.

ARF6 Directs Axon Transport and Traffic of Integrins and Regulates Axon Growth in Adult DRG Neurons. *J Neurosci* 2012; 32: 10352-64

Gao M, Patel R, Ahmad I, Fleming J, Edwards J, McCracken S, Sahadevan K, Seywright M, Norman J, Sansom O, Leung HY. SPRY2 loss enhances ErbB trafficking and PI3K/AKT signalling to drive human and mouse prostate carcinogenesis. *EMBO Mol Med* 2012; 4: 776-90

Margadant C, Kreft M, de Groot DJ, Norman JC, Sonnenberg A. Distinct Roles of Talin and Kindlin in Regulating Integrin alpha5beta1 Function and Trafficking. *Curr Biol* 2012; 22: 1554-63

Muller PA, Trinidad AG, Timpson P, Morton JP, Zanivan S, van den Berghe PV, Nixon C, Karim SA, Caswell PT, Noll JE, Coffill CR, Lane DP, Sansom OJ, Neilsen PM, Norman JC*, Vousden KH*. Mutant p53 enhances MET trafficking and signalling to drive cell scattering and invasion. *Oncogene* doi: 10.1038/onc.2012.148, published online 14 May 2012

*corresponding authors

Onodera Y, Nam JM, Hashimoto A, Norman JC, Shirato H, Hashimoto S, Sabe H. Rab5c promotes AMAP1-PRKD2 complex formation to enhance beta1 integrin recycling in EGF-induced cancer invasion. *J Cell Biol* 2012; 197: 983-96

Rainero E, Caswell PT, Muller PA, Grindlay J, McCaffrey MW, Zhang Q, Wakelam MJ, Vousden KH, Graziani A, Norman JC. Diacylglycerol kinase alpha controls RCP-dependent integrin trafficking to promote invasive migration. *J Cell Biol* 2012; 196: 277-95

Rizzolio S, Rabinowicz N, Rainero E, Lanzetti L, Serini G, Norman JC, Neufeld G, Tamagnone L. Neuropilin-1 dependent regulation of EGF-Receptor signaling. *Cancer Res* 2012; 72: 5801-11

von Thun A, Birtwistle M, Kalna G, Grindlay J, Strachan D, Kolch W, von Kriegsheim A, Norman JC. ERK2 drives tumour cell migration in three-dimensional microenvironments by suppressing expression of Rab17 and liprin-beta2. *J Cell Sci* 2012; 125: 1465-77

Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futo K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrady B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM. N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. *J Cell Biol* 2012; 199: 527-44

Other Publications

Bridgewater RE, Norman JC, Caswell PT. Integrin trafficking at a glance. *J Cell Sci* 2012; 125: 3695-701

Michael Olson (page 36)

Molecular Cell Biology

Primary Research Papers

Jamieson T, Clarke M, Steele CW, Samuel MS, Neumann J, Jung A, Huels D, Olson MF, Das S, Nibbs RJ, Sansom OJ. Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. *J Clin Invest* 2012; 122: 3127-44

Mleczak A, Millar S, Tooze SA, Olson MF, Chan EY. Regulation of autophagosome formation by Rho kinase. *Cell Signal* 2012; 25: 1-11

Other Publications

Mardilovich K, Olson MF, Baugh M. Targeting Rho GTPase signaling for cancer therapy. *Future Oncol* 2012; 8: 165-77

Rath N, Olson MF. Rho-associated kinases in tumorigenesis: re-considering ROCK inhibition for cancer therapy. *EMBO Rep* 2012; 13: 900-8

Wickman G, Julian L, Olson MF. How apoptotic cells aid in the removal of their own cold dead bodies. *Cell Death Differ* 2012; 19: 735-42

Wood JM, Olson MF. Collective migration: spatial tension relief. *Curr Biol* 2012; 22: R125-7

Kevin Ryan (page 20)

Tumour Cell Death

Primary Research Papers

Bennett HL, Stockley J, Fleming JT, Mandal R, O'Prey J, Ryan KM, Robson CN, Leung HY. Does androgen-ablation therapy (AAT) associated autophagy have a pro-survival effect in LNCaP human prostate cancer cells? *BJU Int* doi: 10.1111/j.1464-410X.2012.11409.x, published online 16 Aug 2012

Ladoire S, Chaba K, Martins I, Sukkurwala AQ, Adjemian S, Michaud M, Poirier-Colame V, Andreiuolo F, Galluzzi L, White E, Rosenfeldt M, Ryan KM, Zitvogel L, Kroemer G. Immunohistochemical detection of cytoplasmic LC3 puncta in human cancer specimens. *Autophagy* 2012; 8: 1175-84



Lemarie F, Croft DR, Tate RJ, Ryan KM, Dufes C. Tumor regression following intravenous administration of a tumor-targeted p73 gene delivery system. *Biomaterials* 2012; 33: 2701-9

Macintosh RL, Timpson P, Thorburn J, Anderson KI, Thorburn A, Ryan KM. Inhibition of autophagy impairs tumor cell invasion in an organotypic model. *Cell Cycle* 2012; 11: 2022-9

Rosenfeldt M, Nixon C, Liu E, Mah LY, Ryan KM. Analysis of macroautophagy by immunohistochemistry. *Autophagy* 2012; 8: 963-9

Other Publications

Klionsky DJ *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 2012; 8: 445-544

Liu EY, Ryan KM. Autophagy and cancer – issues we need to digest. *J Cell Sci* 2012; 125: 2349-58

Long JS, Ryan KM. New frontiers in promoting tumour cell death: targeting apoptosis, necroptosis and autophagy. *Oncogene* 2012; 31: 5045-60

Owen Sansom (page 38)

Colorectal Cancer and Wnt Signalling

Primary Research Papers

Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, Ridgway RA, Seo SS, Spee B, Van Rooijen N, Sansom OJ, Iredale JP, Lowell S, Roskams T, Forbes SJ.

Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nat Med* 2012; 18: 572-9

Cordero JB, Stefanatos RK, Scopelliti A, Vidal M*, Sansom OJ*. Inducible progenitor-derived Wingless regulates adult midgut regeneration in Drosophila. *EMBO J* 2012; 31: 3901-17

*corresponding authors

Cordero JB*, Stefanatos RK*, Myant K, Vidal M, Sansom OJ. Non-autonomous Jak/Stat and EGFR signaling crosstalk mediates intestinal stem cell proliferation following Apc loss in the Drosophila adult midgut. *Development* 2012; 139: 4524-35

*these authors contributed equally to this work

Gao M, Patel R, Ahmad I, Fleming J, Edwards J, McCracken S, Sahadevan K, Seywright M, Norman J, Sansom O, Leung HY. SPRY2 loss enhances ErbB trafficking and PI3K/AKT signalling to drive human and mouse prostate carcinogenesis. *EMBO Mol Med* 2012; 4: 776-90

Jamieson NB, Morran DC, Morton JP, Ali A, Dickson EJ, Carter CR, Sansom OJ, Evans TR, McKay CJ, Oien KA. MicroRNA molecular profiles associated with diagnosis, clinicopathologic criteria, and overall survival in patients with resectable pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2012; 18: 534-45

Jamieson T, Clarke M, Steele CW, Samuel MS, Neumann J, Jung A, Huels D, Olson MF, Das S, Nibbs RJ, Sansom OJ. Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. *J Clin Invest* 2012; 122: 3127-44

Klotz DM, Nelson SA, Kroboth K, Newton IP, Radulescu S, Ridgway RA, Sansom OJ, Appleton PL, Nathke IS.
The microtubule poison vinorelbine kills cells independently of mitotic arrest and targets cells lacking the APC tumour suppressor more effectively. *J Cell Sci* 2012; 125: 887-95

Li A, Ma Y, Jin M, Mason S, Mort RL, Blyth K, Larue L, Sansom OJ, Machesky LM.
Activated Mutant NRas(Q61K) Drives Aberrant Melanocyte Signaling, Survival, and Invasiveness via a Rac1-Dependent Mechanism. *J Invest Dermatol* 2012; 132: 2610-21

McCall P, Bennett L, Ahmad I, Mackenzie LM, Forbes IW, Leung HY, Sansom OJ, Orange C, Seywright M, Underwood MA, Edwards J.
NFkappaB signalling is upregulated in a subset of castrate-resistant prostate cancer patients and correlates with disease progression. *Br J Cancer* 2012; 107: 1554-63

Moyes LH, McEwan H, Radulescu S, Pawlikowski J, Lamm CG, Nixon C, Sansom OJ, Going JJ, Fullarton GM, Adams PD.
Activation of Wnt Signalling promotes development of dysplasia in Barrett's Oesophagus. *J Pathol* 2012; 228: 99-112

Muller PA, Trinidad AG, Timpson P, Morton JP, Zanivan S, van den Berghe PV, Nixon C, Karim SA, Caswell PT, Noll JE, Coffill CR, Lane DP, Sansom OJ, Neilsen PM, Norman JC, Vousden KH.
Mutant p53 enhances MET trafficking and signalling to drive cell scattering and invasion. *Oncogene* doi: 10.1038/onc.2012.148, published online 14 May 2012

Munoz J, Stange DE, Schepers AG, van de Wetering M, Koo BK, Itzkovitz S, Volckmann R, Kung KS, Koster J, Radulescu S, Myant K,

Versteeg R, Sansom OJ, van Es JH, Barker N, van Oudenaarden A, Mohammed S, Heck AJ, Clevers H.
The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent '4' cell markers. *EMBO J* 2012; 31: 3079-91

Radulescu S, Brookes MJ, Salgueiro P, Ridgway RA, McGhee E, Anderson K, Ford SJ, Stones DH, Iqbal TH, Tselepis C, Sansom OJ.
Luminal iron levels govern intestinal tumorigenesis after Apc loss *in vivo*. *Cell Rep* 2012; 2: 270-82

Radulescu S, Ridgway RA, Cordero J, Athineos D, Salgueiro P, Poulsom R, Neumann J, Jung A, Patel S, Woodgett J, Barker N, Pritchard DM, Oien K, Sansom OJ.
Acute WNT signalling activation perturbs differentiation within the adult stomach and rapidly leads to tumour formation. *Oncogene* doi: 10.1038/onc.2012.224, published online 4 June 2012

Ridgway RA, Serrels B, Mason S, Kinnaird A, Muir M, Patel H, Muller WJ, Sansom OJ, Brunton VG.
Focal adhesion kinase is required for beta-catenin-induced mobilization of epidermal stem cells. *Carcinogenesis* 2012; 33: 2369-76

Roth S, Franken P, Sacchetti A, Kremer A, Anderson K, Sansom O, Fodde R.
Paneth cells in intestinal homeostasis and tissue injury. *PLoS One* 2012; 7: e38965

Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, Heijmans J, Huels DJ, Moreaux G, Rupec RA, Gerhard M, Schmid RM, Barker N, Clevers H, Lang R, Neumann J, Kirchner T, Taketo MM, van den Brink GR, Sansom OJ, Arkan MC, Greten FR.
Acquisition of stem cell like property by dedifferentiation of intestinal non stem cells initiates tumorigenesis. *Cell* doi: 10.1016/j.cell.2012.12.012, published online 22 Dec 2012



Other Publications
Ahmad I, Sansom OJ, Leung HY.
Exploring molecular genetics of bladder cancer: lessons learned from mouse models. *Dis Model Mech* 2012; 5: 323-32

Machesky LM, Sansom OJ.
Rac1 in the driver's seat for melanoma. *Pigment Cell Melanoma Res* 2012; 25: 762-4

Blyth K, Morton JP, Sansom OJ.
The right time, the right place: will targeting human cancer-associated mutations to the mouse provide the perfect preclinical model? *Curr Opin Genet Dev* 2012; 22: 28-35

Tan EH, Sansom OJ.
A new tumour suppressor enters the network of intestinal progenitor cell homeostasis. *EMBO J* 2012; 31: 2444-5

Douglas Strathdee (page 55)
Transgenic Technology

Primary Research Papers
Schachtner H, Li A, Stevenson D, Calaminus SD, Thomas SG, Watson SP, Sixt M, Wedlich-Soldner R, Strathdee D, Machesky LM.
Tissue inducible Lifeact expression allows visualization of actin dynamics *in vivo* and *ex vivo*. *Eur J Cell Biol* 2012; 91: 923-9

Marcos Vidal (page 40)
Drosophila Approaches to Cancer

Primary Research Papers
Cordero JB, Stefanatos RK, Scopelliti A, Vidal M*, Sansom OJ*.
Inducible progenitor-derived Wingless regulates adult midgut regeneration in Drosophila. *EMBO J* 2012; 31: 3901-17

*corresponding authors

Cordero JB*, Stefanatos RK*, Myant K, Vidal M, Sansom OJ.
Non-autonomous Jak/Stat and EGFR signaling crosstalk mediates intestinal stem cell proliferation following Apc loss in the Drosophila adult midgut. *Development* 2012; 139: 4524-35

*these authors contributed equally to this work

Karen Vousden (page 22)
Tumour Suppression

Primary Research Papers
Chaneton B, Hillmann P, Zheng L, Martin AC, Maddocks OD, Chokkathukalam A, Coyle JE, Jankevics A, Holding FP, Vousden KH, Frezza C, O'Reilly M, Gottlieb E.

Serine is a natural ligand and allosteric activator of pyruvate kinase M2. *Nature* 2012; 491: 458-62

Cheung EC, Ludwig RL, Vousden KH.
Mitochondrial localisation of TIGAR under hypoxia stimulates HK2 and lowers ROS and cell death. *PNAS* 2012; 109: 20491-6

Coffill CR, Muller PA, Oh HK, Neo SP, Hogue KA, Cheok CF, Vousden KH, Lane DP, Blackstock WP, Gunaratne J.
Mutant p53 interactome identifies nardilysin as a p53R273H-specific binding partner that promotes invasion. *EMBO Rep* 2012; 13: 638-44

Dolezelova P, Cetkovska K, Vousden KH, Uldrijan S.
Mutational analysis reveals a dual role of Mdm2 acidic domain in the regulation of p53 stability. *FEBS Lett* 2012; 586: 2225-31

Dolezelova P, Cetkovska K, Vousden KH, Uldrijan S.
Mutational analysis of Mdm2 C-terminal tail suggests an evolutionarily conserved role of its length in Mdm2 activity toward p53 and indicates structural differences between Mdm2 homodimers and Mdm2/MdmX heterodimers. *Cell Cycle* 2012; 11: 953-62

Dou H, Buetow L, Hock A, Sibbet GJ, Vousden KH, Huang DT.
Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. *Nat Struct Mol Biol* 2012; 19: 184-92

Maddocks ODK, Berkers CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH.
Serine starvation induces stress and p53 dependent metabolic remodeling in cancer cells. *Nature* doi: 10.1038/nature11743, published online 16 Dec 2012

Muller PA, Trinidad AG, Timpson P, Morton JP, Zanivan S, van den Berghe PV, Nixon C, Karim SA, Caswell PT, Noll JE, Coffill CR, Lane DP, Sansom OJ, Neilsen PM, Norman JC*, Vousden KH*.
Mutant p53 enhances MET trafficking and signalling to drive cell scattering and invasion. *Oncogene* doi: 10.1038/onc.2012.148, published online 14 May 2012

*corresponding authors

Preisinger C, Schwarz JP, Bleijerveld OB, Corradini E, Muller PJ, Anderson KI, Kolch W, Scholten A, Heck AJ.
Imatinib-dependent tyrosine phosphorylation profiling of Bcr-Abl-positive chronic myeloid leukemia cells. *Leukemia* doi: 10.1038/leu.2012.243, published online 14 Sep 2012

Rainero E, Caswell PT, Muller PA, Grindlay J, McCaffrey MW, Zhang Q, Wakelam MJ, Vousden KH, Graziani A, Norman JC.
Diacylglycerol kinase alpha controls RCP-dependent integrin trafficking to promote invasive migration. *J Cell Biol* 2012; 196: 277-95



Roxburgh P, Hock AK, Dickens MP, Mezna M, Fischer PM, Vousden KH.
Small molecules that bind the Mdm2 RING stabilize and activate p53. *Carcinogenesis* 2012; 33: 791-8

Tucci P, Agostini M, Grespi F, Markert EK, Terrinoni A, Vousden KH, Muller PA, Dotsch V, Kehrlöesser S, Sayan BS, Giaccone G, Lowe SW, Takahashi N, Vandenabeele P, Knight RA, Levine AJ, Melino G.
Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. *Proc Natl Acad Sci U S A* 2012; 109: 15312-7

Other Publications

Hock AK, Vousden KH.
Tumor Suppression by p53: Fall of the Triumvirate? *Cell* 2012; 149: 1183-5

Sara Zanivan (page 42)

Vascular Proteomics

Primary Research Papers

Azimifar SB, Bottcher RT, Zanivan S, Grashoff C, Kruger M, Legate KR, Mann M, Fassler R.
Induction of membrane circular dorsal ruffles requires co-signalling of integrin-ILK-complex and EGF receptor. *J Cell Sci* 2012; 125: 435-48

Muller PA, Trinidad AG, Timpson P, Morton JP, Zanivan S, van den Berghe PV, Nixon C, Karim SA, Caswell PT, Noll JE, Coffill CR, Lane DP, Sansom OJ, Neilsen PM, Norman JC, Vousden KH.
Mutant p53 enhances MET trafficking and signalling to drive cell scattering and invasion. *Oncogene* doi: 10.1038/onc.2012.148, published online 14 May 2012

Other Publications

Hernandez-Fernaund JR, Reid SE, Neilson LJ, Zanivan S.

Quantitative mass spectrometry-based proteomics in angiogenesis. *Proteomics Clin Appl* doi: 10.1002/prca.201200055, published online 17 Nov 2012

Zanivan S, Krueger M, Mann M.
In vivo quantitative proteomics: the SILAC mouse. *Methods Mol Biol* 2012; 757: 435-50

Other Staff

Primary Research Papers

Abualhasan MN, Good JA, Wittayanarakul K, Anthony NG, Berretta G, Rath O, Kozielski F, Sutcliffe OB, Mackay SP.
Doing the methylene shuffle - Further insights into the inhibition of mitotic kinesin Eg5 with S-trityl L-cysteine. *Eur J Med Chem* 2012; 54: 483-98

Kleijnnot M, Kozielski F.
Structural insights into human Kif7, a kinesin involved in Hedgehog signalling. *Acta Crystallogr D Biol Crystallogr* 2012; 68: 154-9

Nagarajan S, Skoufias DA, Kozielski F, Pae AN.
Receptor-ligand interaction-based virtual screening for novel Eg5/kinesin spindle protein inhibitors. *J Med Chem* 2012; 55: 2561-73

Nichols DB, Fournet G, Gurukumar KR, Basu A, Lee JC, Sakamoto N, Kozielski F, Musmuca I, Joseph B, Ragno R, Kaushik-Basu N.
Inhibition of hepatitis C virus NS5B polymerase by S-trityl-L-cysteine derivatives. *Eur J Med Chem* 2012; 49: 191-9

Talapatra SK, Schüttelkopf AW, Kozielski F.
The structure of the ternary Eg5-ADP-ispinesib complex. *Acta Crystallogr D Biol Crystallogr* 2012; 68: 1311-9

Wang F, Good JA, Rath O, Kaan HY, Sutcliffe OB, Mackay SP, Kozielski F.
Triphenylbutanamines: kinesin spindle protein inhibitors with *in vivo* antitumor activity. *J Med Chem* 2012; 55: 1511-25

Other Publications

Rath O, Kozielski F.
Kinesins and cancer. *Nat Rev Cancer* 2012; 12: 527-39

Peter D. Adams (page 58)

Epigenetics of Cancer and Ageing

Primary Research Papers

Dekker P, Gunn G, McBryan T, Dirks RW, van Heemst D, Lim F-L, Jochemsen, AG, Verlaan-de Vries M, Nagel J, Adams PD, Tanke HJ, Westendorp RG, Maier AB.

Microarray-based identification of age-dependent differences in gene expression of human dermal fibroblasts. *Mech Aging and Dev* 2012; 133: 498-507

Moyes LH, McEwan H, Radulescu S, Pawlikowski J, Lamm CG, Nixon C, Sansom OJ, Going JJ, Fullarton GM, Adams PD.
Activation of Wnt Signalling promotes development of dysplasia in Barrett's Oesophagus. *J. Pathology* 2012; 228: 99-112

Tang Y, Puri A, Ricketts M, Rai T, Hoffmann J, Hoi E, Adams PD, Schultz D, Marmorstein R.
Identification of an Ubinuclein 1 region required for stability of the human HIRA/UBN1/CABIN1/ASF1a histone H3.3 chaperone complex. *Biochemistry* 2012; 51: 2366-77

Other publications

Kennedy AL, Adams PD, Morton JP.
Ras, PI3K/Akt and senescence: paradoxes provide clues for pancreatic cancer therapy. *Small GTPases* 2011; 2: 264-7

Rai TS, Adams PD.
Lessons from senescence: chromatin maintenance in non-proliferating cells. *Biochimica et Biophysica Acta* 2012; 1819: 322-31

van Tuyn, J, Adams PD.
Signaling the end of the line. *Nat Cell Biol* 2012; 14: 339-41

Daniel J. Murphy (page 60)

Oncogene-Induced Vulnerabilities

Primary Research Papers

Bommert KS, Effenberger M, Leich E, Küspert M, Murphy D, Langer C, Moll R, Janz S, Mottok A, Weissbach S, Rosenwald A, Bargou R, Bommert K.
The feed-forward loop between YB-1 and MYC is essential for multiple myeloma cell survival. *Leukemia* doi: 10.1038/leu.2012.185, published online 9 Jul 2012

Liu L, Ulbrich J, Müller M, Wüstefeld T, Aeberhard L, Kress TR, Muthalagu N, Moll R, Kempa S, Zender L, Eilers M, Murphy DJ.
Deregulated MYC expression induces dependence upon AMPK-related kinase 5. *Nature* 2012; 483: 608-12

Stephen Tait (page 62)

Mitochondria and Cell Death

Primary Research Papers

Bender CE, Fitzgerald P, Tait SW, Llambi F, McStay GP, Tupper DO, Pellettieri J, Sánchez Alvarado A, Salvesen GS, Green DR.

Mitochondrial pathway of apoptosis is ancestral in metazoans. *Proc Natl Acad Sci USA* 2012; 109: 4904-9

Sano R, Hou YC, Hedvat M, Correa RG, Shu CW, Krajewska M, Diaz PW, Tample CM, Quarato G, Gottlieb RA, Yamaguchi M, Nizet V, Dahl R, Thomas DD, Tait SW, Green DR, Fisher PB, Matsuzawa S, Reed JC.
Endoplasmic reticulum protein Bi-1 regulates Ca²⁺-mediated bioenergetics to promote autophagy. *Genes Dev* 2012; 26: 1041-54

Other Publications

Tait SW, Green DR.
Mitochondria and cell signalling. *J Cell Sci.* 2012; 125: 807-15

John Paul Career Award

All third year PhD students at the Beatson are eligible for this Award, named after Dr John Paul, the founding Director of the Beatson Institute for Cancer Research. Candidates prepare a progress report on their work and give a talk to staff and other students.

The winner of this year's Award was **Andrew Davidson** from Robert Insall's group. Andrew has been utilising Dictyostelium to determine the cellular roles of WASP family proteins, which are known to induce actin polymerisation.

Theses

Good, James (2012) The development of S-trityl L-cysteine based inhibitors of Eg5 as anticancer chemotherapeutics [PhD thesis, University of Glasgow, BICR]

Mah, Li Yen (2012) Characterisation of DRAM-1 in vitro and in vivo [PhD thesis, University of Glasgow, BICR]

Park, Laura (2012) Function and regulation of the WASH complex in the endocytic cycle [PhD thesis, University of Glasgow, BICR]

Stindt, Maren (2012) Regulation of wild-type and mutant p53 activity [PhD thesis, University of Glasgow, BICR]

Talapatra, Sandeep (2012) Mechanistic investigation of small molecule inhibitors of kinesin-5 and kinesin-6 family members in cancer drug development [PhD thesis, University of Glasgow, BICR]

Tang, Haoran (2012) Scar/WAVE complex suppresses cell invasion and cancer cell transformation [PhD thesis, University of Glasgow, BICR]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference Membrane Dynamics in Cancer

1 - 4 July 2012

Bute Hall, University of Glasgow

Scientific Committee: Jim Norman, Kurt Anderson, Robert Insall, Laura Machesky, Kevin Ryan

In the tradition of previous Beatson meetings, this year's conference proved to be a popular and lively event. We were delighted to be able to welcome Pier Paolo Di Fiore (IFOM-IEO, Milan) who described his work on breast cancer stem cells in the Colin Thomson Memorial Keynote Lecture, sponsored by the Association for International Cancer Research. There was also an excellent list of other speakers including Harald Stenmark (Oslo University Hospital) whose talk was sponsored by Nature Publishing Group, and Panomwat Amornphimoltham, Maria Baietti, Edmond Chan, Elena Morelli, Angela Oppelt, Elena Rainero, Joshua Rappoport, Mathias Rosefeldt and Guido Serini who were selected to give short talks. Maria Baietti (VIB, Belgium), who described her work on the biogenesis of exosomes, was awarded the short talk prize, sponsored by Portland Press, while Carine Rossé (Institut Curie, Paris) was awarded the AMSBIO-Trevigen sponsored poster prize for her work on late endosome trafficking. The meeting was generously co-sponsored by Cancer Research UK and the Association for International Cancer Research.

In 2013, the conference will focus on the important topic of the tumour stroma, its influence on cancer and how it might be targeted in anti-cancer therapies.

Beatson Institute Workshop Cold-blooded Cancer: Non-mammalian Models for Oncology Research

2 - 4 September 2012

The Robertson Trust Lecture Theatre,
Beatson Institute

Scientific Committee: Marcos Vidal, Jim Norman, Owen Sansom

The 2012 workshop focused on emerging *in vivo* cancer research systems involving non-mammalian organisms such as *Drosophila* and zebrafish. The meeting was hosted by one of our junior group leaders Marcos Vidal whose group uses the fruit fly as a model to study cancer. The meeting was a great success bringing as it did experts in the field to the Beatson for a series of excellent talks and posters, and plenty of lively discussion.

Open Evening

This year's opening evening, as in previous years, took place during National Science Week on 14th March and attracted around 200 visitors. There was a series of talks by group leaders Kevin Ryan and Hing Leung, clinical fellow Douglas Morran and post-doc Shereen Kadir followed by some demos and lab tours by an enthusiastic group of volunteers (pictured right).

The open evening always generates a lot of interest in our work, especially from school pupils and so for the first time this year we gave a group of them the chance to visit the Beatson for a week in July to get a more hands-on experience of work in a lab. This was a valuable experience for the pupils as many of them are thinking about a career in science or medicine, but it was also a very rewarding one for the researchers who helped make this event such a success.



Volunteers at the Beatson Open Evening in March.

SEMINARS AT THE BEATSON INSTITUTE

The following seminars were held at the Beatson Institute for Cancer Research during 2012.

January

Ian Eperon, Department of Biochemistry, University of Leicester

Kwee Yong, Clinical Haematology, University College London

Francesco Hofmann, Oncology, Novartis Institutes for Biomedical Research, Basel, Switzerland

Muffy Calder, School of Computing Science, University of Glasgow

February

Suresh Alahari, Department of Biochemistry & Molecular Biology, Stanley S Scott Cancer Center, New Orleans, USA

Harry Mellor, School of Biochemistry, University of Bristol

Hans Bos, Cancer Genomics Center, UMC Utrecht, The Netherlands

Roy Bicknell, Cancer Research UK Molecular Angiogenesis Group, University of Birmingham

March

David Elliott, University of Newcastle

Ahmed Ashour Ahmed, Weatherall Institute of Molecular Medicine and Nuffield Department of Obstetrics & Gynaecology, University of Oxford

Ultan McDermott, The Wellcome Trust Sanger Institute, Cambridge

Sean Lawler, Leeds Institute of Molecular Medicine, University of Leeds

Mark Bass, School of Biochemistry, University of Bristol

April

Frances Brodsky, Bioengineering and Therapeutic Sciences, University of California, San Francisco, USA

William Muller, Molecular Oncology Group, McGill Cancer Centre, Quebec, Canada

Iva Navratilova, College of Life Sciences, University of Dundee

May

Cristina Muñoz Pinedo, Bellvitge, Biomedical Research Institute, Barcelona, Spain

Martin Bushell, MRC Toxicology Unit, University of Leicester

Bernard Kelly, Cambridge Institute for Medical Research

June

Richard Lamb, University of Alberta, Canada

Rob Howes, Horizon Discovery, UK

Eduardo Moreno, University of Bern, Switzerland

Trevor Graham, University of California, San Francisco, USA

Patrice Codogno, INSERM U984 Châtenay-Malabry, France

July

Joao Passos, Newcastle University

Mike Chapman, Cambridge University Department of Haematology and Cambridge University Hospitals NHS Trust

Dennis Wilson, University of California Davis, USA

August

Rudd Delwel, Erasmus MC, The Netherlands

Keith Foster, Syntaxin, UK

Triona Chonghaile, Dana-Farber Cancer Institute, USA

Cédric Blanpain, Welbio, Interdisciplinary Research Institute, Université Libre de Bruxelles, Bruxelles, Belgium

Ashraf Ibrahim, Department of Pathology and MRC Laboratory of Molecular Biology, Addenbrooke's Hospital, Cambridge

September

Michael Rout, The Rockefeller University, New York, USA

Li-Zhi Mi, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, USA

Hector Peinado Selgas, Weill Cornell Medical College, New York, USA

Valerie Speirs, University of Leeds

Daan van Aalten, Division of Molecular Microbiology, University of Dundee

October

Laura Greaves, Centre for Brain Ageing and Vitality, Newcastle University

Brian Rudkin, Molecular Biology of the Cell Laboratory, Ecole Normale Supérieure de Lyon, France

Almut Schulze, Gene Expression Analysis Laboratory, Cancer Research UK London Research Institute

Daniel Peeper, The Netherlands Cancer Institute, Amsterdam

Arno Muller, University of Dundee

November

Johnathan Lopez, Cancer Centre, University of Lyon, France

Guowei Fang, Genentech, San Francisco, USA

Irene Miguel-Aliaga, University of Cambridge

Paul Shore, Faculty of Life Sciences, University of Manchester

December

Lionel Larue, Institut Curie, Paris, France

Masashi Narita, Cancer Research UK Cambridge Research Institute

STUDENTSHIPS AND POSTDOCTORAL FELLOWSHIPS

The training and career development of students and staff is essential in our mission to support cancer research of the highest standard. Our aim is to continue to attract enthusiastic scientists and clinicians early in their careers to work with our established staff and to draw on their experience but also to spark new ideas in a stimulating research environment. As well as learning a very wide range of practical and technical skills our junior researchers participate in all intellectual activities and present and discuss their own work at internal seminars and external meetings. We provide support and facilities of the highest standards and scientific interactions are encouraged by our international conference, workshops and seminars and by funding participation in external meetings.

PhD Studentships

The purpose of our clinician/graduate training programme is to give students and clinical fellows starting in research an opportunity to work in state-of-the-art laboratories. This enables them to assess and develop their research talents to the full, to decide whether a research career suits them and to use their period of graduate study as a springboard for their future career path. Our four-year studentships are designed to give graduates who show a strong aptitude for research the opportunity to complete substantial research projects resulting in very good publications. All students receive training in safe working practices, writing project reports and other transferable skills. Training also involves learning to be an independent scientist and students participate fully in the intellectual life of the Institute, attending and giving seminars and actively contributing to scientific discussions. Students are also given the opportunity to present posters on their work at national and international conferences to enhance their network of scientific contacts.

Our students are registered at the University of Glasgow and are allocated a supervisor and an advisor who are jointly responsible for supporting and monitoring their performance and progress. The supervisor is responsible for developing the student's abilities, providing all practical support required for the project and dealing with any

administrative matters required in relation to the University or funding body. The advisor gives additional guidance by providing independent advice on any matters concerning the studentship and by attempting to resolve any problems that may arise.

Postdoctoral Research Scientists and Fellows

We see post-docs as pillars of the research and intellectual activities of their own groups and of the Beatson Institute as a whole. The training programme is designed to promote the development of outstanding and dedicated junior scientists and we hope that by the end of their tenure they will be ready to compete for an independent position. Post-docs are initially employed for three years but outstanding individuals who are developing into independent scientists may be given additional support and responsibility – such as more technical help or mentoring a postgraduate student. At the discretion of their group leader, funding may be extended for two more years.

For further details on Studentships, Postdoctoral and other posts currently available, see our website www.beatson.gla.ac.uk.

www.glasgow.gov.uk and www.seeglasgow.com give general information about Glasgow and other useful links.

ADMINISTRATION

As an independent charity it is important to ensure that the Beatson Institute complies with all relevant regulations and adopts best practice in financial, personnel and corporate governance matters. Our Finance & Human Resources team has been set up to do this, providing the Institute's Board of Governors with necessary financial and legal information, ensuring that funding bodies' administrative requirements are met and that funding for the Institute's research activities is properly managed.

The Beatson Institute also needs to coordinate with the University of Glasgow's central administration over such matters as the administration of grants, payment of suppliers and staffing. Our Finance & Human Resources and Secretarial teams provide that vital link.

Finance & Human Resources

Peter Winckles, Caroline Preacher

Zoe Chen, Jacqui Clare, Karen Connor, Nicki Kolliatsas, Elaine Marshall, Frank McGeoghegan, Lynn Wilson

The Finance & Human Resources team is responsible for all accounting and personnel management issues including banking, payments, grants management, budgeting, pay administration, staff policies and procedures, and reporting financial information for funders, managers and the Board of Governors.

Secretarial

Laraine Kernahan (PA to Professor Vousden),

Barbara Laing, Catriona Lambert, Sarah Price

The Secretarial team provides an extensive range of secretarial and office services. These include assisting with staff recruitment, organising travel and accommodation, seminar arrangements, organisation of our conferences and workshops, database maintenance and the running of the main reception for the Beatson Institute. The team plays an important role in maintaining internal links, and in relationships with Cancer Research UK, the University of Glasgow and many other organisations with which our scientists have contact.

Scientific Administration

Jackie Beesley

The Scientific Administrator supports scientists at the Beatson Institute by taking minutes at a range of scientific and administrative meetings, editing publications such as the Scientific Report, maintaining an up-to-date website, administering the graduate student training programme, and providing reports and answering queries for both internal and external audiences, including Cancer Research UK.

Cancer Research Technology

Fatima Kranc

Cancer Research Technology (CRT) is an oncology-focused technology transfer and development company wholly owned by Cancer Research UK with 130 employees based primarily in London and Cambridge. Since a substantial amount of the funding for the Beatson Institute comes from Cancer Research UK, CRT manages all intellectual property-related matters on behalf of the Institute and the charity. To facilitate this, there is now a CRT Business Manager based full-time at the Institute.

THANKS FOR SUPPORTING US

The work of our various research groups would barely proceed without the substantial grant funding provided by Cancer Research UK to the Beatson Institute and the University of Glasgow, now amounting to over £18 million per annum combined. In addition, £7 million in capital grants from the charity has supported the expansion of our biological services unit, which was completed this year. We are also indebted to a number of other organisations that provide funding to our scientists, usually supporting projects in a particular sphere of special interest, or supporting the careers of talented junior scientists, enabling them to pursue their research interests within our laboratories. These organisations, whose funding we appreciate greatly, are listed below. The additional funding provided by these organisations makes possible much work that we otherwise could not be undertaking and has become integral and indispensable to our operations.

Cancer Research UK Beatson Institute

Kurt Anderson

Carestream, Ian Sunter Charitable Trust (with KV)

Martin Drysdale

Medical Research Council (with MO)

Jeff Evans

Scottish Executive – Chief Scientist Office, Medical Research Council, Pancreatic Cancer Research Fund, University of Glasgow

David Gillespie

Association for International Cancer Research, European Community

Eyal Gottlieb

AIRC (Italian Association for Cancer Research), IAP programme (Belgian Science Policy), Janssen Pharmaceutica NV

Robert Insall

Wellcome Trust

Hing Leung

The Academy of Medical Sciences, Medical Research Council, Newcastle upon Tyne Hospital, Prostate Cancer Charity

Laura Machesky

Association for International Cancer Research, Breast Cancer Campaign, British Heart Foundation, Medical Research Council, Royal College of Surgeons Edinburgh

Jim Norman

Breast Cancer Campaign, CSIC (Spanish National Research Council)

Michael Olson

Breast Cancer Campaign, Medical Research Council (with MD)

Kevin Ryan

Association for International Cancer Research, EMBO

Owen Sansom

Association for International Cancer Research, EMBO, European Community, Medical Research Council, Medimmune, NC3Rs (with MV), Novartis, Pfizer, Royal College of Physicians & Surgeons, Royal College of Surgeons Edinburgh, Royal Society, Wellcome Trust

Emma Shanks

Breast Cancer Campaign

Marcos Vidal

NC3Rs (with OS)

Karen Vousden

Association for International Cancer Research, Canadian Institutes of Health Research, Netherlands Organisation for Scientific Research, NHS Greater Glasgow & Clyde Health Board Endowment Fund, Rubicon, , Ian Sunter Charitable Trust (with KA), West of Scotland Women's Bowling Association

Sara Zanivan

Breast Cancer Campaign

Beatson Associates

Peter Adams

BBSRC, British Heart Foundation, Medical Research Council, NIH with National Institute on Aging & National Cancer Institute (USA)

Stephen Tait

BBSRC, EU Marie Curie Actions, Royal Society

We do not purposefully solicit contributions to our work directly from the general public – we see this as the role of the cancer charities such as those that feature above. We are, however, fortunate to be in the minds of many local people and organisations who give generously of their time and effort to raise funds for good causes. We are also, more poignantly, in the minds of those who are suffering cancer, or who have lost loved ones to this disease. To those who give time and effort to raise funds on our behalf and to those who thoughtfully regard us as suitable beneficiaries of their generosity, thank you.

- 45th Glasgow Girls' Brigade
- 71st Girls' Brigade
- Florence Adams, in memory of her husband
- Adam & Marcelle Allan, in memory of Kenneth & Andy Baldacci
- Staff of Aviva Insurance
- Legacy of Mr John Beattie's Estate
- Janet Black, in memory of her husband Mr Hamish Black
- Mairi Boyde, in memory of the late Mr Duncan

Crawford of Brenchoille Farm

- Elizabeth Brown
- Helen Brown
- Margaret G Brown
- Buchanan Castle Golf Club
- Archie Callander, in memory of the late Mr Gordon McFarlane
- Caltag Medsystems
- Edward Cameron
- Camlab Ltd
- Mary Campbell, in memory of her husband Mr Campbell
- Peter Campbell
- Castle Precision Engineering (Glasgow) Ltd
- Collectis Bioresearch
- Staff of Clyde Travel
- The Clyde Group
- Sarah Colman
- Crail Golfing Society
- In memory of the late Mr Duncan Crawford
- Culcreuch Castle Hotel Staff & Workmates, in memory of Mr John Cable
- Robert A Cumming
- Mr & Mrs Dowling
- Employees of The Edrington Group
- Eppendorf UK Ltd
- Eric Forbes
- Martin Gallagher
- Alan Gill
- Govan & Linthouse Church of Scotland
- May Gow
- Jane Graham, in memory of her husband Mr Iain Graham
- Helenslea Very Sheltered Tenants Association, in memory of Margaret Tominay
- Staff of Hewlett Packard
- R Hornig, in memory of her husband Mr Andrew Hornig
- The James Inglis Trust
- Ipsos MORI Scotland
- E Irvine, in memory of her husband
- Charlotte Johnston
- Dorothy Kelly
- Dr & Mrs J D Olav Kerr's Charitable Trust
- Knightswood St Margaret's Guild



Tesco staff on a tour of the Beatson Institute with PhD student Laura Park (right). Cancer Research UK was Tesco's Charity of the Year for 2012.

- Lanarkshire Women's Bowling Association
- Cameron Lawrence
- Family of Mrs Jean Millar Lawson
- Legacy of Mrs Jean Millar Lawson
- Logie Kirk
- H McCulloch
- Christina McDougall, in memory of her husband John
- In memory of the late Mr Gordon McFarlane
- Fiona McNeill and family
- In memory of the late Miss Rhoda McQuarrie
- Merck Millipore Bioscience
- Merrylea Mother & Toddler Group
- B Millar
- Miltenyi Biotec Ltd
- Mosshead Primary School
- Newton Mearns Baptist Church, in memory of Kathryn Miller
- North View Housing Association
- Anne O'Hare & Hillpark Bowling Club
- Order of the Eastern Star Carluke Violet Chap 66, in memory of Past Worthy Patron Brother John Ellarby
- Pacitti Jones
- Parklands Country Club Ltd
- Polaroid Eyewear Ltd, in memory of Mr Robert Mclean
- Mrs Raeside, in memory of her husband Mr Iain Raeside
- Riverside Drama Club
- The Robertson Trust
- Roche Diagnostics Ltd
- Pupils of Sandaig Primary School
- Scottish Crime & Drug Enforcement Agency, in memory of friend and colleague Mr Iain Graham
- Tom Slimming
- St Luke's Guild, Milngavie
- StarLab (UK) Ltd
- Stratech Scientific Ltd
- The Staypar Charitable Trust, in memory of the late Gail Wylie
- Temple Anniesland Guild
- Gordon Thom, in memory of his brother-in-law Mr Michael McArtney
- Thornhill Gardening Society
- Vector Laboratories
- J Walker
- West of Scotland Women's Bowling Association
- Mrs M White
- Employees of William Grant & Sons

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The Rt Hon. Lord Mackay of Clashfern
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Chief Executive, Cancer Research UK

Mr Craig Anderson

Senior Partner, KPMG

Mr Ian Dickson

Consultant, MacRoberts Solicitors, Glasgow

Dr Iain Foulkes

Executive Director, Strategy and Research Funding, Cancer Research UK

Prof Nic Jones

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Mr Peter Winckles

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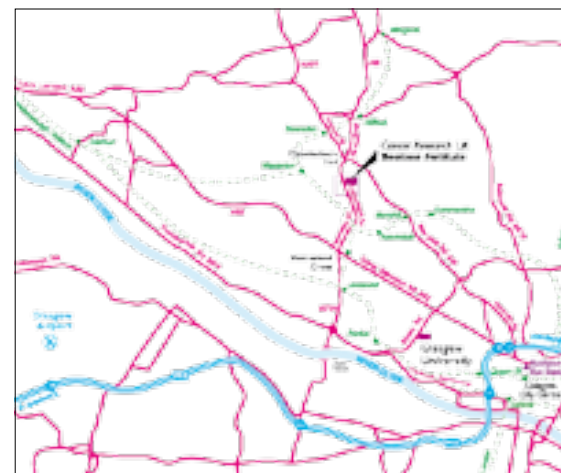
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Cancer Research UK

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