SCIENTIFIC REPORT 2014

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Cover image
PhD student Christin Bauer from Marcos Vidal's lab, who uses the fruit fly as a tool to study cancer biology.

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CONTENTS

INTRODUCTION

Professor Karen Vousden CBE, FRS, FRSE, FMedSci
Director of the Cancer Research UK Beatson Institute

The success of the Beatson Institute continued this year, with outstanding quinquennial reviews for four of our largest groups and continued recruitment into both core funded and University of Glasgow supported positions.

A number of key appointments this year mean that the Institute is now at almost full capacity. We welcomed new group leaders, **Shehab Ismail** and **Alexei Vazquez** along with our final two Beatson Associates, **David Bryant** and **Jurre Kamphorst**. **Heather McKinnon** was also appointed as Head of Biology in Drug Discovery.

Shehab is establishing a programme focused on the structural biology of cilia, while Dave is studying the control cell polarity in cancer. Meanwhile, with their respective expertise in lipid metabolism and computational modelling, Jurre and Alexei considerably strengthen our Cancer Metabolism Research Unit (CaMeRU). The Unit also received vital funding from the Beatson Endowment for equipment.

We said farewell to **Nick Morrice**, head of our proteomics facility and for the time being, **Sergio Lilla** and **David Sumpton** are jointly running this important service for us. My PA **Laraine Kernahan** also retired from the Institute in August and **Sheila McNeill** has joined us to take on this key role.

Four of our senior scientific groups - **Eyal Gottlieb**, **Jim Norman**, **Mike Olson** and Karen Vousden - had highly successful reviews this year, while we were also delighted with **Karen Blyth's** well deserved promotion to Senior Staff Scientist. As head of our transgenic facility, Karen is a vital component of our mouse models team and she was enthusiastically supported by our external expert review panel.

All of the Beatson faculty are now working hard to attract grant funding from various sources to support our work. In several cases this has also involved establishing new collaborations and networks that further broaden our expertise and research areas.

Two of our senior group leaders, **Robert Insall** and **Laura Machesky** were recognised for their considerable contributions to their fields by being elected as Fellows of the Royal Society of Edinburgh, while **Jeff Evans** was made a Fellow of the Royal College of Physicians of Edinburgh. I was also honoured to give the Norman Heatley Lecture at the University of Oxford and the Inaugural Women of Distinction in Science and Medicine Lecture at Queen Mary University of London, while junior group leader **Jurre Kamphorst** was awarded a prestigious Cancer Research Career Development Award. Postdoc **Robert Fordham** was awarded a Royal Commission for the Exhibition of 1851 Fellowship, while postdoc **Kevin Myant** moved across to Edinburgh to establish an independent research group.

To further develop our expertise in cancer metabolism, we were delighted to welcome experts on cancer metabolism to our conference In July. The conference, led by Eyal Gottlieb and Jim Norman, was a great success and allowed us to introduce new and old friends to Glasgow and the Beatson.

Recruitment to the Cancer Research UK Glasgow Centre's infrastructure posts is now also largely complete and includes the appointment of **Liz Musgrove**, the new Synergy Manager for the Centre, who has overall responsibility for coordinating the infrastructure and developing mechanisms through which all Centre members can access it and **Fiona Thomson**, senior research fellow in cancer biomarkers/pharmacology and Director of the Analytical Services Unit.

Professor Robert Insall and Professor Laura Machesky who became Fellows of the Royal Society of Edinburgh in March

The activities supported by the Centre range from curating core resources to developing methods, providing advice and expertise, and building capacity to undertake specific projects. These activities encompass a wide variety of expertise and different operational structures, necessitating the development of individualised models for resource management and access. This is an ongoing process, with a major focus to date on preclinical trials and functional screens. A number of calls for bids to take advantage of these new means of support have led to the approval of several exciting new projects aimed at translating our basic science.

Finally, huge thanks must to go our many supporters who both remember us in the form of legacies and work so hard to raise funds for us, including especially Clyde Travel, Mosshead Primary School and the West of Scotland Women's Bowling Association who do this so tirelessly and to such effect year after year.

RESEARCH HIGHLIGHTS

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.

Beatson Institute

Cordero JB, Ridgway RA, Valeri N, Nixon C, Frame MC, Muller WJ, Vidal M, Sansom OJ.
c-Src drives intestinal regeneration and transformation. ***EMBO J*** 2014; 33: 1474-91

Src is overexpressed or activated in up to 20% of colorectal tumours, although it is unclear what the consequences of this might be. To begin to address this, the authors investigate Src actions within the intestine of the *Drosophila* and the mouse. They show that Src is necessary and sufficient to drive intestinal stem cell proliferation during tissue self-renewal, regeneration and tumourigenesis, and that this occurs via upregulation of EGFR and activation of Ras/MAPK and Stat3 signalling.

Faller WJ, Jackson TJ, Knight JR, Ridgway RA, Jamieson T, Karim SA, Jones C, Radulescu S, Huels DJ, Myant KB, Dudek KM, Casey HA, Scopelliti A, Cordero JB, Vidal M, Pende M, Ryazanov AG, Sonenberg N, Meyuhas O, Hall MN, Bushell M, Willis AE, Sansom OJ.
mTORC1-mediated translational elongation limits intestinal tumour initiation and growth. ***Nature*** 2015; 517: 497-500. doi: 10.1038/nature13896. Epub 2014 Nov 5

In a series of *in vivo* experiments, the authors of this paper demonstrate that mTORC1 is an essential downstream effector of Wnt signalling in the intestine. They show that it is absolutely required for enterocyte proliferation following APC loss and Wnt activation, and that this is as a result of it increasing translation elongation. Thus, the authors suggest that targeting mTOR and translation control might benefit patients at high risk of developing colorectal cancer.

Labuschagne CF, van den Broek NJ, Mackay GM, Vousden KH, Maddocks OD.
Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. ***Cell Rep*** 2014; 7: 1248-58

Building on previous work showing that cancer cells can be highly dependent on serine/glycine uptake for proliferation, this study uses a new technique called Pulse-Stop-Flux, which detects flux into low-abundance metabolites, to determine whether cancer cells can use serine and glycine interchangeably. The authors conclude that serine, rather than glycine consumption supports both nucleotide synthesis and cancer cell proliferation.

Li A, Morton JP, Ma Y, Karim SA, Zhou Y, Faller WJ, Woodham EF, Morris HT, Stevenson RP, Juin A, Jamieson NB, MacKay CJ, Carter CR, Leung HY, Yamashiro S, Blyth K, Sansom OJ, Machesky LM.
Fascin is regulated by slug, promotes progression of pancreatic cancer in mice, and is associated with patient outcomes. ***Gastroenterology*** 2014; 146: 1386-96 e1-17

This paper provides strong evidence that the actin-bundling protein fascin is regulated by the transcription factor slug and involved in pancreatic tumour formation and metastatic spread. Mechanistically, the authors show that fascin promotes filopodia formation and cell invasion. They also find an association between fascin levels and patient outcomes suggesting fascin could be a useful marker or therapeutic target.

Morran DC, Wu J, Jamieson NB, Mrowinska A, Kalna G, Karim SA, Au AY, Scarlett CJ, Chang DK, Pajak MZ, Oien KA, McKay CJ, Carter CR, Gillen G, Champion S, Pimlott SL, Anderson KI, Evans TR, Grimmond SM, Biankin AV, Sansom OJ, Morton JP.
Targeting mTOR dependency in pancreatic cancer. ***Gut*** 2014; 63: 1481-9

In this study, the authors use established preclinical models of pancreatic cancer to demonstrate that tumours driven by activated KRAS and PTEN loss are dependent on mTOR signalling and are uniquely responsive to its inhibition, when compared to tumours driven by activated KRAS and mutant p53. They also show that approximately 20% of human pancreatic tumours have low PTEN expression and a gene expression signature similar to that seen in the preclinical model, leading them to conclude that anti-mTOR therapies may benefit this subset of patients. Overall, this work illustrates how, in the future, genetic signatures of human tumours could be used to personalise cancer treatments, making them more effective.

Muinonen-Martin AJ, Susanto O, Zhang Q, Smethurst E, Faller WJ, Veltman DM, Kalna G, Lindsay C, Bennett DC, Sansom OJ, Herd R, Jones R, Machesky LM, Wakelam MJ, Knecht DA, Insall RH.
Melanoma cells break down LPA to establish local gradients that drive chemotactic dispersal. ***PLoS Biol*** 2014; 12: e1001966

Metastasis is particularly prevalent in melanoma, leading to high mortality rates. This work seeks to understand what drives melanoma cells to migrate out of a tumour with such efficiency and what role, if any chemotaxis plays in this. Using a chamber-based assay developed in the lab, the authors show that melanoma cell dispersal occurs by positive chemotaxis as a result of outward-facing lysophosphatidic acid (LPA) gradients. Importantly, cells *in vivo* and in culture generate these gradients themselves by breaking down LPA. This leads the authors to conclude that melanoma drives its own metastasis.

Rosenfeldt MT, Bell LA, Long JS, O'Prey J, Nixon C, Roberts F, Dufes C, Ryan KM.
E2F1 drives chemotherapeutic drug resistance via ABCG2. ***Oncogene*** 2014; 33: 4164-72

In this paper, the authors report that, in a number of settings, E2F1 regulates the expression of the transporter ABCG2 leading to drug efflux and suppression of chemotherapy-induced cell death. They also find that elevated E2F1 correlates with ABCG2 expression in human lung cancer. Thus, the study highlights an exciting new function for this transcription factor, which is frequently upregulated in cancer, in multidrug resistance and cancer therapy.

Scopelliti A, Cordero JB, Diao F, Strathdee K, White BH, Sansom OJ, Vidal M.
Local control of intestinal stem cell homeostasis by enteroendocrine cells in the adult *Drosophila* midgut. ***Curr Biol*** 2014; 24: 1199-211

Enteroendocrine cells in the intestine are known to translate local cues into systemic responses by releasing hormones into the bloodstream. However, using the *Drosophila* midgut as a model system, the authors of this study identify a novel, local role for these cells as paracrine regulators of intestinal stem cell proliferation. Furthermore, they show that this is mediated via Bursicon/DLGR2 signalling, leading to stem cell quiescence. The authors suggest these results point toward a potential role for LGRs as tumour suppressor genes.

Stindt MH, Muller PA, Ludwig RL, Kehrloesser S, Dotsch V, Vousden KH.
Functional interplay between MDM2, p63/p73 and mutant p53. ***Oncogene*** 2014 Nov 24. doi: 10.1038/onc.2014.359. [Epub ahead of print]

MDM2 binds to and is an important regulator of wild type p53, the tumour suppressor protein. However, this study examines the functional consequences of MDM2's interactions with mutant p53, p63 and p73. As well as confirming that mutant p53 binds to both p63 and p73, the authors show that MDM2 preferentially binds to p73 rather than p63. This leads them to propose a model in which MDM2 competes with p63 for binding to mutant p53 to restore p63 activity, while forming a trimeric complex with p73 and mutant p53 to more strongly inhibit p73 function. The study highlights the complex interactions that can occur between p53 family members and the proteins that regulate them.

van den Biggelaar M, Hernandez-Fernaud JR, van den Eshof BL, Neilson LJ, Meijer AB, Mertens K, Zanivan S.
Quantitative phosphoproteomics unveils temporal dynamics of thrombin signaling in human endothelial cells. ***Blood*** 2014; 123: e22-36

As well as being the key enzyme involved in coagulation, thrombin triggers PAR1 signalling in the endothelium. In this paper, the authors combine SILAC, phosphopeptide enrichment techniques and mass spectrometry to analyse thrombin-induced signalling in human primary endothelial cells. They identify 2224 thrombin-regulated phosphorylation sites, providing a unique resource for future studies of thrombin and PAR signalling. Ultimately, a greater understanding of this signalling could lead to the development of improved PAR1 antagonists, for use as anti-coagulants, that affect platelet but not endothelial cell function.

Institute of Cancer Sciences

Haller M, Hock AK, Giampazolias E, Oberst A, Green DR, Debnath J, Ryan KM, Vousden KH, Tait SWG.
Ubiquitination and proteasomal degradation of ATG12 regulates its pro-apoptotic activity, ***Autophagy*** 2014; 10: 2269-78

This study investigates the regulation of the essential autophagy and ubiquitin-like protein ATG12 when in its free state, not conjugated to

ATG5. The authors find that ATG12 is highly unstable and targeted for proteasomal degradation via ubiquitination. As a consequence of this turnover, ATG12 contributes to proteasome inhibitor-mediated apoptosis. Since proteasome inhibitors are used as anti-cancer agents, this may be a clinically important finding.

Lund K, Cole J, VanderKraats ND, McBryan T, Pchelintsev NA, Clark W, Copland M, Edwards JR, Adams PD.
DNMT inhibitors reverse a specific signature of aberrant promoter DNA methylation and associated gene silencing in Acute Myeloid Leukemia. ***Genome Biol*** 2014; 15: 406

In this paper, the authors use whole genome DNA methylation and RNA sequencing to identify a set of genes whose methylation and silencing in AML is reversed by DNA methyltransferase inhibitors. The authors conclude that, as good candidates for direct regulation by DNA methyltransferase inhibitors, reactivation of these genes might contribute to therapeutic activity as well as making them useful biomarkers.

Muthalagu N, Junttila MR, Wiese KE, Wolf E, Morton J, Bauer B, Evan GI, Eilers M, Murphy DJ.
BIM is the primary mediator of MYC-induced apoptosis in multiple solid tissues. ***Cell Rep*** 2014; 8: 1347-53

MYC, an oncogene frequently overexpressed in cancer, can drive both cell proliferation and apoptosis. The latter limits its oncogenic potential and the authors of this paper set out to determine, using a single transgenic model, what the key mediator of this MYC-induced apoptosis is. They conclude that the BH3-only protein BIM, rather than p19ARF, is required for MYC-induced apoptosis. Thus, BH3 mimetics might prove useful therapeutically in boosting MYC's intrinsic apoptotic response.

Rai TS, Cole JJ, Nelson DM, Dikovskaya D, Faller W, Vizioli MG, Hewitt RN, Anannya O, McBryan T, Manoharan I, van Tuyn J, Morrice N, Pchelintsev NA, Ivanov A, Brock C, Drotar M, Nixon C, Clark W, Sansom OJ, Anderson KI, King A, Blyth K, Adams PD.
HIRA orchestrates a dynamic chromatin landscape in senescence and is required for suppression of neoplasia. ***Genes Dev*** 2014; 28: 2712-25

Cellular senescence acts as an important barrier to proliferation and tumour progression, and extensive changes to chromatin occur in senescent cells. However, the molecular basis and consequences of these changes is not fully understood. The authors of this study show that non-proliferating senescent cells express and incorporate H3.3 and other histones into a dynamic chromatin landscape. Importantly, they also find that the histone chaperone HIRA is required for regulation of this landscape and for suppression of oncogene-induced neoplasia *in vivo*.

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 with us and, in 2013, to the adjoining Wolfson Wohl Cancer Research Centre. 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

Cancer Research UK
Beatson Institute

REGULATION OF CANCER CELL GROWTH METABOLISM AND SURVIVAL

CANCER RESEARCH UK BEATSON INSTITUTE

Eyal Gottlieb - Tumour Metabolism
Danny Huang - Ubiquitin Signalling
Hing Leung - Prostate Cancer Biology
Kevin Ryan - Tumour Cell Death
Alexei Vazquez - Mathematical Models of Metabolism
Karen Vousden - Tumour Suppression

TUMOUR METABOLISM

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Our lab utilises state-of-the-art metabolomics capabilities to study metabolic transformations and identify metabolic vulnerabilities in cancer. An early hallmark of cancer tissues is metabolic reprogramming, first noted by Otto Warburg who found that cancer cells rely on glycolysis under aerobic conditions. More recent research showed that metabolic alterations in cancer involve many additional pathways, potentially increasing the number of clinical targets. In fact, most, if not all tumour suppressors and oncogenes regulate metabolism. Furthermore, tumours are typically placed in a metabolically stressful environment, leading to essential metabolic adaptations. Our major interest is in metabolic enzymes that also function as tumour suppressors or oncogenes or those which regulate the essential metabolic requirements of cancer cells.

Acetyl-CoA synthetase 2 promotes acetate utilisation and maintains cancer cell growth under metabolic stress

The metabolic adaptation of cancer cells to environmental changes often involves an increase in the uptake and metabolism of extracellular resources such as glucose, amino acids and lipids. However, cancer cells can further modify or shift their metabolism during periods of nutrient stress. Over the last two years, we led a consortium of five groups, co-funded by a CRUK Discovery Committee award and AstraZeneca, with the specific purpose of identifying targets in the field of lipid metabolism. We performed siRNA screens on a focused set of 60 genes using a panel of 13 breast and prostate cancer cell lines. Cells were maintained in either 10% or 1% serum, and in both normoxic and hypoxic conditions. Under conditions of 1% serum and hypoxia, the expression of acetyl-CoA synthetase 2 (ACSS2), an enzyme that converts acetate into acetyl-CoA in many of the cancer cell lines, was markedly induced. Accordingly, silencing of ACSS2 strongly inhibited cellular growth under these conditions. Data mining and disease linkage analyses revealed that of our shortlisted targets, ACSS2 exhibited the highest level of amplification in invasive breast carcinomas (7%). The characterisation of acetate metabolism during hypoxia by heavy isotope tracing coupled with liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) technologies confirmed that acetate was consumed by cancer cells in an ACSS2-dependent manner. It also showed that acetate was used to synthesise fatty acids and TCA cycle intermediates. In contrast, the formation of these metabolites from glucose-derived acetyl-CoA was significantly diminished under hypoxia. These results suggested that when mitochondrial glucose oxidation was compromised (hypoxia) or when the availability of lipids was limited (low serum), acetate was needed to generate acetyl-CoA for the production of energy (TCA cycle) and biomass (fatty acids/cholesterols). These mechanistic effects were reinforced by phenotypic studies showing that ACSS2 silencing inhibited the growth in two-dimensional cultures, thre-dimensional spheroids and tumour xenografts (Fig. 1). In summary, our data revealed a previously unappreciated role for acetate as a nutritional source for the growth and survival of cancer cells under metabolically stressful conditions (Schug *et al.*, Cancer Cell 2015; 27: 57).

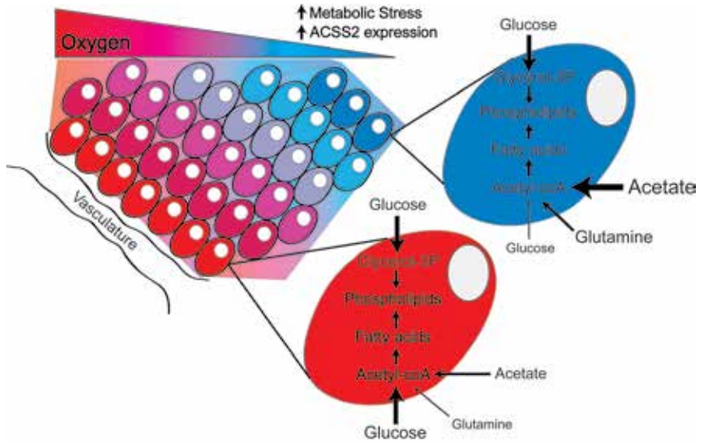

Figure 1
Acetate metabolism under metabolic stress. The depletion of available oxygen and fatty acids to cancer cells growing at a distance from the blood supply induces a hypoxia adaptive response and *de novo* fatty acid biosynthesis, two metabolic hallmarks observed in most human cancers. However, metabolic adaptations to these stressful conditions require a switch of nutrient utilisation, turning from glucose to acetate as a major carbon source for fatty acid biosynthesis. This metabolic switch is mediated by ACSS2.

Glutathione succination by fumarate induces redox stress and cellular senescence and is a molecular barrier that protect FH-deficient renal cells from carcinoma

Mutations in the tricarboxylic acid (TCA) cycle enzyme, fumarate hydratase (FH) are associated with hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome, which is characterised by the formation of mostly benign smooth muscle tumours and, in some patients, a highly malignant form of papillary and collecting duct renal cell cancer. Although the mechanisms of tumourigenesis related to FH mutations have been extensively investigated, little is known about the overall consequences that loss of FH has on cellular metabolism. A mouse model of FH conditional gene deletion in the kidney demonstrated that FH deletion in the kidney causes benign cysts but fails to induce renal cell carcinoma (RCC) (Zheng *et al.*, Cancer Metab 2013; 1: 12). Using primary non-immortalised kidney cells from these mice, we recognised that acute FH deletion leads to redox stress-induced senescence and p21 induction (Fig. 2). We further demonstrated that fumarate accumulation in FH-deficient cells leads to oxidative stress by covalently binding and inactivating reduced glutathione (GSH) forming a succinic-GSH adduct (Fig. 2). This metabolite was found in both human and mouse FH-deficient cells and in kidney cysts of Fh1-deficient mice. We also demonstrated that the oxidative stress induced by GSH succination is sufficient to elicit cellular senescence in non-transformed cells. Importantly, the ablation of p21, a key mediator of senescence, in Fh1-deficient mice resulted in the transformation of benign renal cysts into a hyperplastic lesion, suggesting that fumarate-induced senescence needs to be bypassed for the initiation of renal cancers (Zheng *et al.*, Nature Commun 2015; 6: 6001). These results suggest that fumarate-induced senescence may be a novel tumour suppressive event that must be circumvented for RCC formation. It is worth noting that HLRCC patients also develop multiple benign kidney cysts that then evolve into aggressive malignant carcinoma. Furthermore, in many human RCCs, other than HLRCC, low FH expression is associated with disease progression. Hence, recognising the mechanisms that lead FH-deficient cells to overcome senescence *in vivo* will help us understand and hopefully treat human RCC in general.

Publications listed on page 80

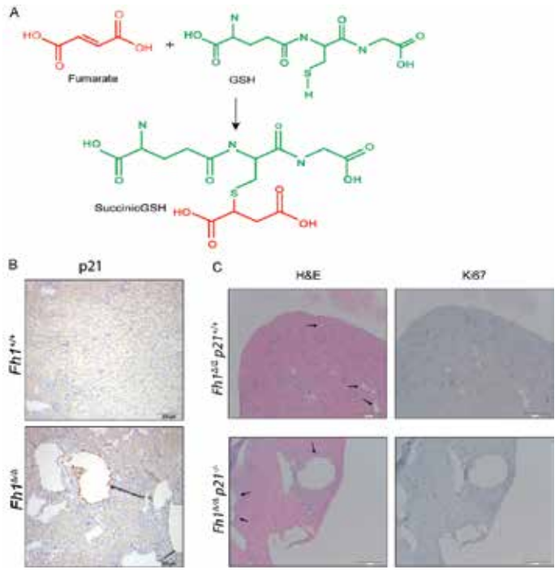

Figure 2
Fumarate induces glutathione succination and senescence. (A) The loss of FH leads to fumarate accumulation, which forms adducts with the thiol group of GSH (succinic-GSH). (B) Redox stress mediated by glutathione succination leads to p21 induction and benign renal cysts in an Fh1-deficient mouse model. (C) The co-ablation of the p21 gene in the Fh1-deficient mouse model leads to hyper-proliferation as indicated by increase Ki67 staining of hyperplastic regions.

UBIQUITIN SIGNALLING

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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 Mg^{2+} 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. 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 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.

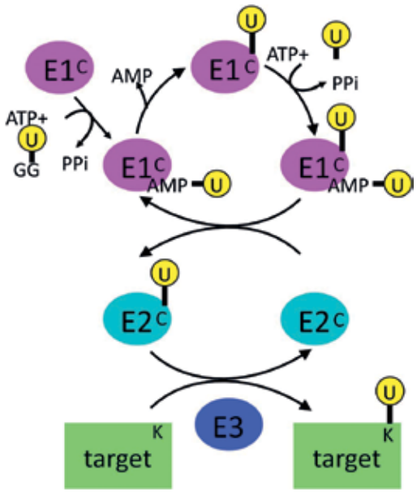

Figure 1
Enzymatic cascade for Ub modifications.

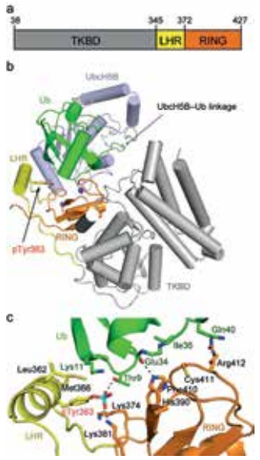

Figure 2
Phosphorylated LHR tyrosine participates activation of E2~Ub. (a) The conserved Cbl's N-terminal domain containing TKBD, LHR and RING domain. (b) Structure Tyr363 phosphorylated Cbl-b bound to an E2~Ub and a TKBD substrate peptide. (c) Close-up view of pTyr363-Ub and RING-Ub interactions. All structures are coloured as in (a). E2 is in light blue, TKBD substrate peptide is in pink and Ub is in green.

All Cbls share a highly conserved N-terminal SH2-containing tyrosine kinase-binding domain (TKBD), a linker helix region (LHR) and a RING domain (Fig. 2a) followed by a variable proline-rich region (PRR). 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. 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. We found that Tyr371 phosphorylation enhances c-Cbl's catalytic efficiency by 1400-fold. Tyr371 phosphorylation activates its 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. 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 (Dou *et al.*, Nat Struct Mol Biol 2012; 19: 184).

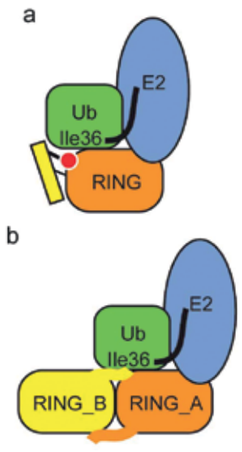

Figure 3
Requirements for E2~Ub activation for transfer by RING E3s. An additional component (highlighted in yellow) outside the RING domain (orange) is required for stabilisation of Ub by Cbl (a) and by the dimeric RING E3s (b).

Although our results showed that LHR Tyr371 phosphorylation could induce dramatic conformational changes to activate c-Cbl, these changes are insufficient to account for the 1400-fold enhancement in catalytic efficiency. More recently we showed that phosphorylation of LHR Tyr plays an additional crucial role. By determining a crystal structure of phosphoTyr363-Cbl-b (Tyr363 is the corresponding LHR Tyr in Cbl-b) bound to a TKBD substrate peptide and an E2 covalently linked to Ub (E2~Ub), we showed that the phosphoTyr-induced E2-binding surface also participates in Ub binding (Fig. 2b). Notably, the phosphate moiety of the phosphoTyr363 directly interacts with Ub's Thr9 sidechain (Fig. 2c). These Ub interactions alone enhance Cbl's catalytic efficiency by ~200-fold, explaining the massive rate enhancement upon phosphorylation (Dou *et al.*, Nat Struct Mol Biol 2013; 20: 982).

c-Cbl's Ty371 is one of the mutational hotspots in patient with myeloid neoplasms. Our results explain why Tyr371 mutants cannot adopt active Cbl configuration and optimise E2~Ub for transfer. We are currently investigating on how c-Cbl mutations could alter c-Cbl's conformation 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. We have now determined two crystal structures of RING E3 bound to E2 UbcH5B covalently linked to Ub at its active site. Both structures reveal extensive noncovalent donor Ub interactions with UbcH5B and the RING domain. Notably an additional Ub-binding element outside the RING domain is crucial in stabilising the Ub's Ile36 surface (Fig. 3). In dimeric RING E3s such as BIRC7 and RNF4, the C-terminal tail of the second RING subunit acts as this additional Ub-binding component, and in Cbl-b, phosphorylation of LHR Tyr363 creates a phosphoTyr-induced element adjacent to the RING domain that functionally mimics that dimeric RING E3 tail in stabilising Ub. Both Ub-binding components optimise k_{cat} and K_m for Ub transfer. These results demonstrate that, in addition to Ub-E2, Ub-RING and E2-RING interactions, an additional Ub-binding component outside the RING domain is essential for stabilisation of the donor Ub configuration, restraining the globular Ub into a closed conformation and allowing optimal positioning of Ub's C-terminus for transfer (Dou *et al.*, Nat Struct Mol Biol 2012; 19: 876; Dou *et al.*, Nat Struct Mol Biol 2013; 20: 982).

PROSTATE CANCER BIOLOGY

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Prostate cancer is a major global health issue and a very common cause of morbidity and mortality in men worldwide. Our research programme builds on ongoing work on aberrant cellular signalling (including androgen receptor, Sprouty2 and ERK5) and novel preclinical models to fast track the development of new treatment agents or strategies for prostate cancer through cross-disciplinary collaborations (Fig. 1).

Here, we select three examples (Fig. 2) where we strive to integrate our laboratory and clinical resources to provide new biologic insight into castration-resistant prostate cancer for future clinical applications.

In vivo sleeping beauty (SB) transposon screen identified *Pparg* activation as a key interacting event with *Pten* lost in driving prostate tumourigenesis through altered lipid metabolism

[Collaborators: Owen Sansom, Dave Adams (Wellcome Trust Sanger Centre, Cambridge) Sioban Fraser and Joanne Edwards (Histopathology, University of Glasgow)]

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 (Ahmad *et al.*, Proc Nat Acad Sci 2011; 108: 16392). Male mice with appropriate SB genotype: *SB:Pten*^{Null} *(Pb-Cre4:Pten*$^{fl/fl}$ *T2Onc3/+Uren/+)* and littermate controls [*Pten*^{Null} *(Pb-Cre4:Pten*$^{fl/fl}$*)* and *SB*^{Control} *(Pb-Cre4:T2Onc3/+Uren/+)*] were generated and aged. *SB:Pten*^{Null} mice exhibited significantly accelerated prostate tumourigenesis with larger tumours and enhanced metastasis.

Overall, 59 unique common insertion sites (CIS) were identified from the *SB:Pten*^{Null} cohort, and using a cross-species oncogenomics approach, six CIS fulfilled a predetermined set of criteria for further validation. *Pparg* (isoform 1), a critical regulator of lipid metabolism, is of particular interest with upregulated mRNA and protein expression levels in the prostates of *SB:Pten*^{Null} mice, which also showed upregulation of the downstream *Pparg* target genes such as Fasn, Acc, Acly (key lipogenic enzymes) at protein and mRNA levels. Similarly, in human PC3 cells, *PPARG*-targeting siRNA suppressed the expression of these *PPARG* targets. Oil Red O staining demonstrated a reduction in levels of triglycerides and cholesterol esters in the siRNA knockdown cell line. Reduced PPARG expression in PC3 cells significantly suppressed proliferation and migration at 24 hours ($p<0.001$ and $p<0.005$, respectively).

In a clinical cohort of prostate cancer, nuclear PPARG expression was upregulated in prostate cancer (all Gleason grades) compared to the benign controls ($p<0.001$). Importantly, the presence of upregulated PPARG expression and low levels of PTEN was associated with a shorter disease specific survival when compared to those patients with only low PTEN (median: 7.05 versus 2.05 years; $p=0.0015$), consistent with data from our *in vivo* screen.

Sprouty2 deficiency promotes castration-resistant prostate cancer through *de novo* androgen synthesis

[Collaborator: Owen Sansom]

We have recently shown that loss of PTEN cooperates with Sprouty2 (SPRY2) deficiency by bypassing key tumour suppressor checkpoints (Patel *et al.*, J Clin Invest 2013; 123: 1157). Analysing a panel of matched hormone naïve and castration-resistant resected prostate tumours, SPRY2 expression was suppressed significantly further in castration-resistant prostate cancer. Applying the androgen receptor positive human CWR22 prostate cancer cell line, we genetically suppressed SPRY2 expression by stably transfecting an shRNA construct to target *SPRY2* mRNA. Using the CWR22 SPRY2 KD cells as orthografts in a nude mouse model, their growth characteristics were determined following surgical castration, with control animals subjected to mock surgery. Orthografts of CWR22 SPRY2 KD cells showed minimal shrinkage despite castration, contrasting to the dramatic response shown by the control, non-silencing shRNA (NSi). Hence, CWR22 SPRY2 KD represents castration-resistant prostate cancer.

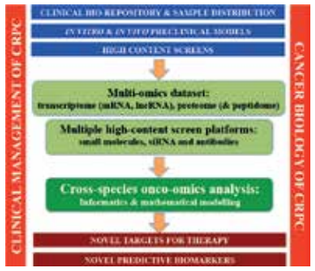

Figure 1

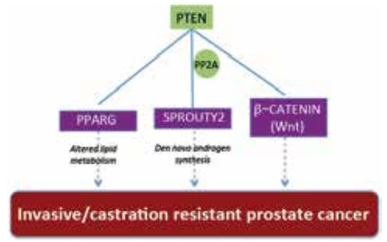

Figure 2

Figure 1
Overview of the interdependent nature of our research methodology and approach.

Figure 2
Summary of three PTEN-interacting events to promote prostate carcinogenesis.

While surgical castration clearly suppressed serum testosterone levels as expected, we found that the intra-tumoural testosterone levels in SPRY2 KD orthografts were maintained. Furthermore, the expression of key enzymes for androgen biosynthesis were significantly elevated in the CWR22 SPRY2 KD orthografts, signifying *de novo* androgen synthesis upon castration. We then subjected orthografts of CWR22 SPRY2 KD cells (along with non-silencing (Nsi) control cells) to castration (as a means to achieve androgen deprivation therapy, ADT) or treatment with simvastatin, alone or in combination, in a four-week treatment regime. Combined ADT and simvastatin resulted in a significantly stronger tumour killing response in CWR22 SPRY2 KD cells, despite their ability to survive ADT alone. The level of nuclear androgen receptor was effectively abolished by combined treatment, along with significant levels of apoptosis (enhanced cleaved caspase 3 expression). Even in the control cells with NSi, which signified hormone responsive disease, the tumour response was enhanced following combined ADT and statins treatment. Based on these exciting early data, we are initiating efforts to design a clinical study to directly test the value of combining statins with currently available ADT.

The Wnt/β-catenin-signalling pathway drives prostate carcinogenesis and may mediate resistance to androgen deprivation therapy

[Collaborators: Chris Ponting (University of Oxford), Owen Sansom]

We undertook quantitative tumour transcriptome profiling pre- and post-ADT by mRNA-Seq on prostate tumours from eight patients with locally advanced/metastatic prostate cancer (Rajan *et al.*, Eur Urol 2014; 66: 32). We identified 774 upregulated and 755 downregulated ADT responsive genes, with enrichment for upregulated genes within the Wnt/β-catenin-signalling pathway. We observed enhanced β-catenin immunoreactivity in a subset of castration-resistant prostate cancer. Using the isogenic LNCaP/LNCaP-AI human prostate cancer cell lines as models for hormone naïve and castration-resistant disease respectively, we demonstrated expression changes consistent with mRNA-Seq data among selected members of the pathway, including the Frizzled family of receptors (*FZD4/FZD5/FZD7/FZD8*) and the downstream effector *JUN*. Upon treatment with the tankyrase inhibitor XAV939, to promote β-catenin degradation, LNCaP-AI cell growth was more potently suppressed when compared to the parental LNCaP cells.

We further genetically engineered the *Pb-Cre:Pten*$^{fl/+}$ *Catnb*$^{\Delta(ex3)/+}$ mice, which form aggressive tumours by 6-8 months and have a significantly poorer survival outcome compared to control mice (*Pb-Cre:Catnb*$^{\Delta(ex3)/+}$). The *Pb-Cre:Pten*$^{fl/fl}$ *Catnb*$^{\Delta(ex3)/+}$ mice with homozygous loss of *Pten* have an even shorter latency, suggesting Pten has a 'dose dependent' effect in driving tumour progression. Importantly, *Pb-Cre:Catnb*$^{\Delta(ex3)/+}$ mice develop prostate cancer by 12 months indicating that stabilisation of β-catenin is sufficient to drive tumourigenesis, while heterozygous loss of *Pten (Pb-Cre:Pten*$^{fl/+}$*)* does not result in prostate cancer.

The molecular basis for upregulated β-catenin expression in clinical prostate cancer remains to be fully investigated. We found that the RNA binding protein, hnRNPA2 may regulate β-catenin protein expression and is itself overexpressed in prostate cancer (Stockley *et al.*, RNA Biol 2014; 11: 755). Both nuclear and cytoplasmic hnRNPA2 protein are present and appear to be functionally important in prostate cancer. Cytoplasmic hnRNPA2 may upregulate β-catenin protein expression and modulate cellular phenotype through 3'-UTR-mediated effects.

Summary

Taken together, we continue to make excellent progress in our understanding of key events in aggressive (and treatment-resistant) prostate cancer. Through this new knowledge and our novel model systems, we are developing and designing new therapeutic strategies.

Publications listed on page 81

TUMOUR CELL DEATH

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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.

Metabolic cell death priming downstream of p73

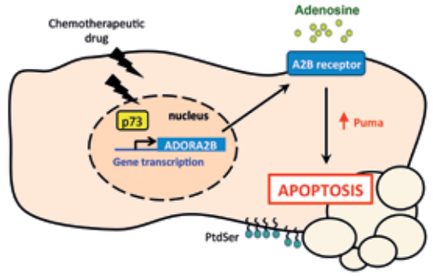
It is now widely accepted that tumour cells undergo changes in metabolism to facilitate growth and mitigate the oxidative stress associated with anabolism. It therefore seemed conceivable that tumour suppressive mechanisms should exist that detect these metabolic changes and invoke a cellular response to either correct the change or eradicate the damaged cell. This idea led to our previous discovery that the tumour suppressor p53 activates the expression of the adenosine receptor, A2B (Long *et al.*, Mol Cell 2013; 50: 394). We found that enhanced expression of A2B alone has little impact on the cell but if expressed in the presence of high levels of extracellular adenosine (the receptor's natural ligand), then a potent cell death response ensues. The importance of this cell death priming mechanism to tumour suppression becomes clear when we consider that high levels of adenosine can be derived from the breakdown of the energy-rich molecule, ATP. Under conditions of metabolic stress, such as occurs in a growing tumour, the energy-rich phosphate bonds in ATP are broken and adenosine is produced. This adenosine accumulates outside the cell, and studies have shown that the extracellular space of solid tumours can be bathed in adenosine.

The tumour suppressor p53 is the most frequently inactivated gene in human cancer. Approximately 50% of tumours have a p53 mutation and it is considered that many other tumours contain mutational events in other genes that affect either p53 activation or downstream effectors of the p53 response. This naturally caused us to consider whether ADORA2B (the gene encoding A2B) was also activated by other p53 family proteins such that this metabolic cell death priming mechanism could be active in the absence of wild type p53.

p73 is a protein closely related to p53 both in structure and function, and our studies revealed that it too can activate expression of ADORA2B. Activation was observed with the three transactivating isoforms - TA-p73α, TA-p73β and TA-p73γ – but not with the dominant-negative form of p73, ΔN-p73. Interesting, activation by the TA-p73s occurred through the same DNA binding element that is activated by p53, indicating that activation of ADORA2B is a conserved response (Fig. 1).

Another indication of the conservation of this response came from our analysis of cell death in the presence of adenosine. This revealed that expression of TA-p73β and TA-p73γ, but interestingly not TA-p73α, sensitised cells to the presence of extracellular adenosine and that this sensitisation was lost upon depletion of A2B.

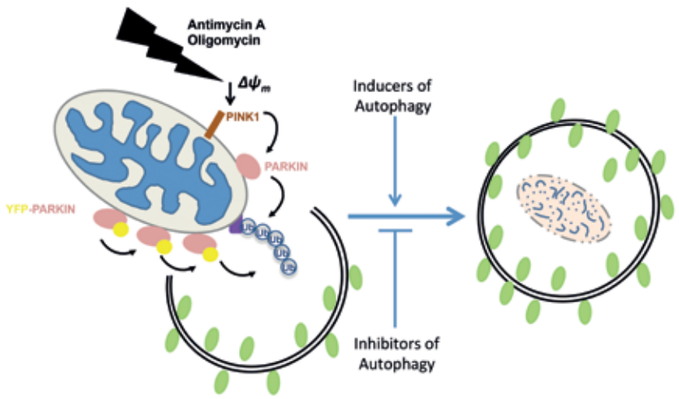
Figure 1
p73-mediated activation of primes cells to die in respor accumulation of extracellu adenosine. Activation of p7 chemotherapeutic drug lea increased expression of the encoding the adenosine receptor, A2B. In the preser elevated levels of extracellu adenosine, expression of A causes upregulation of Pur and cell death by apoptosis



Our further analysis revealed that the cell death response from p73 and adenosine was apoptotic and involved upregulation of the BH3-only protein, PUMA leading to mitochondrial outer membrane permeabilisation (MOMP) (Fig. 1).

In our studies relating to p53 and A2B, we observed that both cisplatin and hypoxia cause accumulation of extracellular adenosine, which can be sensed by elevated levels of A2B to promote cell death. We were therefore keen to identify chemotherapeutic agents or conditions that could activate ADORA2B expression and/or cause accumulation of adenosine that could be sensed by p73-induced A2B. To exclude the involvement of p53, these studies were undertaken in HCT116 cells in which p53 had been deleted by homologous recombination. In these cells, we found that Adriamycin, but not cisplatin causes a p73-dependent induction of ADORA2B expression. In addition, we found that exposure of these cells to ultraviolet light causes a highly significant increase in extracellular adenosine, which enhanced p73-induced death in a manner dependent on A2B. These studies therefore not only broaden the range of transcription factors that can induce metabolic cell death via A2B but they also increase the spectrum of scenarios in which adenosine can be produced to signal tumour cell death.

Figure 2
Enhanced mitophagy can be used to measure autophagy flux. Expression of exogenous Parkin causes autophagic degradation of mitochondria en masse when cells are treated with agents that cause mitochondrial damage. This provides the cell with an unlimited pool of autophagic substrate such that the rate of loss of mitochondrial proteins can be used to assess the autophagy rate/capacity of the cell.



A novel technique to measure autophagic flux

In addition to apoptosis, autophagy is another process that can regulate cell viability. As the name suggests, autophagy is a process of self-eating whereby cytoplasmic constituents are engulfed in double membrane structures called autophagosomes that serve to deliver cargoes to lysosomes for degradation.

Under basal conditions, autophagy acts to preserve cellular integrity by promoting the degradation of damaged and misfolded proteins and organelles. Over time, autophagy is therefore very important for both cellular and organismal viability. Autophagy is, however, also highly adaptable and a variety of internal and external cues can alter the rate of autophagic degradation. For example, a phylogenetically conserved autophagic response occurs when cells are deprived of nutrients. Under these conditions, autophagic degradation is increased and degradation of cellular constituents is enhanced to boost ATP levels during the starvation period. Importantly, this response is also thought to occur during the starvation periods that occur during tumour evolution and there is good evidence that certain tumours depend on autophagy both as a way to respond to metabolic stress and as a means to preserve cellular fidelity.

In light of the key roles of autophagy, it is important to have reliable tools to study the process. For example, since drugs that target autophagy are being developed, it is important to know in any given scenario if autophagy is being enhanced, impaired or even blocked. To facilitate research in this area, we have developed an assay that we consider reliably measures the autophagic flux or capacity of cells. This assay involves ectopic expression of the ubiquitin ligase, Parkin, which enables autophagic degradation of mitochondria en masse when cells are treated with agents that cause mitochondrial damage (Fig. 2). When this occurs, the simple assessment of the rate of loss of certain mitochondrial proteins can be used as a read-out of the underlying autophagic rate within the cell. Using this assay, we have been able to detect the differences in autophagic flux between different cells and the changes in autophagy that occur upon treatment with autophagy modulators or upon genetic ablation of an essential autophagy gene. As a result, we consider this assay to be an important new tool for those investigating the role of autophagy in tumour cell integrity and tumour cell death.

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MATHEMATICAL MODELS OF METABOLISM

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Group Leader
Alexei Vazquez

Metabolism is essential for life and its alteration is implicated in multiple human diseases. The transformation from a normal cell to a cancerous one requires metabolic changes to fuel the high metabolic demands of cancer cells, including but not limited to cell proliferation and cell migration. Our group investigates cancer metabolism from an evolutionary point of view. We hypothesise that given specific microenvironmental conditions and metabolic constraints, there is an optimal mode of cell metabolism to achieve a metabolic objective. This metabolic mode will offer an evolutionary advantage and therefore be selected for during the time course of cancer development. First, we aim to uncover the metabolic objectives and metabolic constraints upon which natural selection is acting. Second, we aim to determine which known (or yet to be discovered) molecular alterations are driving the deterministic or stochastic occurrence of the optimal metabolic modes.

Impact of molecular crowding on cancer cell metabolism

Molecular crowding constrains the abundance of enzymes, ribosomes and organelles in the cell cytoplasm. Our previous work indicates that this constraint limits the metabolic capabilities of cells and shapes the differential utilisation of metabolic pathways. Cancer cells are subject to molecular overcrowding due to the overexpression of amplified genes and the accumulation of mutated protein aggregates. We plan to investigate the impact of this molecular overcrowding on cancer metabolism and determine its relevance to anticancer therapy.

Proliferation and tissue remodelling are two major metabolic programmes in cancer

Our previous work indicates that proliferation and tissue remodelling are major drivers of gene expression patterns in cancer tissues, and they determine cancer subtypes with significant survival differences. Furthermore, analysis of *in vitro* data indicates that the proliferation/remodelling status of cancer cells determines their differential response to anticancer drugs that target metabolism. We plan to investigate the interplay between proliferation and tissue remodelling during cancer evolution.

Drugs targeting cancers with misfolded mutant p53

We have identified a small molecule that specifically targets cancer cells carrying a mutant form of the p53 gene, a master regulator of cellular responses to stress. This molecule acts specifically on a subset of mutant p53 proteins that are partially misfolded. The ability of these misfolded p53 proteins to recover their wild type function is modulated by the small molecule, the zinc levels in cells and, potentially, by metabolic status. We plan to investigate the interplay between the metabolic status of cells and their response to small molecules that modulate misfolded p53 proteins.

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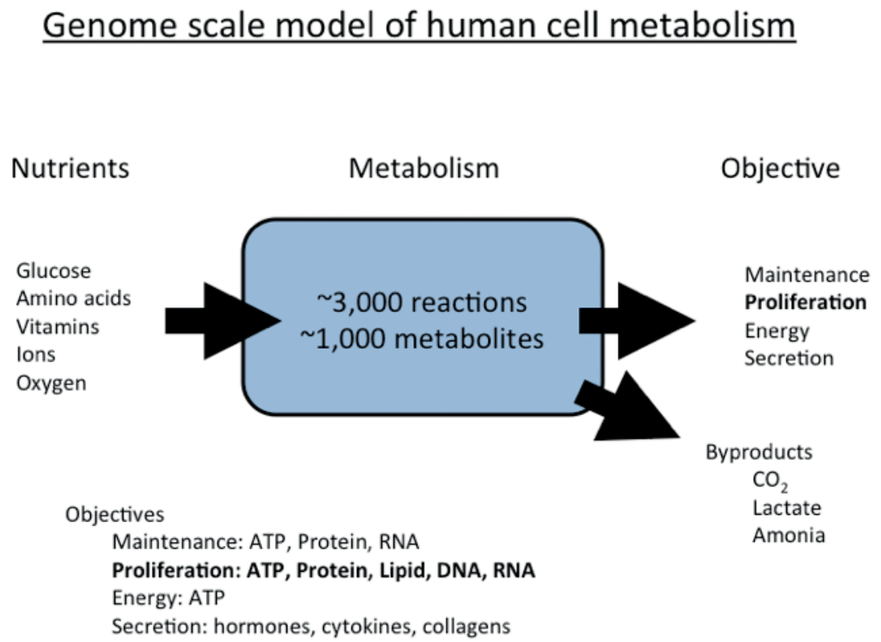
Figure 1
The construction of a genome scale mathematical model of metabolism requires the specification of three major ingredients: the nutrients that are present in the extracellular media, the set of biochemical reactions that are encoded by the genome of the cells under study and the cell metabolic objective. We use this core model to investigate how metabolic constraints and evolutionary principles shape the differential utilisation of metabolic pathways.



TUMOUR SUPPRESSION

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Over the past year, we have extended the analysis of one carbon metabolism in cancer cells and are beginning to develop *in vivo* cancer models to test new therapeutic combinations that include limitation of dietary serine for effects on tumour cell growth.

We have also made progress in understanding the role of MDM2 on the activity of tumour derived mutant p53s. Finally, our work on USP42, a deubiquitinating enzyme that we previously showed targets p53, has expanded to reveal an exciting activity of USP42 in controlling gene expression more generally.

Our past work showing that many tumour cells have a very high demand for exogenous serine has led us to explore in more detail why cancer cells develop this dependency, and whether this can be used for diagnosis or therapy. Serine and glycine can be interconverted by the enzymes SHMT1 (in the cytosol) and SHMT2 (in the mitochondria) and it has been thought that cells can use serine and glycine interchangeably. For this reason, much of our previous work used conditions that starved cells of both serine and glycine. Over the past year, however, we have discovered that this paradigm is not true in many cancer cell lines, and we showed that exogenous glycine, in the absence of serine, cannot support cancer cell proliferation. Using metabolic flux analysis, we showed that cancer cells selectively consume exogenous serine, and under fully fed conditions excrete glycine. The serine that is taken up is converted to glycine, a process that provides a one-carbon unit to support the production of nucleotides, as well as other important metabolic pathways (Fig. 1). However, tracing the fate of glycine in cells starved of serine indicated that while the glycine could be taken up and used for the synthesis of glutathione (GSH), the exogenous glycine was unable to support nucleotide synthesis. The ability of glycine to feed the one carbon cycle depends on the activity of the glycine cleavage system (GCS) to generate Me-THF in mitochondria. However, depletion of the GCS did not affect cell growth, suggesting that this pathway was not functional in tumour cells grown in culture. Interestingly, we also showed that higher concentrations of glycine led to a further inhibition of cell proliferation - an observation that was not fully explained by a non-functional glycine cleavage system. To understand this response we developed a new method for measuring metabolic flux, which we termed 'pulse-stop-flux'. Using this method, we were able to demonstrate that increasing exogenous glycine levels in cells deprived of serine led to an increased conversion of endogenous glycine to serine – a process that would deplete the one carbon cycle (Fig. 1). To confirm our model that glycine inhibited cell growth by exhausting the pool of one-carbon units, we rescued the growth of glycine-only fed cells by adding formate. By following the fate of labelled glycine and formate under these conditions we verified that the use of glycine for nucleotide synthesis was blocked at a step prior to the requirement for one-carbon units, and that this was fully relieved by the addition of formate as a one-carbon donor. Our results suggest that many cancers may depend on exogenous serine for growth but could be inhibited by increased concentrations of glycine. We suggest that the elevation of the activity of the glycine cleavage system, previously described in small cell lung cancers, may play a role in degrading excess glycine that might otherwise accumulate and cause the growth inhibitory effects that we have described.

We are now considering how to use our results for cancer detection or treatment. Our work and that of others has shown that many cancer cells avidly consume serine, and if this characteristic is confirmed in tumours *in vivo*, it may be possible to use radioactive serine to label and image cancers – similarly to the PET scanning methods presently in clinical practice that measure glucose uptake. In initial studies we are testing whether tumours differentially take up serine in various mouse models. Our results would also suggest that depriving cancers of serine would inhibit their growth, and over the past year we have been able to confirm that feeding mice a serine depleted diet resulted in reduced tumour growth in both xenograft and genetically engineered cancer models. While these results validate our general approach, they also showed that – as expected – limiting dietary serine would not completely prevent tumour growth. We are therefore testing additional interventions that might cooperate with serine depletion to kill, rather than inhibit the proliferation of cancer cells. Our previous studies showed that the adaptation to low exogenous serine levels involves a switch to *de novo* serine synthesis, which requires an increased in oxidative phosphorylation and enhanced anti-oxidant support. This work suggests that combining serine depletion with inhibitors of oxidative phosphorylation or increased oxidative stress may lead to a more durable therapeutic response.

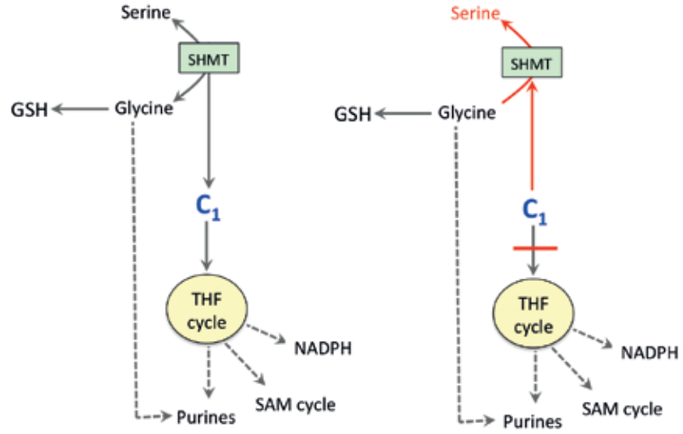

Figure 1
Under fully fed conditions (LHS), cells use serine to produce glycine and feed the one carbon cycle, allowing for the synthesis of purines and NADPH, and providing precursors for the SAM cycle that is important for the methylation of DNA and protein. In the absence of serine (RHS), glycine is converted to serine, depleting the one carbon pool and inhibiting proliferation.

We have shown in the past that the mutant p53 proteins expressed in many cancers can contribute to the invasive and metastatic behaviour of tumour cells, and that this is related to the ability of some of these mutant p53s to bind and inhibit the transcriptional activity of p53 family members such as TAp63 and TAp73. Importantly, wild type p53 does not bind to its protein relatives and the interaction of mutant p53 with p63/p73 is dependent on the type of mutations in the p53 protein. In general, mutations in p53 that affect protein conformation (like p53R175H) show strong binding to p63 and p73, while p53 mutants that only mildly affect the conformation (like p53R273H) bind less well. However, there are many other protein-binding partners for the p53 family, including MDM2, a ubiquitin ligase that targets p53 for ubiquitin dependent degradation. We found that both mutant p53 and p73 bind MDM2 very efficiently – although we have previously shown that this interaction does not result in the ubiquitylation and degradation of p73. In agreement with previous publications, we found that the MDM2/p63 interaction was much weaker. Interestingly, we showed that MDM2 can inhibit p63 binding to p53R175H but enhanced the weaker p53R273H/p73 interaction. These effects on the interactions are reflected in an ability of MDM2 to relieve the inhibition of p63 by p53 R175H, but enhance the inhibition of p73 activity by p53 R175H and R273H. We have therefore proposed a model in which MDM2 competes with p63 for binding to p53 R175H to restore p63 activity, but forms a trimeric complex with p73 and p53 R273H to more strongly inhibit p73 function. These interactions are likely to be affected and modified further by other binding partners, such as HSP70, and the impact on the formation of these different complexes remains to be fully explored. However, our results reveal an interesting differential activity of MDM2 on the activity of mutant p53, suggesting that the ability of different mutant p53s to inhibit p63 and p73 is modulated by the expression of MDM2.

We have been very interested in the regulation of p53 stability and a couple of years ago described the ability of a deubiquitylating enzyme (DUB), USP42 to drive the rapid stabilisation of p53 in response to stress. By removing ubiquitin from p53, USP42 accelerates the activation of p53, although we showed that USP42 is not essential for the induction of p53. However, during the course of these studies we also noted that USP42 could regulate transcription independently of p53, demonstrating that USP42 has p53-independent functions. This was not unexpected - as with other protein modification systems, the potential number of target proteins exceeds the number of DUBs (around 100 in human). It is clear, therefore, that each DUB is likely to target many different proteins. In a continuation of our analysis of USP42 function, we identified mono-ubiquitylated histone H2B as a target for deubiquitylation by USP42. This is an interesting observation because it is known that the dynamic interchange and balance between ubiquitylation and deubiquitylation of histones is critical for the regulation of transcription. USP42 co-localised with RNA pol II in nuclear foci, bound to histone H2B and deubiquitylated H2B. Depletion of USP42 increased H2B ubiquitylation at a model promoter, and decreased both basal and induced transcription from a number of promoters. These results showed that USP42 regulates transcription independently of p53 through the control of histone ubiquitination, and suggest that USP42 may be an important component of this fundamental level of transcriptional activity.

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REGULATION OF CANCER CELL INVASION AND METASTASIS

CANCER RESEARCH UK BEATSON INSTITUTE

Kurt Anderson - Tumour Cell Migration
Jeff Evans - Translational Cancer Therapeutics
Robert Insall - Cell Migration and Chemotaxis
Shehab Ismail - Structural Biology of Cilia
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

TUMOUR CELL MIGRATION

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[2] SINAPSE

Our work is focused on development of imaging approaches to study the cellular and molecular dynamics of metastasis *in vitro* and *in vivo*. Metastasis is linked to mortality in most epithelial cancers. 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 readouts 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 tumour suppressor E-cadherin in tumour cell migration. More recently, we have demonstrated the first *in vivo* use of fluorescence lifetime imaging (FLIM) to study the activation and response to therapy of the small GTPase Rho and the non-receptor tyrosine kinase Src during mutant p53 driven invasion of pancreatic cancer cells.

Nuclear imaging

We continued to develop the use of preclinical imaging in collaboration with colleagues from NHS Greater Glasgow and Clyde, including Gerry Gillen and the clinical team (Glasgow PET Centre), Jonathan Owen (Gartnavel Cyclotron) and Sally Pimlott (Radiopharmaceutical Dispensary). In addition to FDG imaging, two new probes were imaged this year: the proliferation marker ^{18}F-3′-Fluoro-3′-deoxy-L-Thymidine (18FLT) and the metabolic marker ^{18}Fluoro-acetate. ^{18}FLT was synthesised manually and used to measure the response of KRAS-PTEN pancreatic tumours to inhibition of the mTOR pathway by rapamycin. Work is currently underway at the Gartnavel Cyclotron to begin automated FLT synthesis, with preclinical demand serving as a test run for clinical production. We also embarked on a collaborative project with David O'Hagan (University of St Andrews) to produce ^{18}Fluoro-acetate, which was used by Eyal Gottlieb's lab to investigate metabolic changes in colon cancer.

Biosensor development for drug discovery

This year we began a joint project Owen Sansom's group and Novartis to study the role of GEFs in pancreatic and colon cancer. To accomplish this work Novartis have funded a postdoc (Kirsty Martin) to develop the use of FRET biosensors to probe the activation of Rac and Ral GTPases. Kirsty started in June, and has begun to characterise optimal FRET pairs for fluorescence lifetime imaging of FRET. A significant benefit of the biosensor approach is that the same assay will be directly translated from cell-based assays into preclinical studies. We also contributed to the characterisation of a mouse capable of tissue specific expression of the Rac-Raichu probe. The probe was readily expressed and imaged in a variety of tissues including the intestine, pancreas and mammary tissue. Activation with PMA demonstrated the utility of this mouse model for drug development studies (Fig. 1).

Development of adaptive optics for deep tissue multiphoton imaging

Adaptive optics has the potential to increase signal intensity and spatial resolution by sharpening the focus of a laser beam used for multiphoton excitation deep within tissue. This approach can be use to correct for aberrations in focus introduced by both the optics of the imaging system and refractive index variations of the sample. In collaboration with Amanda Wright and Keith Mathieson (Institute of Photonics, University of Strathclyde), Caroline Müllenbroich spent six months working with Ewan McGhee (BAIR) on a fellowship funded by INSPIRE to develop an adaptive optics module for use on one of our LaVision TRIM scopes. The module they developed was based on a deformable mirror placed in the OPO illumination pathway and imaged via a 4f system into the back-focal plane of the microscope objective. The mirror shape was determined using a random search algorithm that was optimised on the basis of signal intensity from the sample. Significantly, we found that mirror shapes optimised using one sample were transferrable to others, meaning that the lengthy optimisation procedure does not need to be performed prior to each imaging session. Also, the signal derived from collagen second harmonic generation (SHG) can be used in place of two-photon excited fluorescence as a signal for mirror optimisation. This is advantageous because unlike fluorescence, the second harmonic signal does not photo-bleach over time. As a result, signal improvements of between two and four fold were achieved in a variety of samples, including organotypic cultures, zebrafish embryos, and freshly excised mouse skin and intestinal tissue (Fig.2). This work is being continued in collaboration with LaVision Biotec using their new adaptive optics module, which has been installed on our new TRIM scope.

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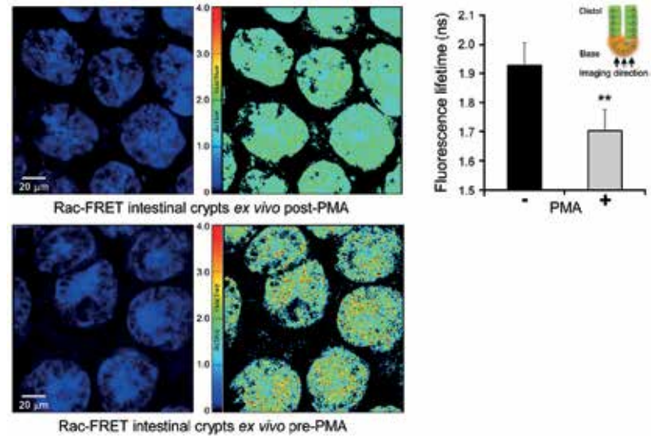

Figure 1
The Rac-Raichu FRET probe was expressed under the control of Villin-Cre in the crypts of mouse intestine. Intensity imaging revealed membrane localisation that delineated cell-cell boundaries (A and D). Fluorescence lifetime imaging (FLIM) revealed a basal activation level having an average lifetime of approximately 1.9 ns (B and C). *Ex vivo* treatment of tissue with PMA resulted in significant ($p < 0.05$) shortening of the fluorescence lifetime to 1.7 ns due to FRET, indicating activation of the probe. (Taken from Johnsson et al., Cell Rep 2014; 6: 1153)

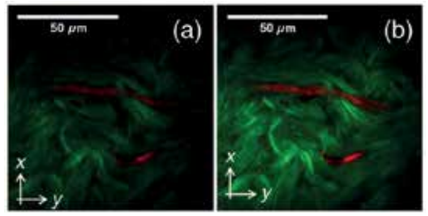

Figure 2
Images of mouse skin showing approximately two fold gain in signal resulting from the use of adaptive optics. SHG from collagen I (green) and fluorescence from Quantum Dots (red) were imaged in mouse skin using either a flat (left) or optimised (right) mirror shape.

TRANSLATIONAL CANCER THERAPEUTICS

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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, and during the development and progression of the invasive and metastatic phenotype, and that are potential therapeutic targets in advanced disease. Using these models, we will determine how potential anticancer agents might best be evaluated in subsequent clinical trials.

Infiltrating ductal carcinoma of the pancreas (PDAC) is the fifth commonest cancer in the UK, and is predicted to become the second commonest cause of cancer-related deaths by the end of this decade. Aggressive invasion and early metastases are characteristic of the disease, such that 90% of patients have surgically unresectable disease at the time of diagnosis. Overall survival remains poor for both resectable and advanced disease using conventional therapies, and has only improved marginally over the last few decades with a preponderance of negative clinical studies using current trial designs. Preclinical modelling has invariably failed to deliver robust results in predicting success or in reducing drug attrition in pivotal clinical trials. Consequently, novel approaches are urgently required.

Our work aims to develop therapeutic interventions for advanced pancreatic cancer by exploiting tumour vulnerabilities in preclinical models with specific genetic backgrounds, and to optimise therapy of localised pancreatic cancer through inhibition of metastases and through optimising control of localised inoperable disease.

Exploiting tumour vulnerabilities: implications for therapeutic intervention in advanced pancreatic cancer

We have 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 *p19*^{ARF}. We are using these models to help us understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours, and to allow us to identify and characterise the signalling pathways that are deregulated at the early stages of pancreatic cancer, during the development and progression of the invasive and metastatic phenotype, and in advanced disease.

We are using these murine models to explore tumour vulnerability *in vivo* to specific targeted agents. In addition to conventional response assessments and survival studies, we also monitor therapeutic activity using a PET/SPECT/CT preclinical imaging system (collaboration with Kurt Anderson and Gaurav Malviya), enabling us to use clinically relevant imaging probes to assess therapeutic efficacy with regard to tumour metabolism and tumour proliferation, and which can then be incorporated into clinical protocols. Our initial focus has been on targeting pathways that are (a) implicated in PTEN deletion (PI3K / AKT, mTORC 1 and 2); (b) downstream of mutant KRAS (e.g. effector pathways); and (c) intra- and peri-tumoural inflammatory pathways that are implicated in tumour initiation and tumour progression.

We will exploit these studies to inform a UK-wide MAMS (multi-arm molecular stratified) clinical trial that is currently in development in collaboration with Juan Valle (University of Manchester), Andrew Biankin and Sean Grimmond (University of Glasgow), and the CRUK Clinical Trials Unit, Glasgow. This study will consist of a series of parallel, early phase, efficacy signal-seeking studies in which patients will be recruited into multiple treatment arms of specific agents based on their molecular profile. Critical to these approaches will be identifying potential genotype-specific biomarker signatures in murine models and confirming the clinical relevance of these in human tissue microarrays, and developing robust assays for patient selection to select or enrich the clinical trial population.

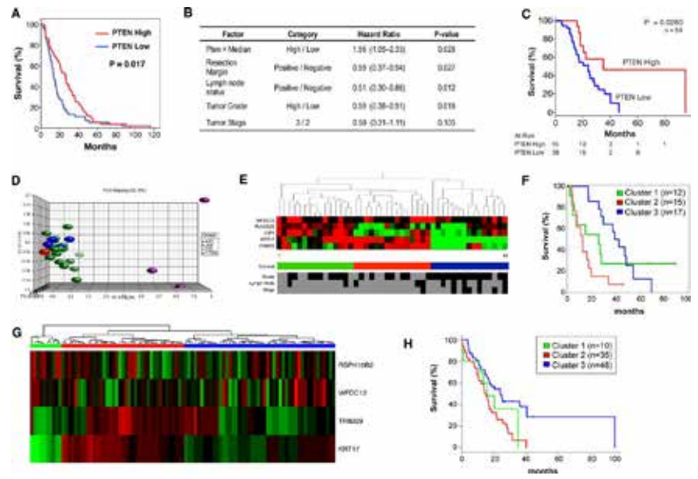

Figure 1
Low PTEN and expression of a low PTEN-associated signature predicts poor survival in human PDAC. (A) Kaplan–Meier analysis of Glasgow cohort showing cases with low PTEN expression have poorer outcomes. (B) Multivariate analysis showing low PTEN expression is an independent predictor of survival. (C) Kaplan–Meier analysis of Australian confirmatory cohort. (D) Principal component analysis of gene expression data generated from tumours in mice. (E) Heat map showing the PTEN-deficient signature could be used to delineate three groups of patients. Black indicates low or negative, while grey indicates high or positive values. (F) Kaplan–Meier analysis showing of Glasgow cohort cases delineated on the basis of gene expression of low PTEN-associated signature. Cases with high expression of signature (red) have significantly decreased survival compared to those with medium (green) or low expression (blue). (G) Heat map showing validation of the PTEN-deficient signature used to delineate three groups of patients. (H) Kaplan–Meier curves showing difference of overall survival between three groups of patients identified by the PTEN-deficient signature in the confirmatory cohort.

This approach is exemplified by our observations that PTEN loss leads to acceleration of KrasG12D-driven PDAC in murine models and these tumours have high levels of mammalian target of rapamycin (mTOR) signalling. We compared response to mTOR inhibition in this model with the response in another established model of pancreatic cancer, KRAS P53. We found that tumours in KRAS PTEN mice exhibit a remarkable dependence on mTOR signalling. In these tumours, mTOR inhibition leads to proliferative arrest and even tumour regression. Importantly, pancreatic tumours driven by activated KRAS and mutant p53 did not respond to treatment.

We also assessed whether there was a subset of human pancreatic cancer patients who may respond to mTOR inhibition. PTEN expression was determined by immunohistochemistry on a tissue microarray of resected human pancreatic tumour specimens, and patients were divided into groups of low and high expression quantified using a histoscore method. Low PTEN expression was associated with significantly poorer survival in these patients. Furthermore, by multivariate analysis, low PTEN expression was an independent predictor of survival. These data were validated in a second group of patients in which low PTEN expression was again associated with significantly poorer survival. In human tumours, approximately 20% of cases demonstrated low PTEN expression and have a gene expression signature that overlaps with murine KRAS PTEN tumours, suggesting that this could be applicable to enrich patient selection in subsequent human trials.

Optimising therapy in localised pancreatic cancer

Two of the key issues in optimising therapy of localised pancreatic cancer are (a) inhibition of metastases and (b) local control of inoperable disease.

One aim of our work is to determine if potentially anti-invasive agents have anti-migratory and hence anti-invasive and/or anti-metastatic properties. Our initial studies used the *Pdx1-Cre*, *Z/EGFP*, *LSL-Kras*$^{G12D/+}$, *LSL-Trp53*$^{R172H/+}$ (KPC) model and cell lines developed from these mice. We demonstrated that dasatinib, an inhibitor of Src family kinases, inhibited cancer cell migration and invasion *in vitro*, and inhibited the development of metastases *in vivo*. An international, multicentre clinical trial based on this work has now completed accrual (Evans, global Chief Investigator), incorporating freedom from distant metastases as a novel exploratory endpoint in patients with inoperable, locally advanced (but without metastases) disease.

Having used this approach to refine the clinical evaluation of existing anticancer agents, we are now using principal component analysis, in collaboration with Owen Sansom and Andrew Biankin (University of Glasgow), to define a functionally relevant signature associated with survival in resected PDAC in order to understand the key pathways that drive poor survival and identify targets for the development of novel anti-invasive agents.

Optimal local control remains an important clinical issue in patients with non-metastatic disease, particularly in those with 'borderline' operable disease, and in whom chemo-radiation is frequently used. Inhibitors of poly(ADP-ribose) polymerase (PARP) may have radio-sensitising effects and a phase I/II study of olaparib in combination with chemo-radiation in locally advanced pancreatic cancer will commence soon (Experimental Cancer Medicine Centres-Industry Alliance).

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CELL MIGRATION AND CHEMOTAXIS

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Crawling movement is fundamental to the behaviour of most eukaryotic cells. In most tissues it is suppressed, so cells remain within the correct organs. However, when tumours metastasise, the regulation of cell movement goes wrong - cancer cells invade other tissues, and spread through the blood and lymph systems to form secondary tumours. This spreading behaviour is one of the most feared features of cancer and a principal driver of death in patients. Despite this, we do not understand what makes cells move or what steers their direction. Our group aims to improve understanding using all means at our disposal, for example *in vivo* models, cancer cells, model organisms and computational simulations. We apply a wide range of techniques, from genetics through biochemistry and technical microscopy to quantitative analysis of microscope movies and computational modelling.

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. About half of the lab has a genetic focus, which varies in detail in response to the mutant phenotypes we see. However, our strategy is always based around chemotaxis and actin.

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, 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 of single cells in collaboration with the Mathematics Department, University of Strathclyde, and of populations of moving cells with the Institute of Biodiversity Animal Health and Comparative Medicine, 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/WAVE, which is a fundamentally important regulator of cell movement. Mutants in a variety of species show that it is required whenever cells need to make large actin-based structures such as lamellipods; without SCAR/WAVE such structures are either small and malformed, or completely absent. It 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 and lipid signalling to actin polymerisation. However, this view seems very simplistic in view of the size of the complex and its dynamic behaviour.

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 the 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.

Mechanisms underlying chemotaxis: Pseudopods and self-generated gradients

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 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.

The most interesting part of a melanoma cell's response is that we find they make their own chemotactic gradients. LPA - which appears to be present at substantial levels in the tissue surrounding tumours - is a strong attractant for all the melanoma cells we have observed. But melanoma cells also break down LPA. This leads to a self-generated gradient, in which cells move out of tumours in response to gradients they are themselves creating. Thus tumours appear to need no external drivers to steer metastasis – they do it themselves.

We are collaborating with the Mathematics Departments of the University of Strathclyde and the University of Glasgow to make different 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. Our theory of self-generated gradients implies that cells behave in a similar way to herds of animals in the wild. We are therefore also collaborating with mathematical ecologists at the University of Glasgow to determine whether this comparison can yield useful predictions about cancer cell behaviour.

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STRUCTURAL BIOLOGY OF CILIA

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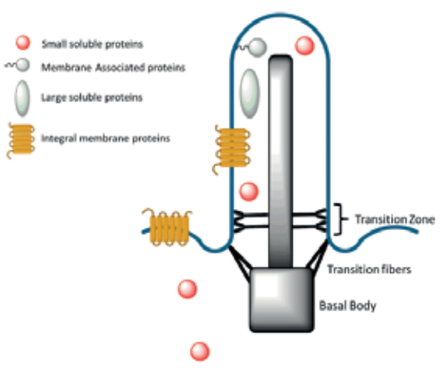
The primary cilium is a microtubule-based sensory organelle that is present on almost all cell types in the body. While lymphocytes lack cilia, due to functional and structural similarities, the immune synapse can be considered as a special type of primary cilium. The assembly of cilia is tightly synchronised with the cell cycle and coordinates several signalling pathways such as the Wnt and Hedgehog pathways, both of which are therapeutic targets for cancer. Furthermore, the immune synapse plays a central role in tumour immunology. Concentrating signalling proteins and receptors inside the cilium is key to its function. Nevertheless the cilium, unlike other organelles, is not separated from the cell body by a membrane barrier and the molecular mechanism for regulating ciliary targeting and access of relevant proteins to cilia is elusive.

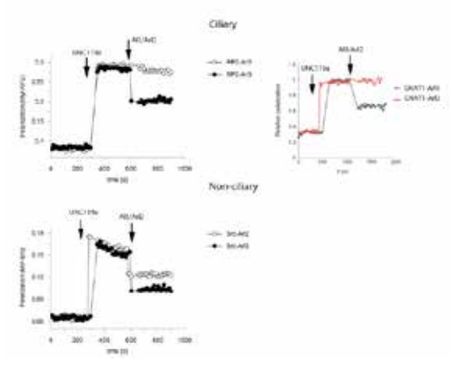
Our group aims to unravel the regulation of ciliary access, for membrane associated, integral membrane and large soluble proteins (Fig. 1), by using an interdisciplinary approach where we combine structural biology, biochemistry and cell biology.

Ciliary access of membrane associated proteins

Several lipid-modified, myristoylated and prenylated proteins are solubilised and targeted to their cellular destination in complex with GDI-like solubilising factors (GSFs). Three homologous GSFs, PDE (delta subunit of phosphodiesterase), UNC119a and UNC119b, contain hydrophobic pockets that accommodate and bury lipid moieties of post-translationally modified, membrane-associated proteins and hence function as solubilising factors (Hanzal-Bayer *et al.*, 2002; Ismail *et al.*, 2011; Wright *et al.*, 2011; Zhang *et al.*, 2011). During my postdoctoral work, I showed that the solubilising function of GSFs is regulated by the small G proteins Arl2 and Arl3 in a GTP-dependent manner (Ismail *et al.*, 2011; Ismail *et al.*, 2013). Currently, my group is trying to answer the question of how GSFs selectively target the now soluble cargo to different destinations in the cell.

Figure 1
A cartoon representation of a cilium showing the different types of protein cargoes, as indicated, sorted to the cilium.





Using biochemical studies, we show that Arl2, localised outside cilia, releases the cargoes destined for the outside of cilia, while Arl3, localised within cilia, releases those cargoes destined for the cilia itself.

We are currently investigating the structural basis of the interaction of several lipid-modified cargoes with GSFs in order to understand the selective release of cargoes by Arl2 or Arl3. Furthermore, we are studying the effect of these interactions on the functions of lipid-modified proteins. Finally, we will vigorously test the structural and biochemical data in cells.

Ciliary access for integral membrane proteins and large soluble proteins

A diffusion barrier regulates access of integral membrane proteins and large soluble proteins to cilia. A septin ring, a protein network that resides in the ciliary transition zone, and the transition fibres connecting the basal body to the ciliary membrane are believed to control access to cilia and be involved in forming the ciliary diffusion barrier (Fig. 1). The protein network in the transition zone is thought to be part of the ciliary diffusion barrier and to have a gating function. Many of the network members are involved in ciliopathies. The exact identity of the ciliary gate, the molecular mechanism of its function and its structural, biochemical and dynamic properties remain elusive. In our group, we are working on obtaining atomic level details of the ciliary gate and its biochemical characterisation by reconstituting multimeric protein complexes of the ciliary transition zone and using X-ray crystallography.

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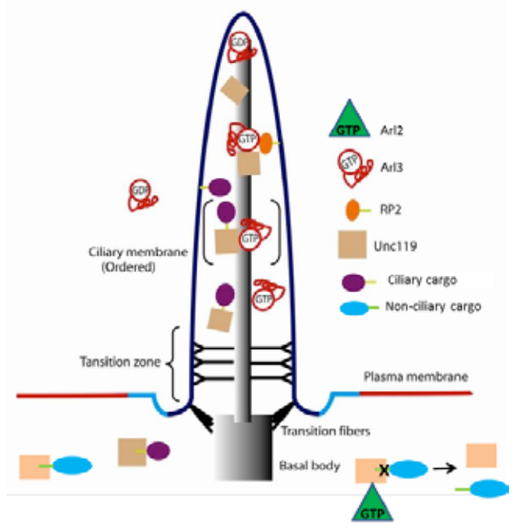
Figure 2
Ciliary and non-ciliary cargo release from UNC119a via Arl2 and Arl3 GTP. a) Fluorescence polarisation measurement where UNC119a is added to fluorescently labelled, myristoylated ciliary or non-ciliary cargo, as indicated, followed by the addition of Arl2 or Arl3 GTP, as indicated. b) Cartoon representation showing our model of the release of lipid-modified cargo inside or outside the cilium by Arl3GTP or Arl2GTP, respectively.



MIGRATION, INVASION AND METASTASIS

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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-1, 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 metastasis 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 family proteins (including WASP/N-WASP, Scar/WAVE, WASH, WHAMM and JMY) transmit signals to the Arp2/3 complex to trigger actin assembly. 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, cell division and assembly of invasive structures such as podosomes and invadopodia. Postdoc Ben Tyrrell is studying the role of the WASH protein complex in the production of actin networks on endocytic vesicles to regulate cell signalling and motility in three-dimensional matrices. New postdoc Iben Veland is studying the role of WASH proteins in invasive migration and the delivery of matrix metalloproteases and receptors into invasive pseudopods. She is also interested in how the microtubule network connects to actin to affect polarised cell invasion (Fig. 1). PhD student Loic Fort is working in collaboration with Jose Batista in Robert Insall's group to discover new regulators of the Scar/WAVE complex and study their role in migration and invasion.

Role of actin regulatory proteins in colorectal and pancreatic cancer

This year, clinical research fellow Hayley Morris continued her study of the role of N-WASP in colorectal cancer (in collaboration with Owen Sansom) and with postdoc Amelie Juin on the role of N-WASP in pancreatic ductal adenocarcinoma (PDAC). High levels of N-WASP have recently been correlated with poor outcome in human patient PDAC (Guo *et al.*, World J Surg 2014; 38: 2126) pointing to it as a potentially interesting new target for PDAC. We will determine whether the role of N-WASP in invadopodia translates into differences in tumour formation, progression and spread. Thus far, we find a strong role of N-WASP in invasion and metastasis and we are working to discover the mechanisms by which the actin cytoskeleton confers invasiveness and metastatic capability on pancreatic cancer cells (Fig. 2).

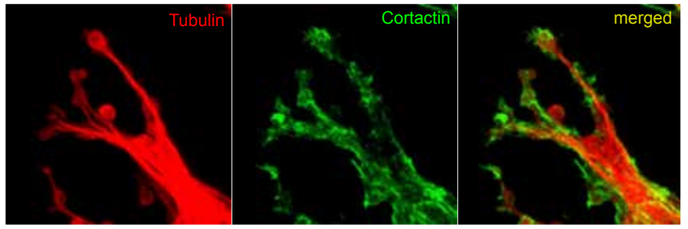

Figure 1
The leading edge of an invading melanoma cell is shown with microtubules in red and the invadopodia protein cortactin in green. Microtubules play an important role in invading pseudopods and coordinate with the actin cytoskeleton to deliver receptors and proteases to aid matrix remodeling. Photo Credit: Iben Veland

We are also studying the role of fascin-1 in PDAC progression, a project initiated by former student Ang Li (now at Rockefeller University in New York) and now continued by Amelie Juin, Hayley Morris and Loic Fort in collaboration with Jennifer Morton and Owen Sansom. This year, we published that fascin-1 is upregulated in human PDAC and correlates with poor outcome and time to recurrence of disease (Li *et al.*, Gastroenterology 2014; 146: 1386). We found that fascin is a target of the epithelial to mesenchymal transition, which allows tumour cells to break away from their neighbours. Surprisingly, fascin-1 has a key role in early tumour formation as well as metastatic dissemination. Fascin mediates metastasis to the peritoneal cavity via the formation of actin-rich filopodia that enable cells to intercalate and migrate through mesothelial cell layers and seed a new tumour. Fascin also contributes to liver metastasis. This work complements our ongoing efforts to develop fascin-1 inhibitor compounds together with Martin Drysdale's Drug Discovery team. We also have a new project funded by the Pancreatic Cancer Research Fund to further probe the mechanism of fascin involvement in PDAC and to test compounds in collaboration with Martin Drysdale and Drug Discovery.

Role of actin regulatory proteins in melanoblast migration and melanoma

We previously showed that loss of Rac1 causes major defects in melanoblast migration and proliferation during development (Li *et al.*, Dev Cell 2011; 21: 722) and now we are studying the roles of RhoA and Cdc42 in melanoblasts with PhD student Emma Woodham and Ben Tyrrell, together with Cord Brakebusch (BRIC, University of Copenhagen, Denmark). Emma has found a major role for Cdc42 in melanoblast migration and proliferation and is currently working with cultured cells to unravel the molecular mechanisms. With Ang Li, we published this year that the Rac1 activator protein pREX-1 also has roles independent of Rac1 in melanoblasts (Lindsay et al., J Invest Dermatol 2015; 135: 314). With former postdoc Yafeng Ma, we also published that fascin is expressed in some melanomas but is not tightly correlated with stage or progression as it is in many epithelial cancers (Ma *et al.*, Melanoma Res 2014, in press). A new postdoc, Karthic Swaminathan joined the team this year and he will study the role of the major actin nucleation promoting complex Scar/WAVE in melanoblast migration and melanoma metastasis. He and Loic Fort are also setting up a model to study melanoma metastasis in collaboration with Jim Bear (UNC, USA) where we will use intravital imaging to track cells escaping from tumours.

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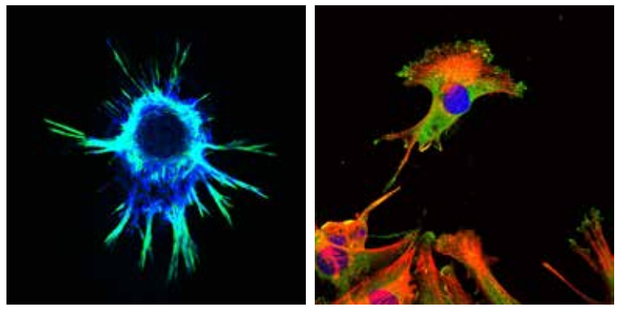

Figure 2
Left- Pancreatic cancer cell spheroids invading into collagen gel (green = actin, blue = DNA). Right- Pancreatic cancer cells invading into Matrigel (red = actin, green = cortactin). Photo Credit: Amelie Juin

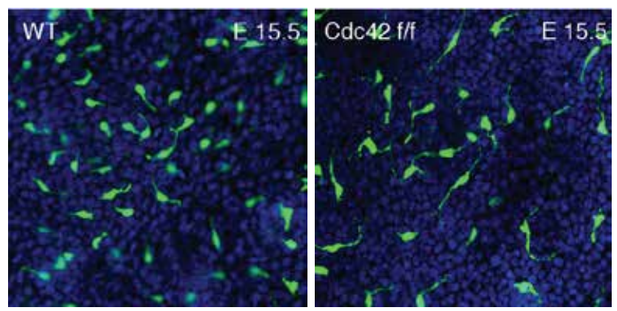

Figure 3
Melanoblast cells migrating in epidermis (green = GFP-melanoblasts, blue = DNA). Left: WT = wild type control, Right: Cdc42 f/f = deleted for Cdc42. Photo Credit: Emma Woodham

INTEGRIN CELL BIOLOGY

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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 drivers 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 hope to reveal which are the most promising components of the pathway to target for cancer therapy.

RCP controls recycling of an Ephrin receptor to promote cell:cell repulsion and tumour cell dissemination

Having characterised the role of the Rab11 effector, Rab-coupling protein (RCP) in integrin recycling, we have been using proteomic and phospho-proteomic approaches to determine whether other receptors might be cargoes of the RCP pathway. We have identified a new trafficking pathway in which RCP, when phosphorylated at Ser^{435} by a kinase called LMTK3, is able to associate with a small GTPase, Rab14 to control trafficking of an Ephrin receptor, EphA2. Importantly, RCP must be phosphorylated by LMTK3 in order for EphA2 to be capable of mediating the cell:cell repulsion events that drive tumour cells away from one another during cancer invasion. Consistently, we have found that genetic disruption of either the EphA2 or the RCP genes in mice opposes dissemination and metastasis of pancreatic adenocarcinomas. We are currently investigating the possibility of developing agents to target LMTK3 kinase to oppose RCP phosphorylation and tumour cell dissemination.

A new role for exosomes in transferring mutant p53-driven invasive phenotype between cells

We have previously shown that mutants of the tumour suppressor p53 can promote cancer invasion via a gain-of-function mechanism. Mutant p53s increase the invasive behaviour of cancer cells by upregulating RCP-dependent trafficking of integrins and receptor tyrosine kinases, such as EGFR1 and cMET. We have now found that the ability of mutant p53 to upregulate integrin trafficking and drive invasive migration is not cell autonomous, and mutant p53-expressing cells can communicate these pro-invasive capabilities to neighbouring cells that do not express p53. This proceeds via a mechanism in which mutant p53 drives Rab35-dependent production of exosomes. These exosomes are then released from mutantp53-expressing cells and can interact with other cells to influence their invasive behaviour. Indeed, we have shown that p53 null cells alter their RCP-dependent integrin trafficking and adopt an invasive migratory phenotype when exposed to exosomes from mutant p53 expressing cells. These findings indicate that a small population of mutant p53 expressing cells are potentially able to influence the aggressiveness of other less invasive cells in a systemic fashion, and indicates the need to look for ways to oppose Rab35-dependent exosome release in cancer therapy.

The role of translation initiation in extracellular matrix synthesis during tumourigenesis

Several RNA polymerase III (Pol III)-associated genes are increased in tumours and tumour metastases. We have generated a number of tools to determine how Pol III may contribute to cancer progression – these include a panel of cell lines in which Pol III and its principal products, including the initiator methionine transfer RNA ($tRNA_i^{Met}$), are up/downregulated and transgenic mice that express additional copies of the $tRNA_i^{Met}$ gene.

A series of experiments conducted using the $tRNA_i^{Met}$ overexpressing mouse indicate that an important component of Pol III's drive to tumour progression is due to the action of increased $tRNA_i^{Met}$ in the tumour stroma, not in the tumour cells themselves. These data indicate that a key role of Pol III and its product $tRNA_i^{Met}$ is to support the synthesis and secretion of stromal collagen II – an event that promotes assembly of an extracellular matrix (ECM), which supports tumour progression *in vivo*. We are currently investigating how altered levels of $tRNA_i^{Met}$ favour synthesis of collagen II-rich pro-tumourigenic ECM, and how control of translation initiation in stromal fibroblasts may be a key factor in determining cancer aggressiveness.

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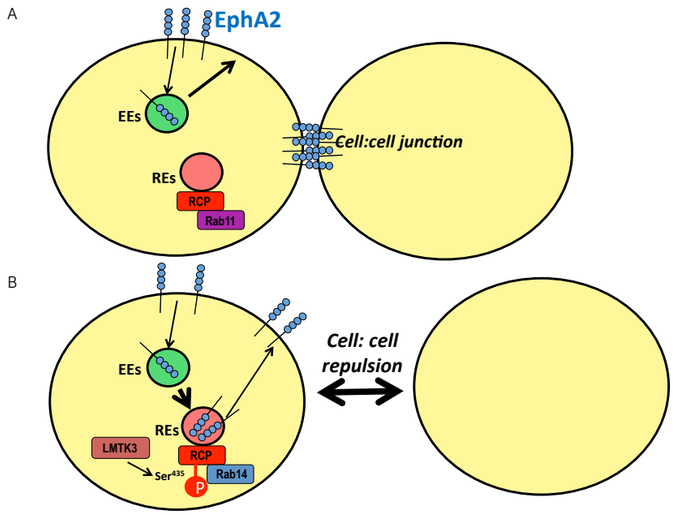

Figure 1
LMTK3 phosphorylates RCP to drive EphA2 trafficking and cell:cell repulsion.(A) Lemur tyrosine kinase-3 (LMTK3) is inactive and the Ephrin receptor, EphA2 is internalised and returns to the plasma membrane from early endosomes (EEs). Under these circumstances, stable cell:cell junctions are maintained. (B) active LMTK3 phosphorylates Rab-coupling protein (RCP) at Ser^{435} enabling RCP to associate with Rab14. EphA2 is then transported, under control of Rab14, to recycling endosomes (REs) and returns to the plasma membrane with delayed kinetics. This process promotes EphA2-dependent cell:cell repulsion leading to cell scattering, and dissemination and metastasis of tumour cells.

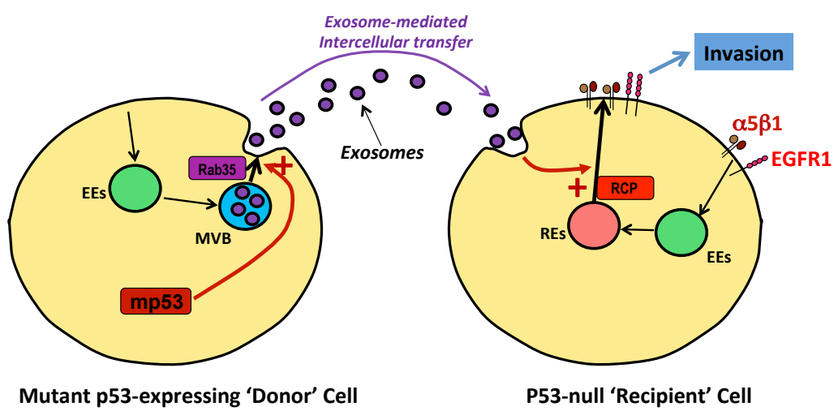

Figure 2
Intercellular transfer of mutant p53's invasive phenotype is mediated by exosomes. The 'donor' cell depicted on the left expresses mutant p53, whereas the 'recipient' cell on the right is p53 null. In the 'donor' cell, mutant p53 activates a Rab35-dependent process that leads to exosome release. Exosomes may then be transferred to 'recipient' cells. p53 null recipient cells respond to these exosomes by activating RCP-dependent α5β1 and EGFR1 recycling to promote tumour cell migration and invasion. EEs, early endosomes; REs, recycling endosomes; MVB, multivesicular body.

MOLECULAR CELL BIOLOGY

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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 including soluble factors and the physical microenvironment. Alterations to the cytoskeletal architecture have significant consequences on the entire cell - such as morphology, cytokinesis, adhesion and motility – and also at the subcellular level. Research in our lab is focused on identifying key regulators of actin cytoskeleton dynamics that contribute to processes that are important for cancer. In particular, we have focused on kinase effectors that act downstream of Rho GTPases. Through these efforts, we aim to uncover proteins that could be potential cancer drug targets.

LIMK inhibitor development

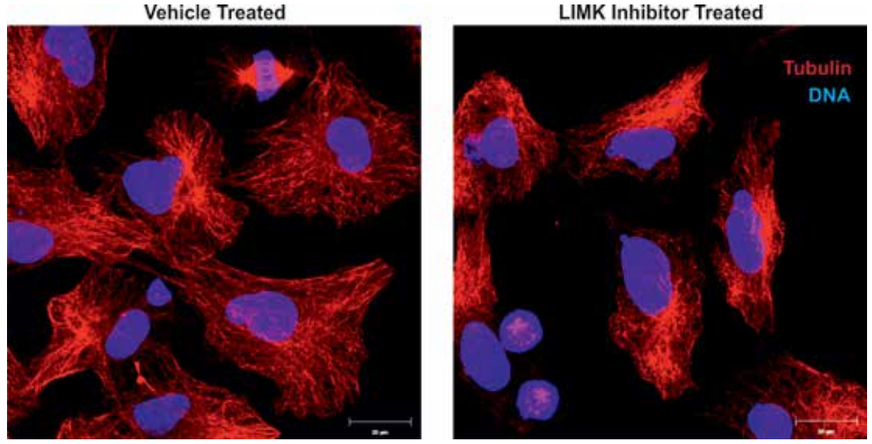
LIM kinases play central roles in Rho GTPase regulation of the actin cytoskeleton by phosphorylating cofilin proteins (cofilin1, cofilin2, destrin/ADF) on Ser[3] and inactivating their F-actin severing activity. The LIM kinase family consists of two members; LIM kinase 1 (LIMK1) and LIM kinase 2 (LIMK2), and their activation results from phosphorylation on specific residues in their kinase domains by Rho GTPase–regulated kinases, including ROCK, PAK and MRCK. There are numerous ways that Rho GTPase activation has been associated with human cancers, particularly with progression to invasive and metastatic stages. Therefore, LIMKs make interesting prospective targets for drug discovery.

CRT's Discovery Laboratories undertook a high throughput screening and medicinal chemistry programme to develop potent and selective LIMK inhibitors. Funding from the CRUK's Discovery Committee enabled us to undertake studies that aimed to identify the tumour types that were most sensitive to growth inhibition induced by LIMK inhibitors. We recently reported that LIM kinases contribute to prostate cancer by enabling the nuclear translocation of the androgen receptor in response to hormone stimulation. In addition, we collaborated with the Wellcome Trust Sanger Institute's Genomics of Drug Sensitivity in Cancer project to screen over 600 tumour cell lines for their sensitivity to two different LIMK inhibitors. These studies revealed that neuroblastoma was the most sensitive to both inhibitors. Cell-based experiments revealed that an important mechanism of action of LIMK inhibitors in blocking proliferation is by inducing mitotic defects.

MRCK inhibitor development

The myotonic dystrophy kinase-related CDC42-binding kinases, MRCKα and MRCKβ regulate actin-myosin contractility and have been implicated in cancer metastasis. Along with the related ROCK1 and ROCK2 kinases, the MRCK proteins initiate signalling events that lead to contractile force generation, which powers cancer cell motility and invasion. A potential strategy for cancer therapy is to reduce metastasis by blocking MRCK activity, either alone or in combination with ROCK inhibition.

Figure 1
Lung cancer cells were fixed and stained for their microtubule structures (red) and nuclear DNA (blue). Cells treated with vehicle alone have well-formed microtubules, while cells treated with a LIMK inhibitor have shorter and more chaotic microtubules. In addition, there is evidence of cells failing to undergo mitosis successfully, which is associated with decreased proliferation.



In collaboration with the Beatson's Drug Discovery Programme, funding was obtained from the Medical Research Council and Worldwide Cancer Research to develop inhibitors for the MRCK proteins. Screening a kinase-focused small molecule chemical library resulted in the identification of compounds with inhibitory activity towards MRCK. Medicinal chemistry combined with *in vitro* enzyme profiling led to the discovery of 4-chloro-1-(4-piperidyl)-N-[5-(2-pyridyl)-1H-pyrazol-4-yl] pyrazole-3-carboxamide (BDP5290) as a potent MRCK inhibitor. X-ray crystallography of the MRCKβ kinase domain in complex with BDP5290 revealed how this ligand interacts with the nucleotide-binding pocket. BDP5290 demonstrated marked selectivity for MRCKβ over ROCK1 or ROCK2 for inhibition of myosin II light chain (MLC) phosphorylation in cells. While BDP5290 was able to block MLC phosphorylation at both cytoplasmic actin stress fibres and peripheral cortical actin bundles, the ROCK selective inhibitor Y27632 primarily reduced MLC phosphorylation on stress fibres. BDP5290 was also more effective at reducing MDA MB 231 breast cancer cell invasion through Matrigel than Y27632. Finally, the ability of human SCC12 squamous cell carcinoma cells to invade a three-dimensional collagen matrix was strongly inhibited by 2 μM BDP5290 but not by the identical concentration of Y27632, despite equivalent inhibition of MLC phosphorylation. These results showed that BDP5290 is a potent MRCK inhibitor with activity in cells, resulting in reduced MLC phosphorylation, cell motility and tumour cell invasion. The discovery of this compound will enable further investigations into the biological activities of MRCK proteins and their contributions to cancer progression.

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COLORECTAL CANCER AND WNT SIGNALLING

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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% of sporadic cancers. Central to our work is the use of novel inducible models of intestinal tumourigenesis that allow us to study the functions of specific tumour suppressor genes.

Elucidating why APC is so frequently mutated in colorectal cancer

A key unanswered question in colorectal cancer is why APC mutations are so frequent (~ 75%) whilst activating mutations in β-catenin are rare (5%) when both activate the WNT pathway. To address this, we have compared the consequences of acute loss of APC and activation of β-catenin within the murine small and large intestine. Compared to *Apc* loss, β-catenin activation took longer to lead to accumulation of β-catenin in the nucleus and acquisition of a crypt progenitor phenotype in the small intestine and, more importantly, was unable to transform the colon. We found that E-cadherin, which is known to interact with β-catenin and hold it at cell junctions hence preventing nuclear accumulation, is expressed at much higher levels in the colon. Moreover, using a proximity ligation assay (PLA), we discovered that there were many more β-catenin-E-cadherin complexes in the colon than in the small intestine (Fig. 1). Deletion of a single copy of E-cadherin (which alone has no impact on intestinal homeostasis) in combination with an activating β-catenin mutation, led to a rapid relocalisation of β-catenin to the nucleus and a crypt progenitor phenotype. There was then rapid transformation of the murine colon. Importantly, these differences were conserved between mouse and man, with humans cancers that carry β-catenin activating mutations all having low levels of E-cadherin.

mTORC1 is required for proliferation of APC deficient cells

mTORC1 is a kinase that is required to coordinate proliferation and cell growth, and has long been viewed as an attractive pathway for cancer therapy. Our work has shown that activation of mTORC1 is a common event following loss of APC. This year, we have shown that APC deficient cells are absolutely dependent on mTORC1 to proliferate (Faller *et al.*, Nature 2015; 517: 497). Genetic inhibition or use of rapamycin, a chemical inhibitor, completely removes the proliferative capacity of APC deficient cells. This is associated with a slowing of translational elongation and hence, given the massive burst of transcriptionally activity of APC deficient cells, cells enter growth arrest (Fig. 2). Although this growth arrest is reversible, since rapamycin is a well-tolerated drug, it is possible that it would be beneficial for those patients that are predisposed to colorectal cancer.

Additional mutation of *KRAS* rewires APC deficient cells to become resistant to mTORC1 and MEK inhibition

In colorectal cancer, *KRAS* is often co-mutated with *APC* (in ~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, we have been investigating the cooperation of *Apc* and *Kras* mutations in models of colorectal cancer. The additional mutation of *Kras* exacerbates

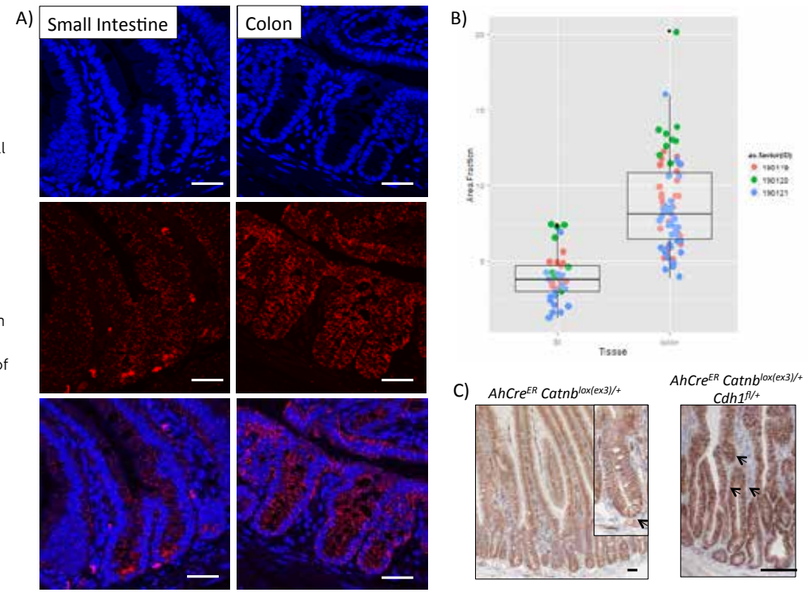

Figure 1
E-cadherin-β-catenin complexes are increased in the colon compared to the small intestine. A) Complexes of β-catenin-E-cadherin (in red), blue stain is DAPI nuclei in small intestine and colon. B) Quantification of complexes in small intestine versus colon (each colour spot represents a different sample). C) β-catenin immunohistochemistry showing 5 days post cre induction, no nuclear β-catenin in *AhCre^{ER} Catnb$^{lox(ex3)/+}$* intestines apart from the base of the crypt (arrowed). When a single copy of E-cadherin is deleted there is now lots of nuclear β-catenin and much larger intestinal crypts.

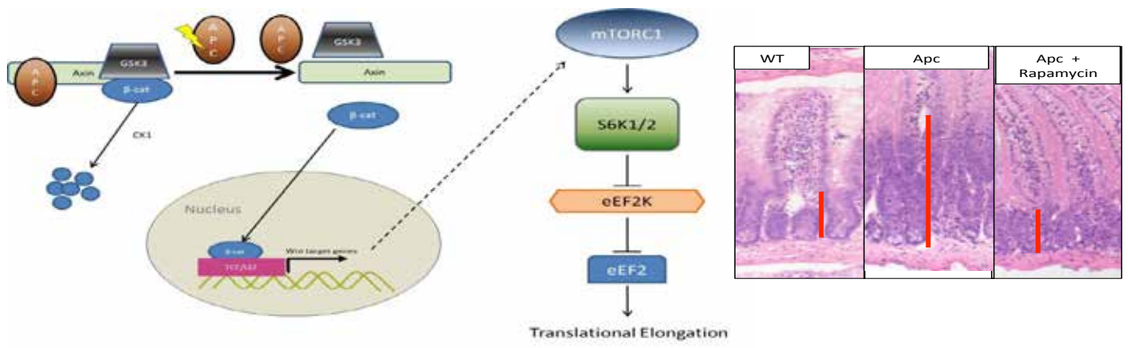

Figure 2
Inhibition of mTORC stops the proliferation of APC deficient cells. A) Schematic of mechanism of how loss of *Apc* causes the activation of mTORC signalling. B) H&E stained sections of intestines, wild type (WT) or APC deficient (APC). As can be seen by the red bar, wild type intestinal crypts are much smaller than APC deficient ones. Treatment with rapamycin returns APC deficient crypts (APC + rapamycin) to the same size as wild type ones.

many of the phenotypes of APC loss, increasing proliferation rates and accelerating tumourigenesis. Importantly, APC deficient cells that also carry a *Kras* mutation are intrinsically resistant to mTORC1 and MEK inhibition. This may help explain why later stage colorectal cancer trials using mTORC1 inhibitors have not worked. Mechanistically, *Kras* mutation causes the activation of mTORC1 targets in an mTORC1-independent manner. 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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DROSOPHILA APPROACHES TO CANCER

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We utilise the fruit fly *Drosophila* to understand fundamental aspects of cancer biology. We have previously reported an inflammatory reaction from the innate immune system to the rise of tumours, comparable to the one observed in cancer patients. Importantly, powerful fly genetic tools allowed us to define for the first time the contexts dictating 'good' versus 'bad' inflammation. Recently, we have dissected a systemic crosstalk between tumours and peripheral energy stores such as the adipose tissue. Remarkably, our data demonstrate that adipocytes can sense tumours at a distance and react by activating proto-inflammatory pathways that act as tissue non-autonomous tumour suppressor factors. We have characterised many aspects of cancer cachexia in our model; these results could shed light on the complex systemic relationships between tumours, chronic inflammation and peripheral tissues in human cancer patients.

Drosophila model of cancer cachexia

Cancer-related cachexia, also known as energy wasting, is perhaps best defined as a 'loss of body weight that cannot be reversed nutritionally'. Nevertheless, whether this clinical wasting syndrome is a passive bystander effect in cancer, or whether it plays a positive or negative effect on cancer progression, is currently unknown. We have characterised key aspects of cancer cachexia in tumour bearing *Drosophila*. These include the mobilisation of triglycerides stored in lipid droplets in the fat body, followed by an increase in free fatty acids and diacylglycerol in the circulation. A transcriptomic profiling of the fat body, a tissue akin the vertebrate liver and adipose tissue, demonstrated the upregulation of several enzymes involved in lipid catabolism. Most importantly, the adipocytes, the cell type that constitutes the fat body, displayed an aberrant morphology and became translucent, consistent with the biochemical and genetic data and pointing towards an evolutionary conserved wasting upon the development of tumours. Moreover, the buoyancy of the animals, as a proxy for lipid content, was altered in the mutant animals (Fig. 1). Most importantly, the fat body phenotypes observed in tumour bearing scribble mutant animals were significantly rescued in double mutants for *scribble* and *eiger/dTNF*, the *Drosophila* homologue of tumour necrosis factor (TNF) (Fig. 1). These results indicate that TNF has an ancestral and evolutionary conserved role in inducing cachexia. This model allows manipulation of lipid metabolism in adipocytes to examine not only tissue autonomous effects in the adipose tissue but also in the epithelial tumours.

Effects in the skeletal muscle

In cachexia, a loss of mass of lean tissue occurs alongside the loss of adipose tissue. Indeed, skeletal muscle displays a severe degeneration in cachectic patients. Our data suggest that the body wall muscle of *Drosophila* larvae, which is analogous to human skeletal muscle, also reacts to high tumour burden and degenerates progressively. We observed induction of autophagy, reduced density of F-actin in myofibrils and, specifically in the case of animals with tumours carrying both a mutation in *scribble* and activation of the Ras oncoprotein, we also observed intramyocellular accumulation of lipid droplets in the muscle (Fig. 2). This latter is a remarkable characteristic of muscle cachexia described recently. The transcriptomic profile of the larval cuticle, enriched for the body wall muscle, indicates the activation of several immune pathways. Our tractable *Drosophila* model will allow manipulations of these pathways in the muscle of tumour bearing animals to probe for effects both in muscle degeneration and tumour size.

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Figure 1
Scribble mutants are bloated and enlarged (top), their fat body appears more translucent (middle panels) and they are more buoyant than control genotypes (bottom). *Eiger* mutation suppressed these phenotypes.

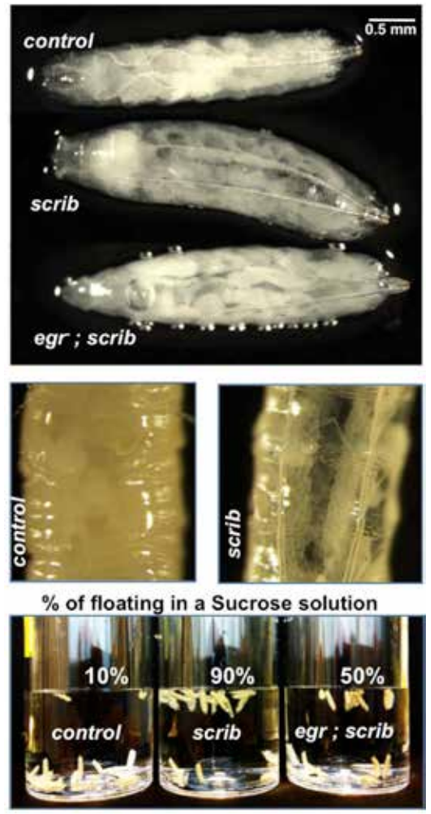
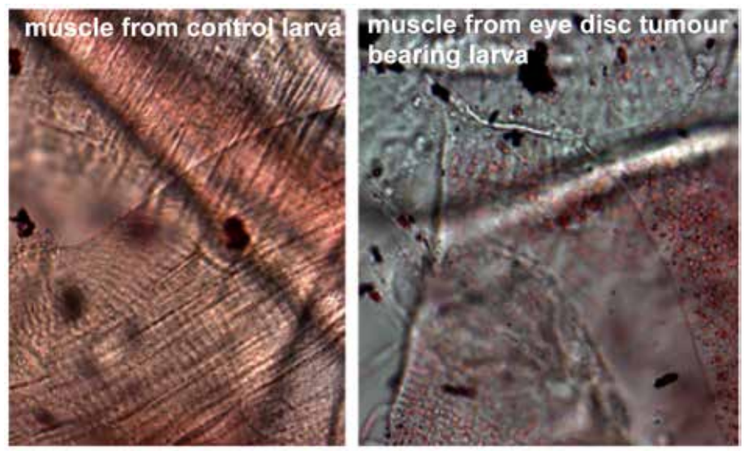


Figure 2
Skeletal muscle from tumour bearing larvae displays increased accumulation of lipid droplets. Note the puncta of Red Oil staining in the experimental genotype (right).



VASCULAR PROTEOMICS

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Angiogenesis is the process of vessel growth from pre-existing ones and an established hallmark of cancer. Many tumours are indeed highly vascularised. When tumour vessels are intact and functional, they provide the oxygen and nutrients necessary to sustain the uncontrolled proliferation of tumour cells as well as a way for tumour cells to escape the primary tumour and form distant metastases. Conversely, when the tumour vasculature is aberrant, vessels are leaky and poorly functional and this results in tumour hypoxia, reduced efficiency of chemotherapeutic drug delivery and limited efficacy of radiotherapy.

For these reasons considerable effort has been devoted to understanding the angiogenic process in cancer in order to develop therapies that interfere with this process and could be used in conjunction with conventional chemo- and radiotherapy. Our group exploits its experience with high resolution mass spectrometry (MS) and accurate quantification methods, including SILAC (stable isotope labelling with amino acids in cell culture), in combination with a variety of cellular and molecular approaches to determine the complex signalling driving (tumour) vessel formation, with a particular focus on endothelial cell metabolism and the impact of tumour stroma surrounding newly forming vessels on this process.

Fatty acid oxidation regulates vascular permeability

Endothelial cells (ECs) line the inner layer of the blood vessel wall and are thus in direct contact with the blood (Fig. 1). This location means that ECs are exposed to the changes in oxygen and nutrient levels that occur in both physiological and, importantly, pathological conditions such as cancer. Notably, oxygen and nutrients fuel cell metabolism, and it is therefore important to understand if and how cell metabolism affects EC function. Currently, the details of how metabolism controls EC function are still largely unknown but a better understanding of this should unravel novel molecular mechanisms regulating angiogenesis and hint at strategies to therapeutically interfere with it.

MS-based proteomics has the potential to contribute to a better understanding of cell metabolism (Reid *et al.*, Methods Enzymol 2014; 543: 235) and we have applied this technology in the context of angiogenesis. We have previously used an *in vitro* model of angiogenesis, which recapitulates aspects of vessel growth *in vivo*, where ECs assemble into a vascular-like network within a day, and measured time-resolved proteomic changes that occur in these cells during this process (Zanivan *et al.*, Mol Cell Proteomics 2013; 12: 3599). We observed that, when cells were assembled into a fully formed network, the levels of the vast majority of metabolic proteins changed. This suggested that major metabolic alterations were occurring during network formation. In collaboration with Eyal Gottlieb's group and Eytan Ruppin's group (University of Tel Aviv), we used our quantitative proteomic data to build up a predictive metabolic model of the cells forming capillary-like structures. We showed that when they are assembled into a fully formed network, ECs increase their fatty acid oxidation (FAO), while decreasing their glycolysis. Moreover, we observed that inhibiting CPT1A, the rate-limiting enzyme for FAO, disrupted the network. Using *in vitro* and *in vivo* functional studies we determined that ECs use FAO to fuel the tricarboxylic acid cycle and that this is required to maintain physiological levels of endothelial and vascular permeability. Importantly, vascular hyperpermeability is typical of diseases such as cancer, thrombosis and atherosclerosis. Therefore, we have identified FAO as a promising metabolic pathway for further investigation as a target to control vascular permeability in pathological conditions.

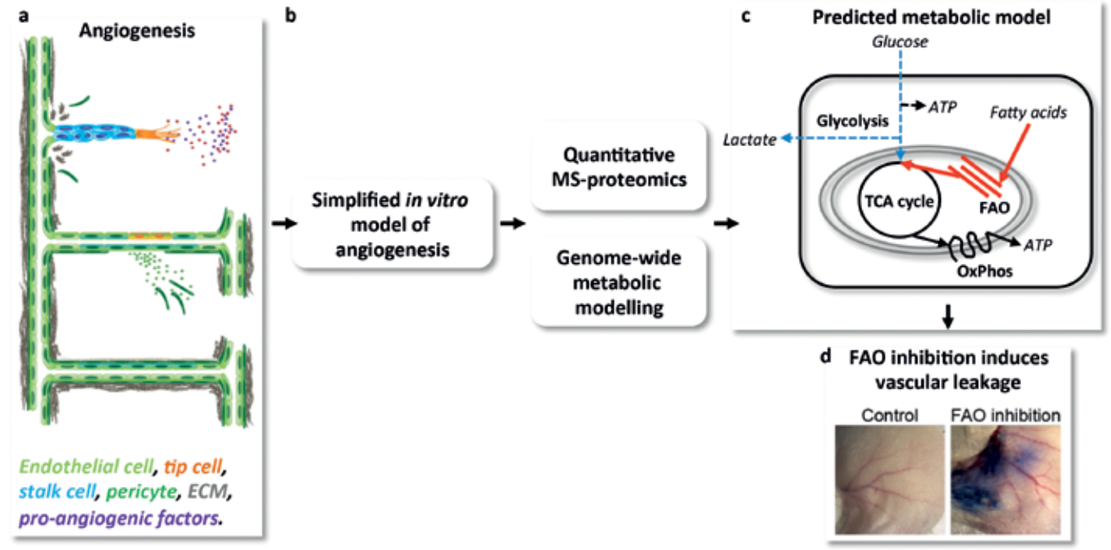

Figure 1
Fatty acid oxidation (FAO) controls endothelial and vascular permeability. (a) Schematic overview of the formation of a new blood vessel from a pre-existing one induced by pro-angiogenic factors. (b) Schematic overview of the approach used to predict metabolic fluxes regulated during the formation of a vascular-like network *in vitro*. The approach integrates quantitative mass spectrometry (MS)-based proteomics data with genome-wide metabolic modelling. (c) The model predicts that when the vascular-like network is fully formed, endothelial cells decrease their glycolysis (blue, dashed line), while increasing their FAO (red, thick line). (d) Hyperpermeability (measured by Evans blue extravasation) observed upon acute inhibition of FAO in established blood vessels of the mouse ear.

Unravelling novel regulators of tumour stiffness (in collaboration with Jim Norman's group)

Tumours are composed of many different cell types in addition to tumour cells. These cells, together with the extracellular matrix (ECM), form what is called the tumour stroma. Among these stromal cells, we are particularly interested in cancer-associated fibroblasts (CAFs). CAFs can secrete a plethora of factors, including ECM components and ECM modifiers that alter the mechanical properties of a tumour. Notably, high stiffness enhances angiogenesis and tumour cell invasion, and has a significant impact on tumour development. Therefore, it is important to better understand the molecular mechanisms regulating tumour stiffness. Our lab uses unbiased proteomic approaches in combination with functional *in vitro* and *in vivo* assays to identify and characterise previously unknown mechanisms through which CAFs regulate the stiffness of the ECM.

We are exploiting established cell lines of human mammary fibroblasts of different origins, normal (iNF) and cancer associated (iCAF), to provide a detailed picture of proteins specifically expressed or with altered levels in the tumour stroma. In particular, we have optimised protocols to perform in-depth quantitative proteomic analysis of soluble proteins and ECM components secreted by fibroblasts in culture. This approach led us to identify more than a thousand proteins secreted by these cells. Among these, we detected ECM components and growth factors, such as collagen, fibronectin and transforming growth factor beta that are well known CAF markers and which were highly abundant in iCAFs compared to iNFs. Unexpectedly, we identified a member of the chloride intracellular channel protein family to be an ECM component secreted by CAFs. Detailed analysis of this protein revealed that it is able to enhance angiogenesis and tumour cell invasion and that this process is mediated, at least in part, by its capability to regulate the stiffness of the ECM. While most of the current studies have been performed *in vitro*, we are now exploring the relevance of these findings *in vivo*. This will hopefully reveal a novel mechanism through which CAFs regulate tumour stiffness.

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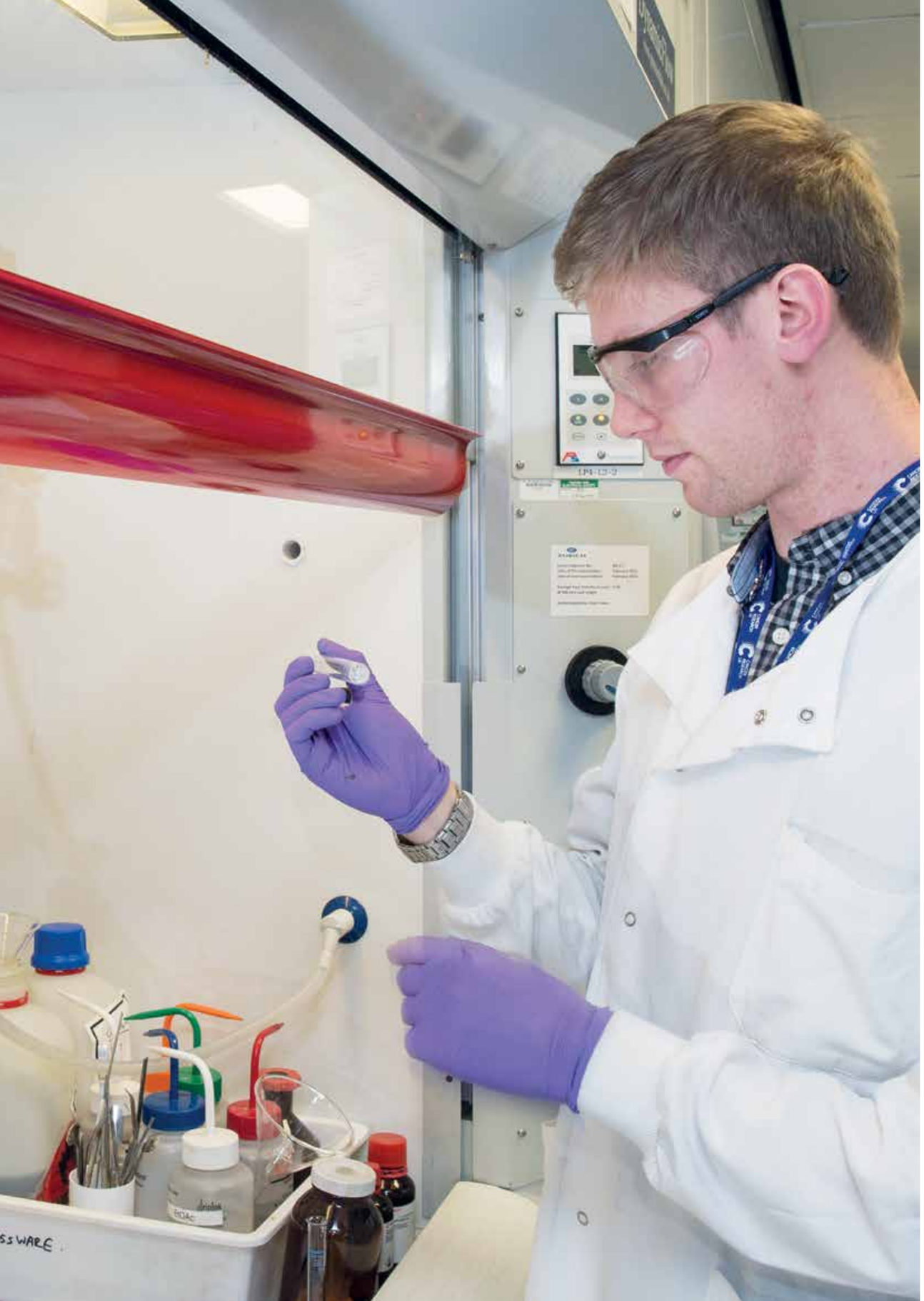

DRUG DISCOVERY

CANCER RESEARCH UK BEATSON INSTITUTE

Martin Drysdale - Drug Discovery Programme

DRUG DISCOVERY PROGRAMME

www.beatson.gla.ac.uk/ddp

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Kenneth Cameron
Gillian Goodwin
Chris Gray
Marta Klejnot
Jen Konczal
Alexander Schuettelkopf

Drug Discovery sits at the interface between bench science and clinical development. Our remit is to provide a mechanism by which we can translate the fundamental biology being undertaken within the Beatson into new medicines for patients where there is a clear unmet medical need.

Exploiting the basic research themes of the Institute's scientists we have made significant advances in our portfolio targeting novel approaches to modulate disease-relevant processes.

Metastasis is responsible for approximately 90% of cancer deaths, however there are currently no therapeutic agents available to combat this process. One of the proteins we have chosen to target is fascin, an actin bundling protein that plays a key role in the movement of cancer cells and whose upregulation is known to correlate with poorer overall survival in severe cases of pancreatic ductal adenocarcinoma. We have continued to utilise our fragment-based hit identification expertise to target other protein-protein interactions since these are very challenging biological targets but with 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 over 30% of all human cancers.

Fascin

Fascin is a migration promoting protein that is frequently upregulated when epithelial tissues become malignant. 80-90% of cancers are of epithelial origin and fascin is overexpressed in a variety of tumour types including bladder, colon, lung and pancreas. Thus, it is not only a prognostic marker for multiple types of cancer but is also a compelling drug target.

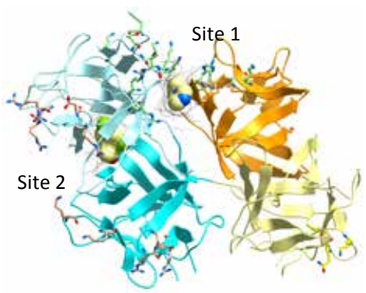

Figure 1
Structure of fascin indicating two ligand binding sites (Site 1 and 2) and their proximity to functionally important residues displayed in green and salmon.

Fascin is a uniquely folded actin bundling protein whose regulation by PKC is tightly coupled with integrins and the extracellular matrix. It exists in equilibrium between the cytoplasm and cytoskeleton where it is bound to actin. It has at least two binding sites for filamentous actin and crosslinks these filaments into tightly packed parallel bundles, oriented with their growing ends toward the plasma membrane. Since fascin is found in actin-rich protrusive membrane structures (microspikes and filopodia) and degradative structures (invadopodia and podosome), which are all pro-migratory, it is proposed that by stabilising actin, fascin provides cells with invasive properties that may confer increased metastatic potential.

We have taken a fragment based approach to identify novel binders of fascin; coupled with a highly successful crystallography campaign this has enabled us to progress initial fragment hits through to compounds with low µM binding. To date, over 100 new small molecule/fascin crystal structures have been generated that have aided hit validation and helped to guide structure-based hit-to-lead chemistry (Fig. 1).

Significant progress has been made in the equally challenging area of biochemical assay development. We have developed two new screens based on highly validated bundling techniques, which has enabled quantitative and reproducible assessment of novel compounds (Fig. 2 and 3). Combining biochemical data with crystallography analysis has enabled us to understand the functional implications of different binding modalities and focus optimisation to the most promising chemical structures.

Figure 2
Optimised robust bundling assay demonstrating concentration response of a BDP fascin inhibitor compound on percentage of bundling. In the absence of inhibitor, >90% of actin is in the pelleted fraction (P), with little in the supernatant (S). This is reversed upon addition of the inhibitor.

Figure 3
Bead-based fascin actin bundling assay. His tagged fascin is pulled down with nickel coated beads and bound actin detected with a fluorescent phalloidin. In the presence of the BDP fascin inhibitor compound, bundling by fascin is inhibited and the amount of phalloidin-labelled actin reduced.

Figure 4
Evolution of a weak binding fragment hit (NMR Kd >1000mM) to more potent analogues (Kd 41 & 30mM); small molecules that directly bind to KRAS (protein in grey).

Figure 5
The GDP-GTP catalytic cycle; b) The KRAS-GDP:SOS1 nucleotide exchange assay; c) Two compounds that inhibit nucleotide exchange in the assay represented in 5b.

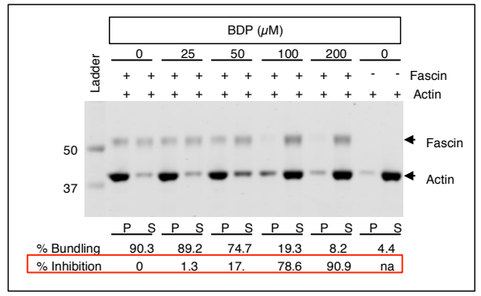

Figure 2

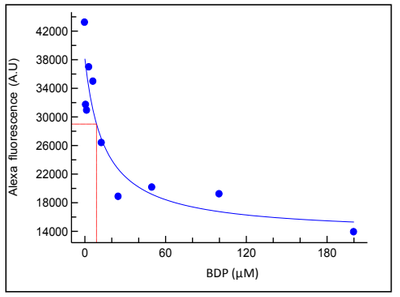

Figure 3

RAS

Approximately a third of all human cancers and the majority of pancreatic, colorectal and lung cancers are driven by mutations in RAS genes. The RAS gene family has three main members; KRAS, HRAS and NRAS, and all play a pivotal role in cell signalling. Under circumstances where RAS genes are mutated, cells multiply uncontrollably and escape cell death signals. The significance of KRAS mutation in driving and maintaining oncogenesis is well recognised. KRAS mutations are associated with over 20% of all human cancers, and in particular are associated with the vast majority of pancreatic ductal adenocarcinomas and a significant number of other tumour types including colon and lung. In the GTP-bound and active state KRAS signals from the plasma membrane through a functionally diverse set of downstream effector proteins including PI3K, RAF and RALGDS to pathways that control cellular growth, apoptosis, survival and differentiation.

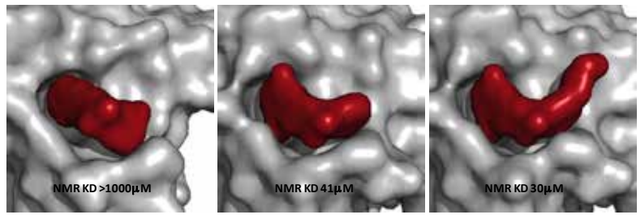

Figure 4

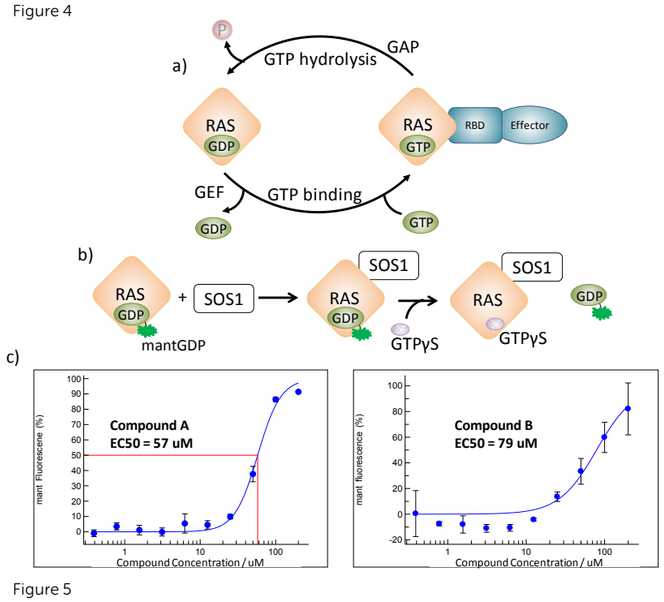

Figure 5

Our approach to identifying start points from which to build a drug discovery project capitalised on our strengths in fragment screening and structure-based drug design. We performed a primary screen of our fragment library against a mutant variant of KRAS using high field NMR experiments, identifying a number of weak binders. After confirmation of binding, crystal structures were obtained that have been instrumental in enabling us to improve potency (Fig. 4) and a strategy to increase this further, towards molecules that will be able to probe inhibition of KRAS effector protein interactions at the cellular level.

In order to assess the ability of our compounds to inhibit one of the functions of KRAS, we have assayed multiple examples in a nucleotide exchange assay (Fig.5).

Through the application of structural biology and medicinal chemistry the project has improved potency of the initial fragment hits, delivering compounds that bind directly to KRAS and inhibit the function of nucleotide exchange. Future strategies will build on this, utilising a combination of chemistry, structural biology and biology to generate potent KRAS binders that can inhibit the interaction between GTP-bound KRAS and its effector proteins.

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ADVANCED TECHNOLOGIES

CANCER RESEARCH UK BEATSON INSTITUTE

Kurt Anderson - Beatson Advanced Imaging Resource (BAIR)
Gabriela Kalna - Bioinformatics and Computational Biology
Gillian Mackay - Metabolomics
Gaurav Malviya - Nuclear Imaging
Sergio Lilla and David Sumpton - 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
Margaret O'Prey
David Strachan

Light microscopy is a fundamental technique in cell biology and cancer research. The development of genetically encoded fluorophores has revolutionised research by enabling direct visualisation of any gene product. There have also been tremendous advances in fluorescence imaging technology to visualise molecular dynamics in living cells, tissues and organisms.

These powerful techniques are increasingly sought 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 and immunofluorescence microscopy. Development of advanced applications requires close work with users to understand their scientific questions and help them develop appropriate imaging strategies. The following have been identified through consultation as important at the Beatson: 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; intravital microscopy; and fluorescence lifetime imaging for the determination of fluorescence resonance energy transfer (FLIM-FRET). More recently PET, SPECT and CT imaging were identified as techniques having great potential to support the extensive use of preclinical cancer models, and preclinical imaging has now become an independent core facility headed by Gaurav Malviya (see page 55). 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*.

This year, one door closed and another opened with the completion of the TSB funded project 'Optimised Lasers for Multi-colour Multiphoton Imaging' and start of the BBSRC funded one 'Super-resolved, three-dimensional, multi-fluorophore tracking of live cell dynamics'. The TSB project helped us to develop a close working relationship with M-squared Lasers and introduced us to new technology for multiphoton excitation in the form of semiconductor disk lasers, which have novel wavelength, pulse widths and repetition rates. We also served as a test site for the new Coherent Discovery Laser, which combines a tuning range of 680 to 1300 nm with a fixed output at 1040 nm. The BBSRC project, in collaboration with Alan Greenaway (Heriot-Watt University), will provide us with a diffraction grating based optical system that can simultaneously focus up to nine planes within a specimen into a 3x3 image array on a CCD camera. This will potentially enable rapid collection of four-dimensional (X, Y, Z, t) image volumes, as well as offering the potential for axial super-resolution of single fluorophores. Our plan is to incorporate this multifocal system into a Warwick Open Source Microscope (WOSM) that we are building in collaboration with Nick Carter and Rob Cross (Institute for Mechanochemistry) to perform PALM/STORM super-resolution microscopy.

Finally, we have continued to 'shake down' our new LaVision TRIM2 multiphoton microscope. This involved taking delivery of two hybrid GaAsP PMTs, which can be used for time correlated single photon counting, and an adaptive optics module with software to enable integration of multiple mirror shapes into multidimensional acquisition routines.

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

www.beatson.gla.ac.uk/advanced_technologies

Head

Gabriela Kalna

Informaticians
Ann Hedley
Matthew Neilson

The Bioinformatics unit provides support for a range of research projects that require computational methods, advanced statistical analyses and mathematical modelling.

Despite the continuing demand for analysing data from high throughput technologies, we strive to ensure that even the smallest task receives our full attention in terms of experimental design, the application of appropriate statistical tests and the clear presentation of results for use in theses and publications.

Our team focuses on exploratory data analysis, with an ultimate goal of providing insights that enhance our understanding of cancer biology. We offer routine processing of data, differential expression, supervised and unsupervised machine learning, and graph and network theory-based analyses. Our data analysis and modelling tasks are performed using the open-source Bioconductor package for R, Fortran and MATLAB (most notably the Bioinformatics Toolbox and the Statistics Toolbox). We make use of analytical routines that have been developed in-house or in collaboration with our colleagues from the areas of mathematics, statistics, computer science and biology. In addition to Ingenuity Pathway Analysis, GeneGo Meta Core and CLC Genomics Workbench, which are available to all researchers at the Beatson, we use the Oncomine Research Premium Edition database and Oncomine Gene Browser to satisfy the demands of researchers who wish to exploit publicly available datasets.

Over the last year, we have been working on a number of projects integrating data from multiple platforms, including RNAseq, microarrays, proteomics and siRNA screens. There has also been a growing interest in exome sequencing and identification of fusion genes. Recently, NextSeq 500 Desktop Sequencer replaced the older Illumina model that had previously been used at the Institute. The new sequencer enables our researchers to perform sequencing at a higher depth. Consequently, we are exploring new analytical approaches and a wide range of open source pipelines with an aim to achieve faster alignment and more extensive downstream data analysis. A webserver for viewing sequenced data through Integrative Genomics Viewer and UCSC Genome browser has been also set up, allowing our scientists to inspect coverage, aligned reads and junction counts for their samples.

Our computational model for pseudopod-centred cell migration and chemotaxis is now capable of simulating phagocytosis, and we have recently been investigating three factors that influence a cell's ability to phagocytose a nearby entity: the size of the entity, the adherence of the entity to the cell and the level of attractant that is secreted by the entity. In a related development, this same computational model can now be used simulate a field of cells interacting with one another via contact inhibition. Our particle-based model for simulating populations of E-cadherin has been calibrated such that it can faithfully reproduce the experimentally observed behaviour of each L Cell / E-cadherin mutant. In an effort to simulate wild type E-cadherin in a consistent fashion, we are using our current model to investigate cooperativity between each of the permitted interaction types.

Finally, we have developed and deployed a set of routines that aim to improve the productivity of researchers in the RNAi Screening facility. Our routines interface the R software environment with the Dotmatics Oracle database, such that researchers can quickly and easily perform calculations on a prescribed set of records and then feed the results back into the database. It is anticipated that this seamless integration will lead to considerable productivity gains within the group.

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METABOLOMICS

www.beatson.gla.ac.uk/advanced_technologies

Head

Gillian Mackay

Scientific Officer

Niels van den Broek

The Metabolomics facility is part of the Cancer Metabolism Research Unit (headed by Eyal Gottlieb) and we work closely with its research scientists. This includes our involvement in several external collaborations. We also work with several other research groups within the Beatson, providing advice on sample preparation and data analysis, as well as measuring metabolite levels in samples.

We have well-established LC-MS methods for targeted metabolomics, analysing approximately 100 metabolites per sample. We are now establishing a workflow for untargeted analysis to identify more metabolites that differ between sample groups.

Using a targeted metabolomics approach, we analyse a range of samples types including cell extracts, medium, plasma, urine, tumour and other tissues, and CSF. This year, we have reduced the analysis time per sample and introduced Thermo's data analysis software, TraceFinder. This enables much faster and more flexible data analysis than our previous LCquan software. We use our own compound databases of over 100 metabolites in routine LC-MS analyses, identifying known metabolites by their accurate mass and their retention time on the LC column. Often a comparison of metabolite levels between samples is sufficient, however we can quantify the concentrations of known metabolites in samples using commercial standards. For metabolic flux analysis, the fate of heavy isotope tracers (often 13C labelled glucose added to medium as a nutrient) can be determined by measuring isotopologues of many intracellular metabolites.

We are starting to become more involved in untargeted analysis for cell culture samples and also extending this to analysing clinical samples, to investigate the clinical relevance of *in vitro* results. Our new Q-Exactive LC-MS/MS instrument, installed in January, is proving very useful, with its fragmentation capability, in particular to help with the identification and structure of unknown metabolites. We have very recently purchased Nonlinear Dynamics' Progenesis software for untargeted data analysis. As well as the MS data for the metabolites, this software uses adducts, isotopes and fragmentation data to identify unknown metabolites.

We are also developing methods for GC-MS and GC-MS/MS analysis using our Agilent triple quad mass spectrometer. We have methods in place for fatty acids and amino acids. The method of sample preparation is more complex than for LC-MS, as samples need to be derivitised to allow them to be volatile in the GC. The aim is to use this as another system for metabolite determination, being able to measure some different metabolites and distinguish between compounds of the same mass that co-elute on the LC systems, to gain a more complete coverage of the human metabolome.

Currently, we have two Thermo Scientific LC-MS systems with Exactive mass spectrometers and a Thermo Scientific Q-Exactive LC-MS/MS system. These are Orbitrap mass spectrometers, which have high resolution and can determine extremely accurate masses. We are in the process of purchasing another Q-Exactive instrument to replace one of the Exactives. These are complemented with our Agilent GC-MS/MS triple quad instrument.

To technically share LC-MS knowledge, the metabolomics team (which includes a member of both Eyal Gottlieb's group and Karen Vousden's) regularly meet with Jurre Kamphorst's lipidomics group and the proteomics teams at the Beatson (David Sumpton, Sergio Lilla and Sara Zanivan).

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NUCLEAR IMAGING

www.beatson.gla.ac.uk/advanced_technologies

Head

Gaurav Malviya

Scientific Officer

Agata Mrowinska

Nuclear imaging techniques have gained wide acceptance in recent years for monitoring response to cancer therapy at the molecular level. Nuclear imaging using specific probes allows non-invasive, longitudinal assessment of biological processes, such as tumour metabolism, cell proliferation, angiogenesis, hypoxia and receptor dynamics.

Nuclear imaging involves the administration of tiny amounts of radioactive tracers followed by functional imaging using PET (positron emission tomography) or SPECT (single photon emission tomography). In addition, anatomic information is often obtained by CT (computed tomography) scanning to localise radioactive tracer uptake within the body. The Nuclear Imaging facility provides PET, SPECT and CT imaging support to a range of *in vivo* research projects. In 2014, we increased our imaging armamentarium beyond ^{18}F-FDG and performed PET/CT and SPECT/CT using a wide variety of radiolabelled probes, including ^{18}F-FLT (for cellular proliferation), ^{18}F-NaF and ^{99m}Tc-MDP (for bone lesions). Through collaboration with Sally Pimlott and Gerry Gillen (NHS Greater Glasgow and Clyde), David O'Hagan (University of St Andrews), Eyal Gottlieb and Kurt Anderson, we have begun PET imaging lipid synthesis using ^{18}F-acetate in a colon cancer model. ^{18}F-acetate, an analogue of acetate, is metabolised to fluoroacetyl-CoA and then fluorocitrate, which cannot be further metabolised to CO_2 and water and is, hence, trapped in the cell in proportion to oxidative metabolism.

Recent studies have demonstrated that well validated radiotracers may help in early stage research of novel therapeutics by providing proof-of-concept for target modulation. Nuclear imaging may assist in testing an underlying hypothesis, inform the rational selection of dose and schedule and aid decision-making, and may explain or predict therapy outcomes. In this area, we have collaborated with Anthony Chalmers and Andrew Sutherland (University of Glasgow), who have developed a novel translocator protein (TSPO) imaging tracer, ^{18}F-AB5186 that is an important target for imaging focal neuroinflammation in diseases such as brain cancer, stroke and neurodegeneration. We have evaluated the tumour imaging potential of this tracer in a glioblastoma mouse model. Additionally, with the same groups, we are evaluating another tracer, ^{123}I- FZ044 for SPECT imaging of poly (ADP-ribose) polymerase (PARP). This tracer is particularly important because PARP inhibitors are being investigated in clinical trials as a means of sensitising tumours to chemotherapy.

In addition, we are at the final stages of negotiation with two commercial companies to obtain novel radiolabelled probes for nuclear imaging. We have recently published a research article with Jennifer Morton (Morran *et al.*, Gut 2014; 63: 1481), where ^{18}F-FLT imaging in KC PTEN mice demonstrated how proliferative arrest following rapamycin treatment could be monitored using ^{18}F-FLT PET scans (Fig. 1). In the future, we look forward to using further novel radiotracers to image the tumour microenvironment, angiogenesis, hypoxia and apoptosis.

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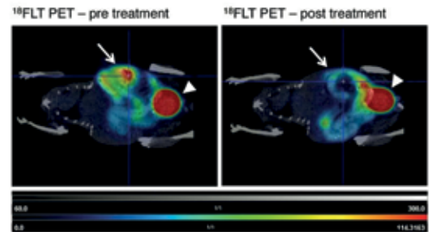

Figure 1
Representative ^{18}FLT PET/CT images show pancreatic tumour uptake (white arrows) as well as excreted tracer in the bladder (arrowheads) in a KC PTEN mouse at time of presentation (left panel) and after 4 days of rapamycin treatment (right panel).

PROTEOMICS AND MASS SPECTROMETRY

www.beatson.gla.ac.uk/advanced_technologies

Sergio Lilla

David Sumpton

Research Scientist
Emma Carrick

Scientific Officers
Sergio Lilla
David Sumpton

The proteomics facility has provided expertise in the characterisation of proteins by mass spectrometry since its launch in 2005. Our key goal is to facilitate proteomic analysis within the Institute by offering access to state-of-the-art instrumentation with assistance in sample preparation, analysis and interpretation of experimental data.

The facility is well equipped with two mass spectrometers, an Orbitrap Velos system coupled to an Easy-LC nano HPLC and an AB-Sciex 5600 triple TOF system with Eskigent UPLC. The facility also houses a number of dedicated software platforms. These include: a Mascot server for protein identification; a dedicated MaxQuant server for the analysis of SILAC data; Progenesis and Skyline for the analysis of label-free, SWATH or pRM data; and Scaffold for data compilation and dissemination. These instruments and software packages together allow the facility to provide a broad range of proteomic workflows, from simple single protein identification to complex proteome-wide protein identifications. More targeted experiments such as the characterisation of both post-translational and chemical protein modifications can be undertaken. With the incorporation of SILAC or with a well-designed label-free experiment relative protein quantitation can also be performed across all workflows. The facility stores all informatics data (raw data, login forms, etc.) submitted to the facility since 2006, making it available to Beatson users for consultation, method development or publication.

We are continually striving to improve the crucial methods currently in place to enrich the quality of the results the facility can provide. The recent introduction of parallel reaction monitoring (pRM) encapsulates this concept. The pRM approach provides at least two orders of magnitude more sensitivity than conventional LC-MS. This boost in sensitivity allows us to identify and quantify protein and their modifications within samples that otherwise would be lost. Another recent improvement, the use of high pH reverse phase LC fractionation, has allowed us to delve deeper into the proteome, identifying more proteins than was previously possible in our hands.

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RNAi SCREENING

www.beatson.gla.ac.uk/advanced_technologies

Head
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Scientific Officers
Kay Hewit[1]
Lynn McGarry

Informatics Manager
Daniel James

Graduate Student
Matthew Davidson

[1] CRUK Glasgow Centre

The RNAi Screening facility couples high throughput RNA interference (RNAi) screening and drug repurposing approaches with high content imaging (HCI) to translate fundamental cancer research towards new therapies.

Collectively, these approaches provide a powerful tool for identifying novel or key players in a system of interest and elucidating novel drug targets and/or drug partners to improve existing cancer therapeutic approaches.

Our activities include development of high throughput RNAi screening (HTS) assays, which involves scaling up existing cell-based assays to produce robust, appropriately controlled screening assays, and refining the use of additional outputs to provide multiparametric HCI analysis. We run HTS assays using robotics and automation to bring precision, accuracy and throughput to the process. Data analysis is an important part of large-scale HTS work; we have data management solutions and databases in place to support this, and carefully evaluate and deconvolute data to give quality assurance.

During 2014, we ran 13 screening campaigns in collaboration with eight groups across the CRUK Glasgow Centre. These encompassed both large-scale projects to support target identification, and more tailored, focused approaches to provide validation to support other observations. We have addressed the following questions in multiple cancer backgrounds of both mouse and human origin:

- Genes exerting selective lethality under hypoxic conditions versus normoxic conditions
- Mediators and effectors of oncogenic Myc
- Potential combinatorial partners for cell lines demonstrating resistance to an innovative therapeutic agent
- Selective mediators of response to different p53 genomic backgrounds
- Mediators of chemotherapeutic resistance in Her2+ breast cancer
- Drug repurposing approaches in pancreatic, glioblastoma and oral-derived cell lines

In early 2014, our lab relocated to the Wolfson Wohl Cancer Research Centre, where we have additional space to incorporate our expanding suite of instrumentation and a more substantial dedicated tissue culture resource. We also received funding for a second HCI instrument, which has provided much needed extra resource to support our throughput but has also brought us additional confocality capability for use within screens and in supporting hit validation and deconvolution.

Through collaboration, we have supported development, purchasing and implementation of a large metabolism-focused siRNA library as a Centre-wide resource, as well as some smaller bespoke sets. These new sets have already been widely utilised. We continue to work to implement prebuilt and develop bespoke algorithms to allow calculation of additional parameters from screening assays. Notably this includes cell/nuclear shape, size, area, intensity and proximity, as these phenotypes can be indicative of additional mechanistic processes. We are also developing algorithms to quantify organoids within screening platforms. In September, we welcomed Kay Hewit to the group, as a screening scientist.

We are currently working to advance the scope of phenotypic analysis we can offer. Further to this, we intend to implement more detailed morphological analysis pertaining to re-distribution and re-organisation of cellular shape in response to siRNA knockdown/drug treatment. Such phenotypic changes have been demonstrated in response to siRNA treatment in a cancer background (Yin *et al.*, Nat Cell Biol 2013; 15: 860), and implementation of this within the facility will be of great utility.

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TRANSGENIC MODELS OF CANCER

www.beatson.gla.ac.uk/advanced_technologies

Head

Karen Blyth

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Kirsteen Campbell[1]
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Scientific Officers
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Susan Mason

Clinical Research Fellow
Anne McKillop[2]

Graduate Students
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Alessandra Riggio

Intern
Hannah Lawson[3]

[1] Royal Society Dorothy Hodgkin Fellowship

[2] CRUK Glasgow Centre (joint with School of Veterinary Medicine)

[3] from University of Glasgow

Our lab capitalises on the use of *in vivo* models to investigate how deregulation of signalling and metabolic pathways contributes to cancer development, using these tools to explore new therapeutic targets which could benefit cancer patients. The lab has a particular interest in the role of *RUNX* genes in breast and prostate cancer.

Preclinical murine models are fundamental tools for the researchers at the Beatson where biological questions can be interrogated in the context of a physiologically relevant system. With genetic models we can accurately recreate tissue specific changes to mimic the alterations found in common cancers such as colorectal and breast cancer to better understand these diseases. In addition, we have good models for melanoma and pancreatic cancer, disease types currently associated with poor outcome and limited treatment options; so we can use our models to investigate associated signalling pathways and novel therapies. A distinct advantage to these *in vivo* models is their representative tumour pathology and an intact tumour microenvironment that can be lacking in tissue culture systems but which affects how tumours develop and respond to treatment. The lab leads the way in exploiting these resources and collaborates with the Beatson groups to dissect all stages of disease progression. In particular, we have been working closely on several projects with Karen Vousden and Eyal Gottlieb on strategies to better understand cancer cell metabolism and its relevance *in vivo*, such as assessing how loss of the TCA cycle enzyme fumarate hydratase predisposes to renal cell carcinoma (Zheng *et al.*, Nat Commun 2015; 6: 6001) and how serine depletion may be a novel therapeutic approach (Maddocks *et al.*, Nature 2013; 493: 542).

Defining the role of the *RUNX* genes in breast cancer

Members of the lab have been specifically interested in elucidating the role of RUNX1 and RUNX2 in epithelial cancer. We have shown that RUNX1, renowned for its importance in human leukaemia, is commonly expressed in breast cancer and that its expression correlates with poor prognosis in the triple-negative subtype (Ferrari *et al.*, PLoS One 2014; 9: e100759). While these studies point to a pro-oncogenic role for *RUNX1*, this gene may also have tumour constraining properties and we are currently exploring the paradox of this behaviour and its relevance in breast cancer. We have found that the related gene, *RUNX2* is also expressed in breast cancer, albeit in a more restricted subgroup of triple-negative patients, but is associated with reduced cancer-specific survival (McDonald *et al.*, Dis Model Mech 2014; 7: 525). The WNT/β-catenin pathway, an important signalling node in epithelial cancer, is also associated with triple-negative breast cancer and RUNX2 is strongly expressed in WNT-driven models of breast cancer. RUNX2 contributes to the regenerative potential of the mammary stem cell, where interestingly WNT signalling is also important, and demonstrates a commonality between these pathways in the regulation of mammary tissue. We are also interested in the role of *MCL1* in breast cancer and were awarded Breast Cancer Campaign funding this year to explore this further.

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TRANSGENIC TECHNOLOGY

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Head

Douglas Strathdee

Scientific Officers
Laurence Cadalbert
Farah Naz Ghaffar

Graduate Student
Nicola Laprano

The Transgenic Technology Laboratory employs molecular genetic approaches to analyse the role of genetic alterations to cancer. The use of this technology allows the generation of more precise models of human cancer.

Using gene targeting and genome editing, it is possible to make controlled alterations in specific genes in the germline. Combining a variety of these alterations commonly observed in human cancer allows the generation of more sophisticated and accurate cancer models.

The use of stem cells to generate cancer models

Embryonic stem (ES) cells provide a very useful tool for the analysis of gene function in cancer. Firstly, these cells have relatively high rates of DNA recombination and this allows us to make controlled alterations in specific genes, which mimic those detected in human cancers. Secondly, ES cells can be differentiated into a wide variety of different tissue types, which allows the consequences of any genetic alteration to be tested not only in the stem cells but also in the specific tissues in which the cancer arises. We are currently collaborating on a number of projects using strategies such as point mutations or conditional knockouts to uncover the roles of a variety of genes in the progression of different cancers.

Adopting a flexible approach to improving efficiency

As well as making the most of current technology, we are also trying to improve and expand the range of different techniques we employ to refine existing models and generate new ones. We are continuing to develop shRNA technology to employ this directly in models. This will allow control of gene expression directly in tumours *in situ*, allowing, for example, potential therapeutic targets to be effectively validated in tumour models.

While shRNA technology may increase the range of model types we can generate the rise of genome editing technology (CRISPRs and TALENs) has the potential to significantly increase the efficiency with which models can be generated (Fig. 1). This may not only impact the speed with which we can generate models but also has the potential to allow the creation of multiple genetic alterations simultaneously. If this technique proves to be as powerful as suggested by initial experiments, this could have a substantial impact on how models are generated and how cohorts are produced for ongoing experiments. We are currently trying to implement this technology effectively and also adapt aspects of it to make it more directly applicable to developing the types of tumour models most frequently used here.

This year, in order to improve efficiency, welfare and distribution of models, we have been establishing methods for cryopreservation and re-derivation. Now up and running, both methods will facilitate the exchange of reagents between the Beatson and other labs. In the same vein, we are also trying to make the most use of resources available from a variety of external sources. Where possible, we are continuing to use cell lines from the IMPC and are now importing GEMM-ESCs from the Netherlands Cancer Institute via Infrafrontier. These stem cell lines already carry a number of genetically modified alleles and we will further modify them to allow them to be effectively employed here for the analysis of cancer development.

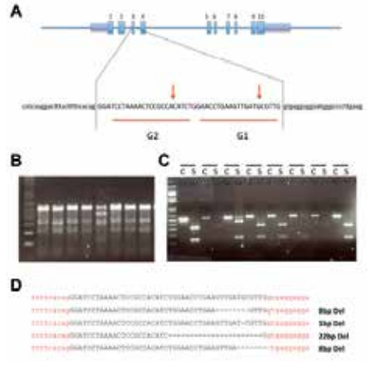

Figure 1
CRISPR-mediated genetic alteration in mouse embryonic stem cells. (A) Diagram of mouse Taz genes and design of CRISPR guide oligos to exon 3. Red arrows indicate the target sites. (B) Screening ES cell clones by Surveyor nuclease assay detected mismatches in the modified alleles. (C) Confirmation of Surveyor results using restriction enzyme analysis of PCR product. From each clone, control (C) and SfaN1 digested (S) PCR products are visualised. (D) Sequence of mutations recovered in exon 3 of the Taz gene following CRISPR transfection of mouse embryonic stem cells.

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EPIGENETICS OF CANCER AND AGEING

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The Adams lab investigates the impact of chromatin structure and epigenetics on cell proliferation, ageing and cancer. In particular, we hypothesise that age-associated changes in chromatin structure, function and regulation contribute to the dramatic age-associated increase in incidence of cancer. While age is the biggest single risk factor for most cancers, the reason for this is current poorly understood.

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.

Genome-wide analysis of chromatin structure and chromatin regulators in senescent cells

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 in proliferating and senescent cells. To do this, we are using next generation sequencing (ChIP-seq), microarray and proteomic approaches and whole genome single nucleotide bisulphite modified DNA sequencing. To complement this analysis of epigenetic marks in senescence, we are also exploring the genome-wide distribution of histone chaperones in senescent cells, again using state-of-the-art approaches. We have also collected gene expression data to build a comprehensive, integrated view of the epigenetic control of senescent cell function.

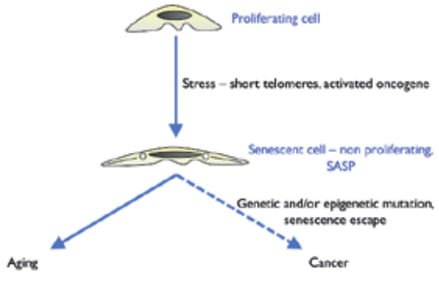
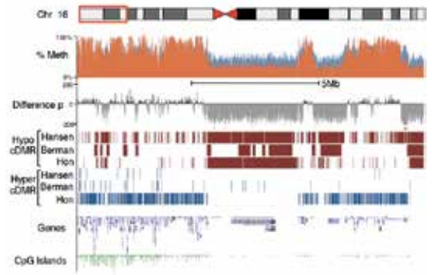


Figure 1
Senescence as a tumour suppressor mechanism. Acquisition of an activated oncogene or inactivation of a tumour suppressor initially causes a proliferative burst. Ultimately, senescence kicks in to arrest proliferation of the cells harbouring the oncogenic event. Proliferation arrest is reinforced 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.



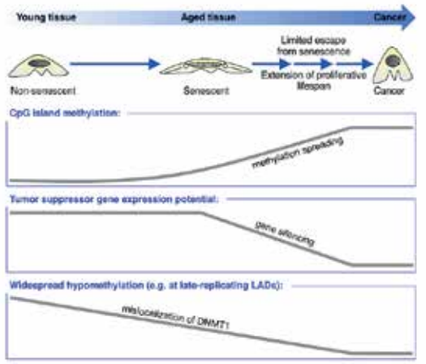


Figure 2
DNA methylation gains and losses in senescent cells overlap with hyper- and hypomethylation in cancer. Paradoxically, this suggests that the epigenome of senescent cells might be primed for progression to cancer. In turn, this suggests that senescence might be an imperfect tumour suppressor mechanism, and accumulation of senescent cells with age might predispose to cancer. The reason(s) why cancer increases with age in humans is poorly understood, at present (Cruickshanks *et al.*, Nat Cell Biol 2013; 15:1495).

Figure 3
The altered epigenome of senescent cells might promote age-associated increase in incidence of human cancers. Our analyses and previous studies and ideas lead to the following model: Senescent cells accumulate with age in human tissues. These senescent cells harbour low-level methylation of CpG islands of tumour suppressor genes that is insufficient to silence gene expression. However, even a limited/transient escape from senescence, for example due to genetic inactivation of *PTEN* confers additional rounds of cell division that permits proliferation-dependent spreading of DNA methylation. Hence, an initial 'seed' of methylation in a senescent cell in an aged tissue can, in conjunction with other genetic alterations and clonal selection, grow to full CpG island hypermethylation and tumour suppressor gene silencing, thereby facilitating progression to late-life cancer. Global hypomethylation in senescent cells may also promote genome instability and dysfunction (Cruickshanks *et al.*, Nat Cell Biol 2013; 15:1495).

To initiate studies in this area, we have analysed the HIRA histone chaperone complex. This complex, comprised of HIRA, UBN1 and CABIN1, collaborates with histone-binding protein ASF1a to incorporate histone variant H3.3 into chromatin in a DNA replication-independent manner. Consistent with this role in DNA replication-independent chromatin metabolism, we have previously implicated this chaperone in regulation of chromatin in non-proliferating cells. To better understand HIRA's function and mechanism, we integrated HIRA, UBN1, ASF1a and histone H3.3 ChIP-seq and gene expression analyses. Most HIRA-binding sites co-localise with UBN1, ASF1a and H3.3 at active promoters, and active and weak/poised enhancers. At promoters, binding of HIRA/UBN1/ASF1a correlates with the level of gene expression. HIRA is required for deposition of histone H3.3 at its binding sites. There are marked differences in nucleosome and co-regulator composition at different classes of HIRA-bound regulatory site. Underscoring this, we report novel physical interactions between the HIRA complex and transcription factors, a chromatin insulator and an ATP-dependent chromatin-remodelling complex. Our results map the distribution of the HIRA chaperone across the chromatin landscape and point to different interacting partners at functionally distinct regulatory sites (Pchelintsev *et al.*, Cell Rep 2013; 3: 1012).

These studies are being extended to an analysis of HIRA, histone H3.3 and other chromatin features in senescent cells. We have performed ChIP-seq and DNA methyl-seq of several histone modifications and DNA methylation in proliferating and senescent cells (e.g. Shah *et al.*, Genes Dev 2013; 27: 1787). These studies have revealed remarkable and paradoxical insights into chromatin in senescence, and the role of senescence as a tumour suppressor and its contribution to tissue ageing. Mechanistic hypotheses are being tested, and these analyses are being extended to studies of human and mouse aged and premalignant tissues to define the mechanism by which age-associated chromatin changes predispose to cancer.

Senescent cells appear 'epigenetically primed' to form cancer cells

Altered DNA methylation and associated destabilisation of genome integrity and function is a hallmark of cancer. Replicative senescence is a tumour suppressor process that imposes a limit on the proliferative potential of normal cells that all cancer cells must bypass. Here we show by whole-genome single-nucleotide bisulfite sequencing that replicative senescent human cells exhibit widespread DNA hypomethylation and focal hypermethylation. Hypomethylation occurs preferentially at gene-poor, late-replicating, lamin-associated domains and is linked to mis-localisation of the maintenance DNA methyltransferase (DNMT1) in cells approaching senescence. Low-level gains of methylation are enriched in CpG islands, including at genes whose methylation and silencing is thought to promote cancer. Gains and losses of methylation in replicative senescence are thus qualitatively similar to those in cancer (Fig. 2), and this 'reprogrammed' methylation landscape is largely retained when cells bypass senescence. Consequently, the DNA methylome of senescent cells might promote malignancy, if these cells escape the proliferative barrier. Since senescent cells appear to be imperfect tumour suppressors, at least from an epigenetic perspective, accumulation of senescent cells in aged tissues might contribute to increased incidence of cancer with age (Fig. 3) (Cruickshanks *et al.*, Nat Cell Biol 2013; 15:1495).

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MOLECULAR CONTROL OF EPITHELIAL POLARITY

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Cells of many tissues are polarised, collectively forming configurations tailored to the needs of a tissue. A common feature of most cancers of epithelial origin is the loss of normal tissue architecture. Despite advances in identifying signalling pathways and genetic alterations involved in driving cancer progression, little is known about this very basic, yet fundamental question: how are highly organised tissues turned into disarrayed tumours?

Our group focuses on how cell polarity is controlled in epithelial cells, particularly in prostate tumours. Our efforts are focused on two molecular pathways: the role of ARF GTPases (and their regulators and effectors, which we call the 'ARF regulome'), and the role of the cell surface protein, podocalyxin. Both molecules are highly overexpressed in metastatic prostate cancer tumours. Our lab focuses on molecular imaging studies to characterise how these molecules control normal and aberrant prostate epithelial polarisation. Our ultimate aim is to investigate these molecules as potential biomarkers of prostate cancer in patients and possible targets for future therapeutic intervention.

Three-dimensional culture models

Three-dimensional spheroid culture, where single epithelial cells are grown in extracellular matrix (ECM)-containing gels to form lumen-surrounding spheroids, can be used to model tissue organisation *in vitro* (Fig. 1). This basic polarised unit is reiterated to build the branched epithelial organs that make up many of the epithelial and endothelial tissues in our bodies (Fig. 2). Three-dimensional culture has received much attention in recent years as methods for making spheroids out of a variety of sources have become available, including from cell lines, stem cells and tumour cells from patients. Three-dimensional culture thus allows for formation and growth of 'mini-organs' from such sources. This presents an exciting new possibility to examine, in a rapid fashion, the factors that regulate tissue formation and its disruption in cancer.

Our group is developing systems for examining gene manipulation in spheroids in a medium-to-high throughput fashion, to model genetic changes seen in patients with metastatic cancer. This will allow us to rapidly determine the genetic changes that facilitate disruption to tissue organisation. Our future work aims to develop such systems for prostate, lung, breast and kidney spheroids.

GTPase regulation of cell polarity

Polarised epithelial cells are the basic building block of many organs. A typical polarised epithelium is composed of a highly organised mono- or bilayer of cells surrounding a single, central lumen. This organisation requires distinct plasma membrane domains, consisting of the apical surface facing the lumen and the basal-lateral side contacting neighbouring cells and the underlying ECM. Such organisation is often lost as cancer progresses, though the exact mechanisms as to why this is remain elusive. By using viral genetic tools to manipulate gene expression in spheroids, we have determined that specific membrane trafficking proteins of the Rab GTPase family are critically important for the normal polarisation of epithelial cells and spheroids. In contrast, specific members of the Arf GTPase family are involved in the disruption of polarity induced by oncogene expression. Notably, many of these genes are altered in metastatic tumours of prostate cancer patients. Our current studies are aimed at investigating how Arf and Rab GTPases regulate normal cell polarisation in spheroids, and how they participate in the acquisition of invasive behaviours when oncogenes are expressed.

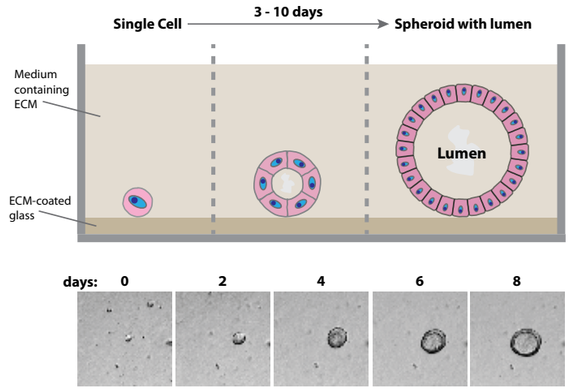

Figure 1

Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we are able to direct single cells to self-assemble into multicellular spheroid structures with a single, central lumen. This process occurs over a number (10) days, and allows us to study the dynamics of tissue formation.

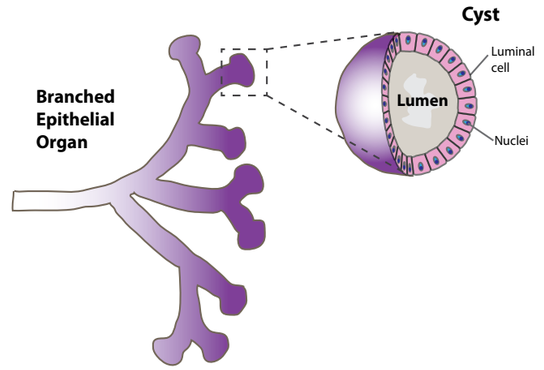

Figure 2

Figure 2
Three-dimensional cultures of cells to form cysts (also called spheroids) allow us to model the basic structure of epithelial organs in the laboratory. This allows us to understand and manipulate factors known to be altered in cancer patients and model in the laboratory what the effect would be on a tissue's organisation.

Knowing up from down: control of cell polarity orientation

A fundamental feature of an epithelial tissue is that the apical surface faces, and forms, the lumen. We recently uncovered a molecular switch that allows cells to sense the orientation of where their apical surfaces will be located, i.e. how to know up from down (Bryant *et al.*, Dev Cell 2014; 31: 171). This pathway involves cells sensing the ECM, whereby cells repress the localisation of the apical protein, podocalyxin at the ECM interface and redirect it to the proper luminal site. Notably, inhibition of ECM-derived signals causes the development of collective migration and invasion, led by the apical surface protein podocalyxin. This represents a completely novel, three-dimensional model of collective cell motility. Podocalyxin expression is highly amplified in prostate, breast and pancreatic tumours, and is mutated in familial forms of prostate cancer. The mechanisms of how podocalyxin contributes to cancer migration and invasion are largely unclear. Our future work aims to examine the molecular basis by which podocalyxin controls polarisation of spheroids, and potentially drives invasion and metastasis in prostate cancer.

We will be using proteomic approaches to understand the interacting network of proteins that regulate podocalyxin function, viral genetic techniques to manipulate its function and ultimately mouse models of podocalyxin loss. These studies are aimed at providing a detailed molecular dissection of podocalyxin function and its contribution to cancer progression *in vitro* and *in vivo*.

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CANCER METABOLOMICS

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Cancer is a disease of uncontrolled cell proliferation. To enable rapid growth, cancer cells need to alter their metabolism to generate the required energy and cellular building blocks. However, while trying to satisfy the metabolic demands of growth, cancer cells often face a limited supply of primary nutrients and oxygen, creating metabolic stress. Recent studies show that cancer cells can switch to consuming the abundant extracellular proteins and lipids to support growth in these stressful conditions. As these metabolic 'scavenging' pathways are tumour-specific we hypothesise that they are promising candidates for therapeutic intervention. Therefore, our primary aim is to elucidate the enzymes involved in scavenging, and their regulation. For this we combine metabolomics, lipidomics and proteomics approaches with sophisticated cancer models.

Acetate is a substrate for fatty acid synthesis in hypoxic cancer cells

Cell growth requires fatty acids for membrane synthesis. Fatty acids are assembled from two-carbon units in the form of acetyl-CoA (AcCoA). In nutrient and oxygen-replete conditions AcCoA is predominantly derived from glucose: pyruvate dehydrogenase produces AcCoA from glucose-derived pyruvate in the mitochondria, followed by ligation of the acetyl group to oxaloacetate to produce citrate. Citrate is then transported into the cytosol and cytosolic AcCoA produced by ATP citrate lyase. In hypoxic conditions (which frequently occur in tumours), however, most of the glucose-carbon is shunted toward lactate as its entry into the TCA cycle is blocked (Fig. 1). How this affects AcCoA and consequently fatty acid production in hypoxic cancer cells remains an area of active investigation. It has been postulated that reductive carboxylation of glutamine-derived α-ketoglutarate enables hypoxic cells to maintain citrate and AcCoA production. However, it was later noted that dropping citrate levels in hypoxic cells makes the α-ketoglutarate to citrate conversion more reversible. An alternative explanation of the extensive citrate and fatty acid labelling from glutamine in hypoxia is isotope exchange without a net contribution of carbon. By feeding hypoxic cancer cells ^{13}C-tracers and then analysing the fatty acids with mass spectrometry, we were able to identify a substantial contribution (20-50% of the AcCoA pool, depending on the cell line) from an unknown carbon source, i.e. not coming from glucose or glutamine (Kamphorst *et al.*, Cancer Metab 2014; 2: 23). Follow up work revealed only a minor contribution of non-glutamine amino acids and of fatty acids to acetyl-CoA in hypoxia. Instead, acetate is the major, previously unaccounted for, carbon donor. Thus, acetate assimilation is a route by which hypoxic cells can maintain lipogenesis and thus proliferation. Conversion of acetate to AcCoA in the cytosol is catalysed by the enzyme acetyl-CoA synthetase 2 (ACSS2), and complementary work by Eyal Gottlieb's group and others has shown that its inhibition does indeed have potent anti-growth effects *in vivo*.

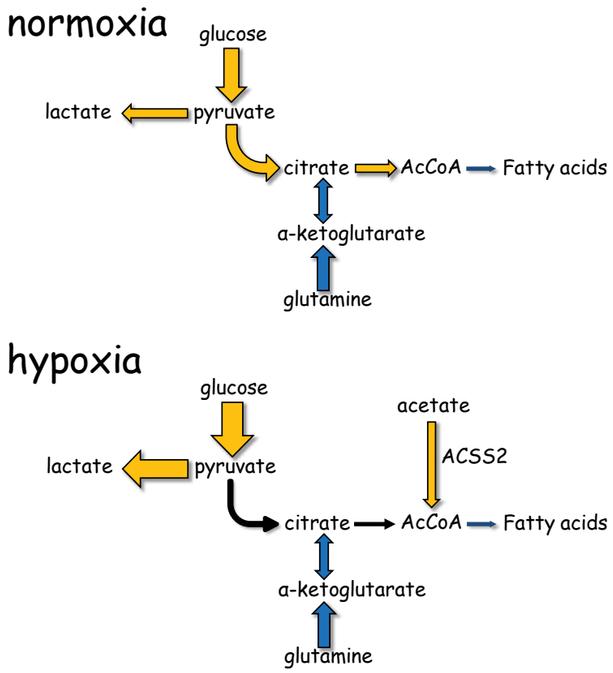

Figure 1
Acetate maintains lipogenesis in hypoxia. In normoxic conditions glucose is the primary carbon donor for acetyl-CoA production, the substrate for fatty acid synthesis. In a low oxygen (hypoxic) environment, which typically occurs in tumours, glucose is instead mainly shunted towards lactate, creating a lack of carbon to maintain fatty acid synthesis and hence growth. By performing stable isotope experiments, we showed that acetate contributes substantially to acetyl-CoA, and thus fatty acid, production in hypoxic conditions. Acetate assimilation may be an attractive target to inhibit the growth of hypoxic tumours.

Human pancreatic tumours are nutrient poor and tumour cells actively scavenge extracellular protein

We recently contributed to the discovery that a cellular process by which cells can internalise part of their microenvironment, called macropinocytosis, enables cancer cells to scavenge and degrade extracellular protein to support their metabolism (Commisso *et al.*, Nature 2013; 497: 633). Macropinocytosis is especially prominent in cell culture and *in vivo* models of pancreatic ductal adenocarcinoma (PDAC), a poorly vascularised, KRAS-driven malignancy. However, whether it actually occurred in human pancreatic tumours and to what degree it could support the amino acid requirements of pancreatic cancer cells, was still unknown. We therefore performed metabolomic comparisons of human PDAC and benign adjacent tissues, which revealed that tumour tissue was low in glucose, upper glycolytic intermediates, creatine phosphate and the amino acids glutamine and serine, two major metabolic substrates. Surprisingly, PDAC accumulated essential amino acids. Such accumulation could arise from extracellular proteins being degraded through macropinocytosis in quantities necessary to meet glutamine requirements, which in turn would produce an excess of most other amino acids. Entirely consistent with this, TMR-dextran (an established marker for macropinocytosis) assays performed on freshly resected human PDAC tissues demonstrated active macropinocytosis. When investigating the metabolic consequences of macropinocytosis further, we found that cultured murine PDAC cells can grow indefinitely in media lacking single essential amino acids, when cultured in the presence of physiological levels of albumin (Fig. 2). Moreover, PDAC cells were able to replicate in the total absence of free amino acids. That PDAC cells actually degrade extracellular protein to derive amino acids was further demonstrated using isotope-tracing experiments. Growth in amino acid-deficient medium supplemented with albumin was characterised by simultaneous glutamine depletion and essential amino acid accumulation, in accordance with the human tumour metabolomics results. Hence, protein scavenging through macropinocytosis occurs in human PDAC and is likely an important supply route for amino acids, when primary nutrients and oxygen supplies are low. This makes it an attractive target for therapeutic intervention.

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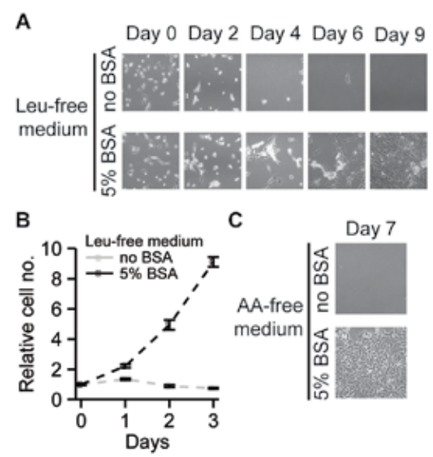

Figure 2
Pancreatic cancer cells can proliferate in medium lacking amino acids when supplemented with physiological levels of serum protein. (A) Mouse-derived pancreatic cancer cells were cultured in medium lacking the essential amino acid leucine and additionally with or without supplementation of additional 5% of bovine serum albumin (BSA). (B) Growth curve for the same conditions as (A). (C) The same cells were cultured in medium lacking all amino acids, with or without physiological (5%) BSA. For (B), data are means ± SE, n ≥ 3.

ONCOGENE-INDUCED VULNERABILITIES

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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 reorganise 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.

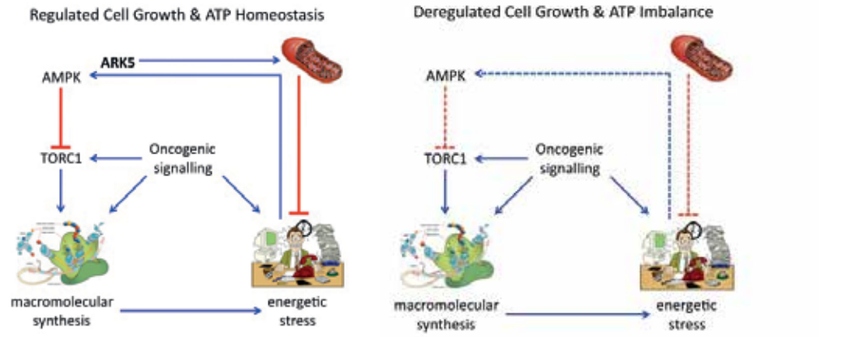
MYC in cancer

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 tumourigenesis 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.

Bim mediates Myc-induced apoptosis

Expression of supra-physiological levels of Myc triggers apoptosis in otherwise non-transformed cells, and many tumour cells can be induced to die upon forced expression of exogenous Myc. A large number of publications link Myc-driven apoptosis to induction of the tumour suppressor p19Arf, followed by accumulation of p53, however Myc can also trigger apoptosis in the absence of a functional p19-p53 pathway. Recent evidence has suggested that direct regulation of BH3 proteins presents an alternative pathway to Myc-induced apoptosis. The stoichiometric balance between pro-apoptotic and anti-apoptotic BH3 family proteins directly determines if a cell will undergo mitochondrial outer membrane permeabilisation (MOMP), widely considered to be a point of no return, in response to death signalling. In a study that we published this year in *Cell Reports*, we demonstrated a prominent role for the pro-apoptotic protein Bim during Myc-induced apoptosis in multiple settings. Acute overexpression of Myc in the intestine drives abundant apoptosis that is abrogated by deletion of Bim but unaffected by deletion of either Puma (BBC3) or p19Arf. Conversely, apoptosis induced by the DNA damaging agent doxorubicin requires Puma but not Bim. Strikingly, we found no requirement for p19Arf during Myc-induced apoptosis under any of the conditions tested, suggesting that the major tumour suppressive activity of p19Arf derives from a non-apoptotic function. Encouragingly, these observations suggest the possibility of augmenting Myc's pro-apoptotic signal, for instance through the use of BH3 mimetics, even in cancers that lack functional p53.

Figure 1
Induced dependencies need not reflect direct molecular interactions. Oncogene-induced cell growth, typically requiring signal transduction via the mechanistic target of rapamycin (mTOR) pathway, drives rampant ATP consumption that must be compensated for through increased cellular intake of fuel (e.g. glucose, amino and fatty acids) combined with AMPK-mediated attenuation of macromolecular synthesis. Upon suppression of Ark5, this feedback mechanism is impaired, leading to ATP depletion and bioenergetic catastrophe. In principle, any intervention that similarly impairs bioenergetic homeostasis may selectively kill tumour cells.



Myc-induced metabolic vulnerability

As part of a coordinated programme of cell growth required for cell division, Myc engages a number of biosynthetic programmes, prominently including ribosome assembly and protein translation, placing tremendous energetic demand upon the cell. In order to maintain energetic homeostasis, Myc upregulates glucose transporters and glycolytic enzymes, promoting the Warburg effect of limited glucose breakdown, and in parallel induces expression of glutamine transporters and exploits this pathway to maintain the citric acid cycle. The energetic strain that Myc deregulation thus places upon the cell is evident in progressive activation of the AMP-activated protein kinase AMPK, which plays a key role in maintaining energetic homeostasis. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with that of ATP production. For reasons that are presently unclear, an AMPK-related kinase, Ark5 is required for this homeostatic feedback mechanism under circumstances of Myc-driven energetic stress. Depletion of either AMPK or Ark5 thus leads to ATP collapse and consequently loss of viability, selectively in cells overexpressing Myc, suggesting that targeting these kinases may be therapeutically effective against Myc overexpressing cancers. We are using genetic models to investigate the therapeutic potential of targeting Ark5 in cancers that typically overexpress Myc, such as colorectal and pancreatic cancer.

Myc-induced lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for some 18% of all cancer-related deaths, and its incidence 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 mouse model for early lung cancer progression using tractable combinations of conditional alleles, including *Kras* and *Myc*. We have used laser capture micro-dissection combined with RNA-SEQ gene expression analysis to identify a cluster of genes whose expression increases with progression from low- to high-grade lung cancer, many of which are frequently amplified and/or overexpressed in human NSCLC. We are presently combining functional screening *in vitro* and *in vivo* to validate the top 50 progression-associated genes, with pharmacological approaches to investigate the potential therapeutic impact of suppressing specific pathways involved in progression to high-grade disease. This work aims to identify new candidates for targeted therapy as well as indicators/biomarkers of early disease progression.

As part of our efforts to further develop lung cancer research in Glasgow, we have established a Lung Cancer Research Steering Group in collaboration with Anthony Chalmers and a group of clinician scientists from Gartnavel and the Southern General Hospital, spanning disciplines of Radiation Oncology, Pulmonary Thoracoscopy and Pathology. The group currently meets four times a year to stimulate multidisciplinary discussion, collaboration and knowledge exchange, with multiple one-to-one interactions ongoing on an *ad hoc* basis.

Major developments in 2014

We published our first independent work since our arrival in Glasgow in 2012 and collaborated on two other manuscripts that are presently under review. Excellent progress was made in our analysis of lung cancer progression and the *in vivo* requirement for Ark5 for colorectal tumourigenesis. In collaboration with Owen Sansom and Jennifer Morton, we secured funding from Worldwide Cancer Research to investigate the functional role of Myc in pancreatic tumourigenesis. I served as co-organiser of the Beatson International Cancer Conference 'Powering the Cancer Machine' and co-authored a subsequent meeting report, published in *Cancer and Metabolism*, with Jurre Kamphorst.

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MITOCHONDRIA AND CELL DEATH

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[1] EMBO
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Cell death inhibits tumourigenesis at multiple stages, ranging from transformation to metastasis. Consequently, in order for cancer to develop, cell death must be inhibited. Moreover, the best way to treat a cancer cell is to kill it, indeed many cancer therapies do just this. Therefore, cell death sensitivity also affects how well anticancer therapy works. Mitochondria, the cellular organelles that power life, are also essential for the major form of programmed cell death called apoptosis. Our overall aim is to understand how mitochondria regulate cell death and define how this process is deregulated in cancer. The ultimate goal is to clinically translate these findings to improve existing anticancer therapies and develop new means to selectively kill cancer cells.

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 inter-membrane 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 anticancer therapies target these apoptotic blocks. For example, BH3 mimetic compounds exploit the Bcl-2 addiction of certain cancer cells leading to tumour specific killing.

Switchable Bcl-XL provides new insight into the commitment to die

We have developed a new approach that conditionally blocks mitochondrial permeabilisation and apoptosis. In this method, a destabilisation domain is fused to the N-terminus of Bcl-XL, resulting in its constitutive degradation. Addition of stabilising ligand effectively upregulates Bcl-XL expression within a matter of minutes, causing cellular resistance to MOMP and apoptosis. In collaboration with Frank Edlich (University of Freiburg), we find that switching on Bcl-XL expression leads to shuttling of Bak, a key pro-apoptotic effector protein, from the mitochondria into the cytoplasm. This demonstrates that so called retro-translocation is a conserved process for pro-apoptotic Bcl-2 proteins. Moreover, it highlights that enhancement of retro-translocation by anti-apoptotic Bcl-2 proteins provides an additional level of protection against cell death.

ATG12 connects autophagy and proteasome function to cell death

Autophagic degradation and proteasome-dependent degradation constitute the cell's two main protein degradation mechanisms. The ubiquitin-like protein ATG12 is an essential autophagy protein. In an ubiquitination-like cascade, ATG12 is covalently conjugated to ATG5 and the ATG12-5 conjugate is required for autophagosome biogenesis. While investigating the role of ATG12 in mitochondrial clearance, we found that the unconjugated, free form of ATG12 is rapidly degraded in a proteasome-dependent manner. Strikingly, ATG12, itself an ubiquitin-like protein, is directly targeted for ubiquitination and this promotes its proteasome-dependent degradation (Fig. 1). As a functional consequence of this turnover, ATG12 contributes to proteasome-mediated cell death - this may be clinically important given the use of proteasome inhibitors as anticancer therapies. Our data develop an emerging paradigm that ubiquitin-like proteins themselves can be directly modified by ubiquitin. Moreover, our results unveil a novel interconnection between autophagy, proteasome activity and cell death mediated by the ubiquitin-like properties of ATG12 (Fig. 2).

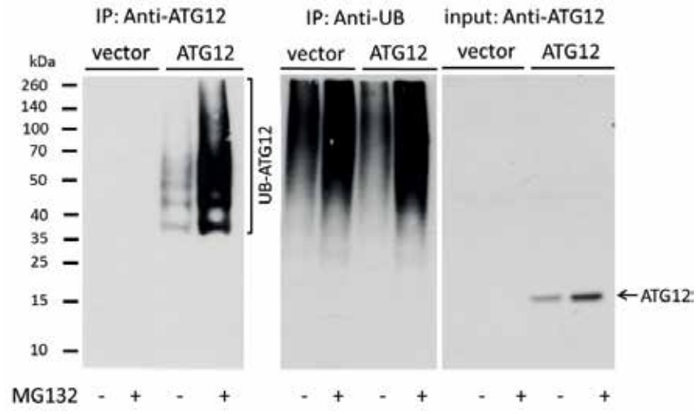

Figure 1
The ubiquitin-like protein ATG12 is itself targeted for ubiquitination. ATG12 was co-expressed with His-tagged ubiquitin in the presence or absence of proteasome inhibitor MG132. Left: ubiquitinated proteins were pulled down and blotted with anti-ATG12 antibody revealing an immunoreactive smear, demonstrating direct ATG12 ubiquitination. Right: cell lysates were probed with ubiquitin or ATG12 antibody showing an increase in ATG12 and polyubiquitination levels following proteasome inhibition.

Mitochondria and non-apoptotic routes to cancer cell death

Various other forms of programmed cell death exist besides apoptosis, including caspase-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. Mitochondria have been implicated as major effectors in necroptosis, a form of regulated cell death that requires activation of the kinase RIPK3. We have directly tested a role for mitochondria in necroptosis by generating mitochondria-free cells through Parkin-mediated mitophagy. Using this method, we find that the kinetics and extent of necroptosis are not affected by mitochondrial depletion, thereby ruling out a major role for mitochondria in executing necroptosis. Our current studies focus upon investigating whether mitochondrial dysfunction can serve to initiate necroptosis, in addition to defining the role for necroptosis in tumour suppression and therapy-induced cell death.

Publications listed on page 88

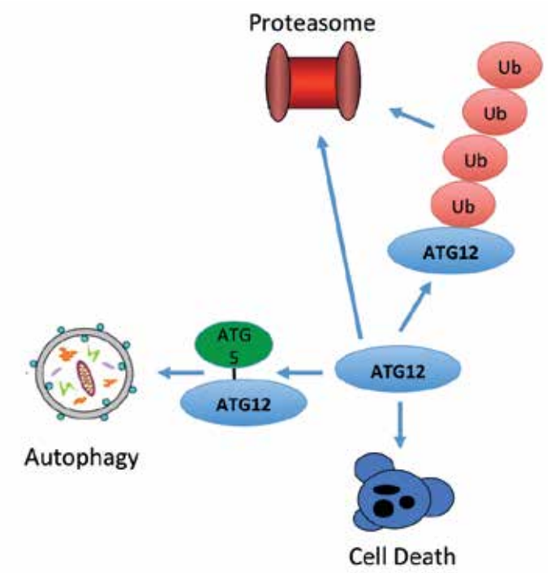

Figure 2
ATG12 connects autophagy and proteasome function to cell death. Conjugation of ATG12 to ATG5 is required for autophagy. Free ATG12 is rapidly degraded by the proteasome through ubiquitin-dependent and independent means. Stabilisation of ATG12, for example by proteasome inhibition, promotes cell death.

RESEARCH SUPPORT AND MANAGEMENT

CANCER RESEARCH UK BEATSON INSTITUTE

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, there has been investment in new equipment for Molecular Technology, which replaced its Illumina GAIIx next generation sequencer with an Illumina NextSeq500 platform. This will enable the sequencing of libraries at a lower cost with increased data output and a faster turnaround time. Building Facilities has been active with a number of projects to refurbish essential services and adapt space to accommodate additional staff.

Building Facilities

Alistair Wilson, Alex Kernahan, Michael Daly

Building Facilities manages the outsourced services provision for catering, cleaning and janitorial services. We provide maintenance support for the Beatson building and manage alterations and refurbishments. This year there have been two major projects to replace an ageing steam and chiller system in one of the services areas. In addition, the ventilation in the cryostore has been upgraded to increase the number of air changes in the room.

Use of the online helpdesk facility continues to be an effective means of logging reactive calls for maintenance and repair. Minor project work continues at a fairly high level. A number of building services have been altered or extended to accommodate new equipment. This year, fixed wire installation electrical testing was completed across the buildings.

Central Services

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

Central Services performs 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, Brenda McGuire, Fiona McGregor, Gemma Thomson, Mark Hughes, Saira Ghafoor, Vivienne Morrison, Wendy Lambie

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 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. If suitable material is available, mouse tissue microarrays (TMAs) can also be constructed using paraffin-embedded tissue.

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 DNA/RNA investigation, PCR analysis and immunofluorescence staining can also be sectioned from both paraffin-embedded material and frozen tissue.

A comprehensive immunohistochemistry service is offered 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 can understand the rationale and techniques available to allow them to perform the staining to an acceptable and consistent standard.

Where there is no antibody available for immunohistochemistry analysis or a more specific conclusive technique is required, the service can provide an *in situ* hybridisation technique using a reagent system designed to visualise cellular RNA targets in formalin-fixed, paraffin-embedded tissue sections using bright-field microscopy. Specific probes can be purchased or designed to exact specifications by external companies such as Advanced Cell Diagnostics along with the necessary retrieval and amplification kits, allowing the *in situ* technique to be performed.

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. Both DNA and RNA material can be retrieved from the tissue sections for downstream analysis.

A fully automated large capacity Leica SCN400F slide scanner has been installed in the facility, which is capable of capturing bright-field or fluorescent images. This allows high quality digital images to be scanned, stored and, if required, quantitative interpretation performed. The image analysis software allows staining techniques to be scored using algorithms designed specifically for that staining, using the researchers input to designate what specific areas are to be scored.

Information Services

Peter McHardy, Iain White

Information Services provides 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 400 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 500 TB of online storage with nightly backups and tapes stored off-site, to provide support for microscopy, DNA sequencing and mass spectrometry data.

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 Yosemite is being rolled out across the site and we have completed the upgrade of relevant Windows computers to Windows 7. All e-mail services run on Microsoft Exchange, which allows 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 continue to migrate over as many physical servers as possible to virtual servers using VMware®. We provide access to virtualised servers for research groups allowing them greater flexibility for both 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 spectrometry analysis or other computationally intense applications. We currently provide virtual desktops for OS X users requiring access to Windows-based packages.

Significant investment has been put into providing documentation and procedures to allow us to run the service in a manner commensurate with ITIL. This has been used as the foundation of our business continuity documentation and has led to the revision and ongoing improvement of many of our day-to-day working practices.

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

Laura Bence, Richard Selkirk, Michael McTaggart, Joe McFadden, George Monteith

Laboratory Management is responsible for providing a number of vital support roles to the Institute. This includes the provision of advice, training and information to all staff on health and safety issues, especially with regard to risk assessments and appropriate control measures necessary for laboratory work involving biological, chemical and genetic modification processes. Safety in regard to fire risk is also managed. As safety plays an important part of everyday life in the laboratory, and in running building services, it is essential that health and safety processes are reviewed and monitored regularly, that any training needs are rectified and that adequate provision is made to fulfil the Institute's legal obligations to staff. All staff and students are required to attend a safety update seminar once a year and new starts attend a series of safety induction talks. This year some of the first aiders were trained in oxygen administration and a defibrillator will be acquired soon for use within the Institute. In addition, a new risk assessment form has been created to include the new harmonised hazard codes.

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. The servicing and maintenance of core equipment and any systems that these require, such as carbon dioxide or nitrogen gas, is carefully managed and coordinated to ensure equipment breakdowns are kept to a minimum. Any equipment repairs are coordinated to ensure these are dealt with as efficiently and effectively as possible. Service contracts for core equipment are reviewed and procured centrally to ensure costs are kept as low as reasonably practical. 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 those 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 outgoing samples or materials are processed by stores for courier collection. We continue to review the services provided by stores to improve what is offered to scientific staff. This includes negotiating free samples from suppliers to enable the scientific staff to assess new or alternative products. By maintaining a good relationship with suppliers preferential pricing is obtained and, 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

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 hours. This year, we replaced our Illumina GAIIx with an Illumina NextSeq500 platform. This will enable us to sequence libraries at a lower cost with increased data output and a faster turnaround time. We also offer library production for next generation sequencing. Protocols currently in use 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 Promega Geneprint 10 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 Mediaclave (Integra Biosciences AG) to sterilise and dispense into the plates.

RESEARCH PUBLICATIONS

Kurt Anderson (page 26)
Tumour Cell Migration

Primary Research Papers

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Targeting mTOR dependency in pancreatic cancer. ***Gut*** 2014; 63: 1481-9

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HIRA orchestrates a dynamic chromatin landscape in senescence and is required for suppression of neoplasia. ***Genes Dev*** 2014; 28: 2712-25

Karen Blyth (page 58)
Transgenic Models of Cancer

Primary Research Papers

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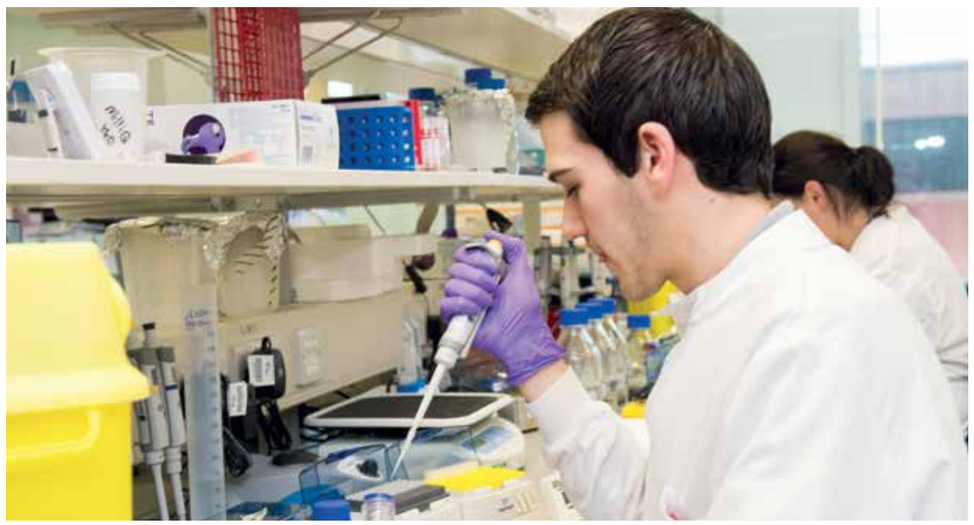

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Martin Drysdale (page 48)
Drug Discovery Programme

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Jeff Evans (page 28)
Translational Cancer Therapeutics

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Eyal Gottlieb (page 12)

Tumour Metabolism

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Robert Insall (page 30)

Cell Migration and Chemotaxis

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Shehab Ismail (page 32)

Structural Biology of Cilia

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Hing Leung (page 16)

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Karen Vousden (page 22)
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Epigenetics of Cancer and Ageing

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Rai TS, Cole JJ, Nelson DM, Dikovskaya D, Faller W, Vizioli MG, Hewitt RN, Anannya O, McBryan T, Manoharan I, van Tuyn J, Morrice N, Pchelintsev NA, Ivanov A, Brock C, Drotar M, Nixon C, Clark W, Sansom OJ, Anderson KI, King A, Blyth K, Adams PD. HIRA orchestrates a dynamic chromatin landscape in senescence and is required for suppression of neoplasia. ***Genes Dev*** 2014; 28: 2712-25

Soni S, Pchelintsev N, Adams PD, Bieker JJ. Transcription factor EKLF (KLF1) recruitment of the histone chaperone HIRA is essential for beta-globin gene expression. ***Proc Natl Acad Sci U S A*** 2014; 111: 13337-42

Other publications

Adams P, Antebi A, Cuervo AM, Kennedy B, Sedivy J. Aging Cell: open access to all. ***Aging Cell*** 2014; 13: 1

David Bryant (page 64)

Molecular Control of Epithelial Polarity

Primary Research Papers

Bryant DM, Roignot J, Datta A, Overeem AW, Kim M, Yu W, Peng X, Eastburn DJ, Ewald AJ, Werb Z, Mostov KE. A molecular switch for the orientation of epithelial cell polarization. ***Dev Cell*** 2014; 31: 171-87

Tran CS, Eran Y, Ruch TR, Bryant DM, Datta A, Brakeman P, Kierbel A, Wittmann T, Metzger RJ, Mostov KE, Engel JN. Host cell polarity proteins participate in innate immunity to Pseudomonas aeruginosa infection. ***Cell Host Microbe*** 2014; 15: 636-43

Jurre Kamphorst (page 66)

Cancer Metabolomics

Primary Research Papers

Fan J, Ye J, Kamphorst JJ, Shlomi T, Thompson CB, Rabinowitz JD. Quantitative flux analysis reveals folate-dependent NADPH production. ***Nature*** 2014; 510: 298-302

Kamphorst JJ, Chung MK, Fan J, Rabinowitz JD. Quantitative analysis of acetyl-CoA production in hypoxic cancer cells reveals substantial contribution from acetate. ***Cancer Metab*** 2014; 2: 23

Other Publications

Kamphorst JJ, Murphy DJ. The Beatson International Cancer Conference: Powering the Cancer Machine. ***Cancer Metab*** 2014; 2: 25

Daniel J. Murphy (page 68)

Oncogene-Induced Vulnerabilities

Primary Research Papers

Muthalagu N, Junttila MR, Wiese KE, Wolf E, Morton J, Bauer B, Evan GI, Eilers M, Murphy DJ. BIM is the primary mediator of MYC-induced apoptosis in multiple solid tissues. ***Cell Rep*** 2014; 8: 1347-53

Other Publications

Kamphorst JJ, Murphy DJ. The Beatson International Cancer Conference: Powering the Cancer Machine. ***Cancer Metab*** 2014; 2: 25

Stephen Tait (page 70)

Mitochondria and Cell Death

Primary Research Papers

Baudot AD, Haller M, Mrschtik M, Tait SW, Ryan KM. Using enhanced-mitophagy to measure autophagic flux. ***Methods*** 2015; 75: 105-11. doi: 10.1016/j.ymeth.2014.11.014. Epub 2014 Dec 9

Haller M, Hock AK, Giampazolias E, Oberst A, Green DR, Debnath J, Ryan KM, Vousden KH, Tait SWG. Ubiquitination and proteasomal degradation of ATG12 regulates its pro-apoptotic activity, ***Autophagy*** 2014; 10: 2269-78

Orozco S, Yatim N, Werner MR, Tran H, Gunja SY, Tait SW, Albert ML, Green DR, Oberst A. RIPK1 both positively and negatively regulates RIPK3 oligomerization and necroptosis. ***Cell Death Differ*** 2014; 21: 1511-21

Todt F, Cakir Z, Reichenbach F, Emschermann F, Lauterwasser J, Kaiser A, Ichim G, Tait SWG, Frank S, Langer HF, Edlich F. Differential retrotranslocation of mitochondrial Bax and Bak. ***EMBO J*** 2015; 34: 67-80. doi: 10.15252/embj.201488806. Epub 2014 Nov 5

Other Publications

Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, Alnemri ES, Altucci L, Andrews D, Annicchiarico-Petruzzelli M, Baehrecke EH, Bazan NG, Bertrand MJ, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Bredesen DE, Brenner C, Campanella M, Candi E, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, Di Daniele N, Dixit VM, Dynlacht BD, El-Deiry WS, Fimia GM, Flavell RA, Fulda S, Garrido C, Gougeon ML, Green DR, Gronemeyer H, Hajnoczky G, Hardwick JM, Hengartner MO, Ichijo H, Joseph B, Jost PJ, Kaufmann T, Kepp O, Klionsky DJ, Knight RA, Kumar S, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, Lopez-Otin C, Lugli E, Madeo F, Malorni W, Marine JC, Martin SJ, Martinou JC, Medema JP, Meier P, Melino S, Mizushima N, Moll U, Munoz-Pinedo C, Nunez G, Oberst A, Panaretakis T, Penninger JM, Peter ME, Piacentini M, Pinton P, Prehn JH, Puthalakath H, Rabinovich GA, Ravichandran KS, Rizzuto R, Rodrigues CM, Rubinsztein DC, Rudel T, Shi Y, Simon HU, Stockwell BR, Szabadkai G, Tait SW, Tang HL, Tavernarakis N, Tsujimoto Y, Vanden Berghe T, Vandenabeele P, Villunger A, Wagner EF, Walczak H, White E, Wood WG, Yuan J, Zakeri Z, Zhivotovsky B, Melino G, Kroemer G. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. ***Cell Death Differ*** 2015; 22: 58-73. doi: 10.1038/cdd.2014.137. Epub 2014 Sep 19

Ichim G, Tait SW. Necroptosis: fifty shades of RIPKs. ***Mol Cell Oncol*** 2015; 2. doi: 10.4161/23723548.2014.965638. Epub 2014 Nov 7

Lopez J, Tait SW. Killing the Killer: PARC/CUL9 promotes cell survival by destroying cytochrome C. ***Sci Signal*** 2014; 7: pe17

Tait SW, Ichim G, Green DR. Die another way - non-apoptotic mechanisms of cell death. ***J Cell Sci*** 2014; 127: 2135-44

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 Institute. 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 Cassie Clarke from Jim Norman's group. Cassie is studying the role of RNA polymerase III in extracellular matrix remodelling and cell migration.

Theses

Chaneton, Barbara (2014) Targeting cancer cell metabolism as a therapeutic strategy [PhD thesis, University of Glasgow, Beatson Institute]

Cuesta Garcia, Nerea (2013) P53 loss and KRAS mutation in an invasive murine model of colorectal cancer [MRes thesis, University of Glasgow, Beatson Institute]

Davidson, Andrew (2014) The role of WASP family members in Dictyostelium discoideum cell migration [PhD thesis, University of Glasgow, Beatson Institute]

Foth, Mona (2014) The role of FGFR3 mutation in tumour initiation, progression and invasion of urothelial cell carcinoma in mice [PhD thesis, University of Glasgow]

Leach, Joshua (2014) Characterising the tumour microenvironment in pancreatic cancer and the changes elicited by targeted therapies [MRes thesis, University of Glasgow, Beatson Institute]

Liu, Emma Yu (2014) Characterisation of the role of autophagy in DNA damage repair [PhD thesis, University of Glasgow, Beatson Institute]

Manoharan, Indrani (2014) Identification and characterization of novel histone modifications during cellular senescence [PhD thesis, University of Glasgow, Institute of Cancer Sciences]

Rud-Majani, Zahra Erami (2014) Investigation of E-cadherin dynamics in cancer cell adhesion and metastasis [PhD thesis, University of Glasgow, Beatson Institute]

Steele, Colin (2014) Investigating the role of CXCR2 signalling in pancreatic inflammation and cancer [PhD thesis, University of Glasgow, Beatson Institute]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Powering the Cancer Machine
6 - 9 July 2014
Bute Hall, University of Glasgow
Scientific Committee: Jim Norman, Eyal Gottlieb, Daniel Murphy, Karen Vousden

This year's conference focused on cancer metabolism, an increasing important area of research for the Beatson as well as for the wider cancer research community. The participants heard from a range of excellent speakers including David Sabatini (Cambridge, MA) who gave a comprehensive overview of what is known about the regulation and functions of the mTOR pathway in the Colin Thomson Memorial Keynote Lecture on the first evening. This and the preceding opening session were sponsored by Worldwide Cancer Research (WCR, formerly known as AICR). Selected short talks were also given by Julianna Blagih, Maria José Bueno, Brooke Emerling, Sarah-Maria Fendt, Maria Georgiadou, Hannah Johnston, Patricia Sancho, Darren Saunders, Saverio Tardito, Caroline Treins (sponsored by WCR) and Hans-Guido Wendel (sponsored by Boehringer Ingelheim). Caroline Treins (Paris) won the Portland Press sponsored prize for her talk describing the identification of novel molecular targets of S6Ks, while Katarzyna Grzes (Dundee) was awarded the AMSBIO-Trevigen sponsored poster prize for her work investigating metabolic regulation in PTEN null T lymphoma cells. The meeting was generously co-sponsored by Cancer Research UK and Worldwide Cancer Research. A report of the meeting was published in *Cancer & Metabolism* (Kamphorst & Murphy. Cancer Metab 2014; 2: 25).

The 2015 meeting will highlight recent exciting research into the molecular and cellular events that contribute to loss of epithelial polarity during carcinogenesis, and how cancer cells acquire different types of polarity that enable them to migrate and invade. The development of agents to target cells with aberrant polarity as a potential route to treatment of metastatic disease will also be discussed (see www.beatson.gla.ac.uk/conf for more details and to register).

In addition, we will be hosting a workshop entitled 'Next Generation Cancer Biology' in September, which will focus on the growing role of systems analysis and data models in modern bioscience research.

Fluorescence Lifetime and FRET Microscopy Workshop

3 & 4 September 2014
CRUK Beatson Institute

This meeting, sponsored by Nikon UK and the Royal Microscopical Society, consisted of presentations by invited speaker Simon Ameer-Beg (Kings College London), head of the Beatson Advanced Imaging Resource Kurt Anderson, Nikon Microscopy UK, LaVision Biotec and Lambert Instruments along with a series of practical hands-on workshops and demonstrations on the use of FLIM-FRET.

Postdoc Meeting

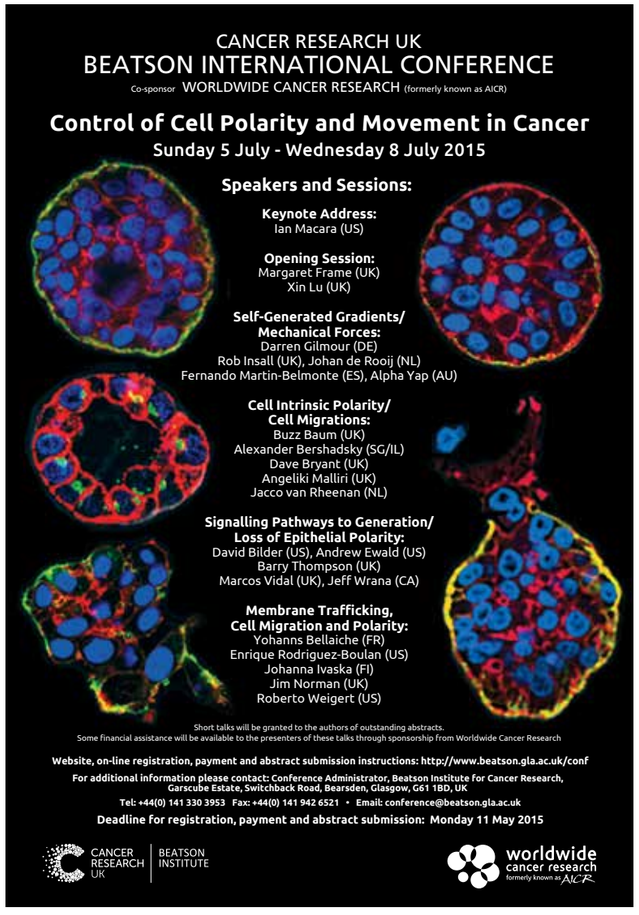
Visualizing Cancer: Microscopy and Beyond
12 September 2014
CRUK Beatson Institute
Organisers: Amelie Juin (Beatson), Martin Lee (Edinburgh), Daniel Soong (Edinburgh), Haoran Tang (Manchester), Ben Tyrrell (Beatson)

In September, our postdocs, along with a number from Edinburgh and Manchester, hosted a one-day meeting at the Institute with funding from the American Society for Cell Biology. This included talks and poster presentations by postdocs and students as well as keynote lectures by Jeff Pollard (University of Edinburgh) and Erik Sahai (London Research Institute). Following the success of this meeting, our postdocs hope to follow up with another meeting next year.

Open Evening

Our opening evening, held in March during National Science and Engineering Week, was again very well attended. There were talks, given by Stephen Tait, Emma Woodham, Colin Steele and Anthony Chalmers, covering topics that ranged from studying cell death and cell migration to treating pancreatic cancer and brain tumours. These were followed a series of lab tours and demos by our hardworking volunteers.

Poster for 2015 conference



SEMINARS AT THE BEATSON INSTITUTE

The following seminars were held at the CRUK Beatson Institute during 2014.

January

Brian Stramer, Randall Division of Cell and Molecular Biophysics, King's College London

Robert Kay, MRC Laboratory of Molecular Biology, University of Cambridge

Martin Humphries, Faculty of Life Sciences, University of Manchester

Lena Claesson-Welsh, Department of Immunology, Genetics and Pathology, Uppsala University, Sweden

Thomas Radimerski, Novartis, Switzerland

February

Nitzan Rosenfeld, Cancer Research UK Cambridge Institute

March

Michael Lisanti, Breakthrough Breast Cancer Research Unit, University of Manchester

Robert Gillies, Chair, Cancer Imaging and Metabolism, Moffitt Cancer Centre, Tampa, FL, USA

Andrew Renault, School of Life Sciences, University of Nottingham

Martin Schwartz, R.W. Berliner Professor of Medicine and Cell Biology, Yale School of Medicine, New Haven, CT, USA

James Bear, Department of Cell Biology and Physiology, Lineberger Comprehensive Cancer Center, NC, USA

Federica Maione, Institute for Cancer Research, Candiolo, Turin, Italy

April

Rob Liskamp, School of Chemistry, University of Glasgow

Thomas Tuetling, University of Bonn, Germany

Tor Henrik Semb, The Danish Center for Stem Cell Research, Denmark

Bruno Galy, European Molecular Biology Laboratory, Heidelberg, Germany

Alberto Signore, Nuclear Medicine Unit, 'Sapienza' University of Rome, Italy

May

Eytan Ruppin, School of Computer Sciences & School of Medicine, Tel Aviv University, Israel

Joel Leverson, Abbvie Pharmaceuticals, Chicago, IL, USA

Oliver Bischof, CNRS-CR1, Institut Pasteur, Paris

Peter Vandenabeele, Flanders Institute for Biotechnology, Belgium

June

Vassiliki Kostourou, Biomedical Sciences Research Centre 'Al. Fleming' Vari, Attiki, Greece

Lorraine O'Reilly, The Walter and Eliza Hall Institute (WEHI), Melbourne, Australia

Andreas Strasser, Walter and Eliza Hall Institute, Melbourne, Australia

July

Helfrid Hochegger, Genome Damage and Stability Centre, University of Sussex

Jason Carroll, Cancer Research UK Cambridge Institute

September

Andrea Musacchio, Max Planck Institute of Molecular Physiology, Dortmund, Germany

Markus Rehm, Royal College of Surgeons, Ireland

October

Michael Overholtzer, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Mathew Coleman, School of Cancer Sciences, University of Birmingham

Antony Braithwaite, Otago University, New Zealand

Bader Al-Anzi, California Institute of Technology, Pasadena, CA, USA

Darren Gilmour, Cell Biology and Biophysics Unit at EMBL, Heidelberg, Germany

November

Christopher Kemp, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Alex Gould, MRC National Institute for Medical Research, London

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 postdocs 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. Postdocs 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
Jacqui Clare, Karen Connor, Nicki Kolliatsas, Elaine Marshall, Frank McGeoghegan, Gary Niven, Lynn Wilson, Patricia Wylie

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 Directors.

Secretarial

Sheila McNeill *(PA to Professor Vousden), Catriona Entwistle, Rebecca Gebbie, Barbara Laing, 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 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, *Catherine Winchester*

The Scientific Administration team 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. The team also assists researchers in identifying and applying for external grant funding and has a role in providing oversight , training and advice in all aspects of research integrity.

Cancer Research Technology

Maria Lopalco

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 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. 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
Bruker, Technology Strategy Board, Novartis

Karen Blyth
Breast Cancer Campaign, Royal Society

Martin Drysdale
Medical Research Council

Jeff Evans
Scottish Executive - Chief Scientist Office, Medical Research Council, Pancreatic Cancer Research Fund

Eyal Gottlieb
AIRC (Italian Association for Cancer Research), FEBS, Janssen Pharmaceutica NV, Metabomed

Hing Leung
Academy of Medical Sciences, Medical Research Council, Prostate Cancer Charity

Laura Machesky
Danish Council, Medical Research Council

Jim Norman
Breast Cancer Campaign)

Michael Olson
Breast Cancer Campaign, Medical Research Council, Worldwide Cancer Research

Kevin Ryan
Astellas Pharma Inc, EMBO, Worldwide Cancer Research

Owen Sansom
Bioven, European Community, Institute of Cancer Research, Janssen Pharmaceutica NV, Medical Research Council, Novartis, Royal Commission for the Exhibition of 1851, Wellcome Trust, Worldwide Cancer Research

Douglas Strathdee
Barth Syndrome Foundation

Karen Vousden
Astex, European Community, NHS Greater Glasgow & Clyde Health Board Endowment Fund, West of Scotland Women's Bowling Association, Worldwide Cancer Research

Beatson Associates

Peter Adams
BBSRC, Medical Research Council, NIH with National Institute on Aging & National Cancer Institute (USA)

Daniel Murphy
British Lung Foundation, European Community, Merck Sharp & Dohme, Worldwide Cancer Research

Stephen Tait
ARC, BBSRC, EMBO, 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.

- Aggreko UK Ltd
- Balfour & Manson LLP
- S3 and S5 pupils of Bellahouston Academy
- Biogenes GmbH
- Bioline Reagents
- Margaret G Brown
- Buchanan Castle Golf Club
- Brian Burns
- Cambridge Bioscience Ltd
- Elizabeth M Campbell, in memory of Mr Alasdair Campbell
- Jane Carmichael, in memory of Miss Elizabeth (Bunty) Cameron
- Cathcart Castle Golf Club Ladies Section
- Citywire
- Clyde Travel
- Legacy from the Estate of the Late Mrs Sarah Colman
- Ann P Coulson, in memory of Bunty Cameron
- Irene Craig
- J Allan Denholm, in memory of the Late Mr Jim Colraine
- Edrington
- Fisher Scientific UK
- May Gow, Rozelle House, Ayr
- Avril Haddow
- B Herbert, in memory of Miss Elizabeth (Bunty) Cameron
- Hodder & Stoughton Ltd (Hachette), re Royalties
- Hyndland Parish Church
- The James Inglis Trust
- Sameena Kelly, Glasgow Kelvin College
- David Kerr
- Kaitlin Kimmins, Glasgow Kelvin College
- Kings Park Parish Church, Ladies Badminton Club
- Janetta Helen Kinloch, in memory of Bunty Cameron
- Lanarkshire Women's Bowling Association
- Agnes MacDonald
- Marks & Clerk
- Legacy from the Estate of the Late Mr & Mrs Finlay McCulloch
- Helen McCulloch
- Christina McDougall, in memory of her husband John
- Liz McGinniss
- Fiona McNeill and family
- McQuarrie, in memory of their daughter Rhoda
- Legacy from the Estate of the Late Mrs Jean Merry
- Legacy from the Estate of the Late Mrs Agnes Middleton
- B Millar
- Jim Millar
- Legacy from the Estate of the Late Mrs Helen S M Mitchell
- MOD Police, RNAD Coulport
- Mosshead Primary School
- North View Housing Association, Castlemilk Family Day
- Anne O'Hare, Hillpark Bowling Club
- Dr & Mrs J D Olav Kerr's Charitable Trust
- Sarah Percy and Irene Kennedy
- The Worthy Matron, Worthy Patron, Officebearers and Members, Order Of The Eastern Star - Lily of the Valley
- Mabel M Reid, in memory of her friend Bunty Cameron

Laraine Kernahan visited Clyde Travel, Hillington to be presented with a cheque for over £5000

- In memory of Andy Robertson, from family and friends
- Sarstedt Ltd
- Scullion
- Eileen Smillie
- St Andrew of Glasgow Royal Arch Chapter No.69
- The Staypar Charitable Trust, in memory of the Late Gail Wylie
- St Isidore's Catholic Church
- Members, friends and family of St Rollox Bowling Club
- St Vincent Bowling Club
- George Taggart, on behalf of Mr & Mrs Ian •
- McDonald's Golden Wedding Anniversary
- John Teevan, in memory of their late mother
- Thermo Fisher
- Thornhill Gardening Society
- Vivienne Triseliotis, in memory of Bunty Cameron
- Peter Vardy Foundation
- VWR
- J Walker
- West of Scotland Women's Bowling Association
- Frank Wilson
- Legacy from the Estate of the Late Mr William Cluggie Wilson

PATRONS AND BOARD OF GOVERNORS

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Board of Directors

The Beatson Institute is an autonomous charity, constituted as a company limited by guarantee, registered in Scotland. The Institute is governed by its Board of Directors who are the directors of the company and trustees of the charity. The Board is ultimately responsible for all aspects of the Institute, including its scientific strategy, operational policies, regulatory compliance and financial stewardship and accountability. On a day-to-day basis, many of these responsibilities are delegated to the Institute's Management Team.

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Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).
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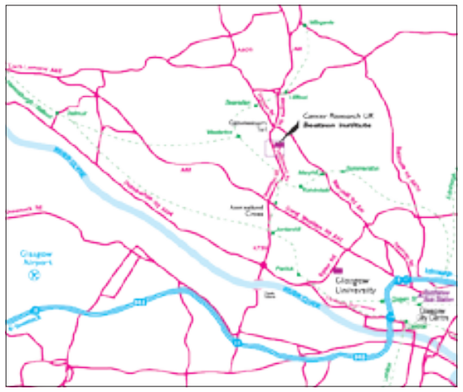

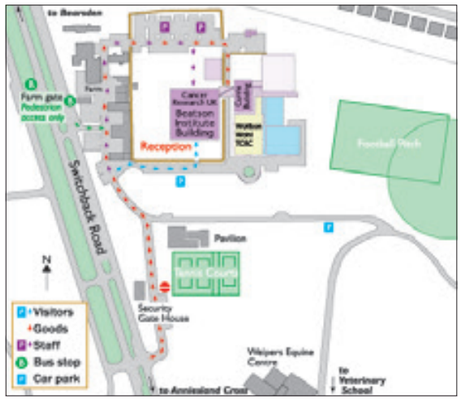

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