SCIENTIFIC REPORT 2015

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Cover image
Duncan McArthur, Chemistry Team Leader within our Drug Discovery Programme.

SCIENTIFIC REPORT 2015

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Institute building

CONTENTS

INTRODUCTION

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

A number of key appointments in recent years mean that the Institute is now at almost full capacity and able to build fully on its considerable strengths. The success of our researchers has continued apace this year, with a number of groups having outstanding quinquennial and tenure reviews as well as being awarded significant grant funding.

Owen Sansom and **Jeff Evans**' QQRs were held in June, the outcomes of which were extremely positive. **Danny Huang** was also considered for promotion to Senior Group Leader in March and was unanimously recommended by the panel of external experts. The review panel was overwhelmingly supportive and subsequently, Danny was promoted to Professor at the University of Glasgow. This year, we held our first advisory group meeting for the Drug Discovery Unit, head by **Martin Drysdale**, and are planning to hold regular follow up meetings. The external experts were very positive about the programme and were able to offer some very useful feedback on current projects.

All of the Beatson faculty are now working hard to attract grant funding from various sources to provide additional support for our work. There were a number of large programme awards, including an ERC Consolidator grant to **Danny Huang** and a CRUK Programme Foundation Award to one of our Beatson Associates, **Stephen Tait**. Our group leaders continue to build important cross-disciplinary collaborations reflected by **Robert Insall** being awarded, along with John Mackenzie (Mathematics Department, University of Strathclyde), a CRUK Multidisciplinary Award and **Martin Drysdale** being a successful co-applicant on an MRC Biomedical Catalyst DPFS Project grant with a group at the University of Oxford. Huge thanks must also go to our many supporters who both remember us in the form of legacies and work so hard to raise funds for us.

We also continue to contribute to the CRUK Glasgow Centre, which is establishing the infrastructure necessary to allow each of the Centre's cancer teams to develop a precision medicine programme that will ultimately help guide the treatment of patients on a much more individual basis. The Institute, in particular, has had considerable input into the areas of pre-clinical trials, histology and high-throughput screens.

This year, I was delighted to be elected to the Academia Europaea, while one of our former, very talented clinical research fellows, **Mathias Rosenfeldt** was awarded the Bellahouston Medal by the University of Glasgow for his PhD thesis. Our postdocs continue to win fellowships with **Gabriel Ichim** receiving a prestigious EMBO Advanced Fellowship and **Rene Jackstadt** a Marie Sklodowska Curie Fellowship, while postdoc **Oliver Maddocks** moved across to the University of Glasgow with a CRUK Career Development Fellowship to establish his own independent research group.

In May, we said farewell to **Caroline Preacher**, our HR manager who retired from the Institute. Meanwhile, **Angela Stuart** has joined us to take on this important role.

New HR Manager, Angela Stuart

We were delighted to welcome experts on cell polarity to our conference in July. The conference, led by **Jim Norman**, was a great success and allowed us to introduce the participants to Glasgow and the Beatson Institute, and all they have to offer.

Professor Marcos Vidal, 1974-2016

Ahead of this annual report going to print, very early in January 2016, we received the dreadful news of the death of our dear friend and colleague Professor Marcos Vidal.

Since establishing his research group here at the Beatson in 2009, Marcos developed an outstanding programme of work focused on tumour-host interactions in the fruit fly. His publications include important studies on the critical role of Ras in the response of epithelia cells to TNF (*Developmental Cell* 2010), the role of TNF/Eiger in tumourigenesis (*Cell Reports* 2014) and the regulation of intestinal cell stem proliferation by Bursicon/Lgr2 (*Current Biology* 2014).

During the last few years, Marcos also became widely acknowledged as the leading expert in using *Drosophila* as a model to study cancer. He was always very willing to share this knowledge and expertise and, as well as being a highly valued colleague to everyone here at the Institute, he was also a very active member of the wider *Drosophila* community, contributing to numerous meetings. In addition, he was a member of the Royal Society of Scotland Young Academy of Scotland and was active in teaching undergraduates at both the Universities of Glasgow and Edinburgh.

In recognition of his work and standing in the field, in July 2015 Marcos was unanimously recommended for promotion to Senior Group Leader by a panel of external experts, and was also subsequently promoted to Professor at the University of Glasgow.

Marcos was an extraordinarily talented and creative scientist, who had already made groundbreaking contributions to the field. He will be hugely missed by everyone here both as a mentor and colleague but also as a friend.

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

Buetow L, Gabrielsen M, Anthony NG, Dou H, Patel A, Aitkenhead H, Sibbet GJ, Smith BO, Huang DT.
Activation of a primed RING E3-E2-ubiquitin complex by non-covalent ubiquitin. ***Mol Cell*** 2015; 58: 297-310

Ubiquitin modification of proteins within cells is a crucial process, which involves RING E3 ubiquitin ligases and ubiquitin conjugated E2. In this structural biology study, the authors focus on the binding of ubiquitin to the backside of E2, which is known to be important for polyubiquitin chain formation. Significantly, they find that backside bound ubiquitin enhances donor ubiquitin transfer, particularly in the presence of RING E3, and does this by stabilising the RING E3-E2-ubiquitin complex.

Cameron JM, Gabrielsen M, Chim YH, Munro J, McGhee EJ, Sumpton D, Eaton P, Anderson KI, Yin H, Olson MF.
Polarized cell motility induces hydrogen peroxide to inhibit cofilin via cysteine oxidation. ***Curr Biol*** 2015; 25: 1520-5

Using fluorescence lifetime imaging microscopy, the authors of this paper show that hydrogen peroxide is generated at the membranes and protrusions of migrating cells. Furthermore, hydrogen peroxide inhibits cofilin activity and binding to actin through oxidation of its cysteine residues. Expression of oxidation resistant cofilin also impairs cell spreading, adhesion and migration, leading the authors to conclude that oxidation of cofilin, and possibly other proteins, influences cell motility.

Cardaci S, Zheng L, MacKay G, van den Broek NJ, MacKenzie ED, Nixon C, Stevenson D, Tumanov S, Bulusu V, Kamphorst JJ, Vazquez A, Fleming S, Schiavi F, Kalna G, Blyth K, Strathdee D, Gottlieb E.
Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. ***Nat Cell Biol*** 2015; 17: 1317-26

Succinate dehydrogenase (SDH) is a key component of the TCA cycle, converting succinate to fumarate. Its loss is associated with the formation of a number of cancers, including renal cell cancer. This study addresses the important question of how SDH deficient cells adapt their metabolism in order to maintain their growth. Using comparative metabolomics and stable isotope labelling, the authors show that SDH deficient cells support their proliferation by consuming extracellular pyruvate - needed to maintain glycolytic flux - and diverting glucose-derived carbons into aspartate biosynthesis via pyruvate carboxylase. Since pyruvate carboxylase is essential for SDH deficient but not normal cell growth, this might be exploited therapeutically.

Huels DJ, Ridgway RA, Radulescu S, Leushacke M, Campbell AD, Biswas S, Leedham S, Serra S, Chetty R, Moreaux G, Parry L, Matthews J, Song F, Hedley A, Kalna G, Ceteci F, Reed KR, Meniel VS, Maguire A, Doyle B, Soderberg O, Barker N, Watson A, Larue L, Clarke AR, Sansom OJ.
E-cadherin can limit the transforming properties of activating beta-catenin mutations. ***EMBO J*** 2015; 34: 2321-33

Many cancers arise as a result of disruption of the Wnt signalling pathway. However, in colorectal cancer, unlike in other tumour types, this is usually linked to APC loss rather than activating mutations in beta-catenin. In this paper, the authors set out to determine why this is the case by comparing the ability of mutated beta-catenin to transform the small intestine versus the colon. They demonstrate that mutated beta-catenin is unable to transform the colon. Mechanistically this is due to high levels of the transmembrane protein E-cadherin in the colon, which acts as a buffer to sequester mutated beta-catenin thus preventing it from driving transformation.

Liu EY, Xu N, O'Prey J, Lao LY, Joshi S, Long JS, O'Prey M, Croft DR, Beaumatin F, Baudot AD, Mrschtik M, Rosenfeldt M, Zhang Y, Gillespie DA, Ryan KM.
Loss of autophagy causes a synthetic lethal deficiency in DNA repair. ***Proc Natl Acad Sci U S A*** 2015; 112: 773-8

In this paper, the authors explore the mechanisms by which autophagy might protect cells from genomic damage. They show that inhibition of autophagy leads to degradation of checkpoint kinase 1, impairment of the DNA repair process, homologous recombination, and ultimately reduced genomic integrity. As a result, autophagy deficient cells also become dependent on the error prone process of non-homologous end joining for repair of DNA double strand breaks. The authors suggest that these critical links between autophagy and DNA repair could be exploited therapeutically in diseases, including some cancers, where autophagy is deficient.

Rainero E, Howe JD, Caswell PT, Jamieson NB, Anderson K, Critchley DR, Machesky L, Norman JC.
Ligand-occupied integrin internalization links nutrient signaling to invasive migration. Cell Rep 2015 pii: S2211-1247(14)01066-3. doi: 10.1016/j.celrep.2014.12.037. Epub 2015 Jan 15

Integrin trafficking is key to cell migration invasion and is known to be important in cancer progression. In this paper, the authors use novel photoactivation-based microscopy techniques to visualise internalisation of ligand-engaged integrins, and to identify a role for actin-regulated processes and an Arf GTPase (Arf4) in this process. These new approaches have allowed the authors to show that reduced levels of glucose drive endocytosis of ligand-engaged integrins via inhibition of mTORC1. Consequently, this report is the first to establish a link between nutrient metabolism and an endosomal trafficking pathway that promotes invasion.

Schug ZT, Peck B, Jones DT, Zhang Q, Grosskurth S, Alam IS, Goodwin LM, Smethurst E, Mason S, Blyth K, McGarry L, James D, Shanks E, Kalna G, Saunders RE, Jiang M, Howell M, Lassailly F, Thin MZ, Spencer-Dene B, Stamp G, van den Broek NJ, Mackay G, Bulusu V, Kamphorst JJ, Tardito S, Strachan D, Harris AL, Aboagye EO, Critchlow SE, Wakelam MJ, Schulze A, Gottlieb E.
Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. ***Cancer Cell*** 2015; 27: 57-71

Acetyl-CoA synthetase 2 (ACSS2) converts acetate to acetyl CoA, which is required for lipid synthesis. This work uses functional genomics and comparative metabolomics to show that ACSS2 provides cancer cells with a growth advantage in conditions of metabolic stress (when oxygen and lipid levels are low) by allowing them to use acetate as an alternative carbon source. ACSS2 expression is also upregulated under these conditions and its silencing reduces the growth of tumour xenografts, leading the authors to speculate on the possibility of targeting ASCC2 as an anti-cancer therapy.

Tardito S, Oudin A, Ahmed SU, Fack F, Keunen O, Zheng L, Miletic H, Sakariassen PØ, Weinstock A, Wagner A, Lindsay SL, Hock AK, Barnett SC, Ruppin E, Mørkve SH, Lund-Johansen M, Chalmers AJ, Bjerkvig R, Niclou SP, Gottlieb E. Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. ***Nat Cell Biol*** 2015; 17: 1556-68

It has been proposed that accelerated anabolism in cancer cells is sustained by glutamine-derived carbons, which replenish the TCA cycle (so-called anaplerosis). However, this paper clearly demonstrates that in glioblastoma cells, glutamine-derived carbon is not essential for anaplerosis. Instead, glutamine synthetase (GS) converts glutamate to glutamine, fuelling purine biosynthesis and maintaining cell growth. In addition, using 13C-glucose tracing, the authors show that GS produces glutamine from TCA cycle-derived carbons, and determine that glutamine is either synthesised by GS-positive glioma cells or supplied by neighbouring astrocytes.

Zheng L, Cardaci S, Jerby L, MacKenzie ED, Sciacovelli M, Johnson TI, Gaude E, King A, Leach JD, Edrada-Ebel R, Hedley A, Morrice NA, Kalna G, Blyth K, Ruppin E, Frezza C, Gottlieb E.
Fumarate induces redox-dependent senescence by modifying glutathione metabolism. ***Nat Commun*** 2015; 6: 6001

In this paper, the authors employ analytical chemistry and metabolic computational models to investigate the metabolic implications of loss of the TCA cycle enzyme fumarate hydratase (FH), mutations in which are associated with aggressive renal cancer. They show that accumulation of fumarate, caused by inactivation of FH, leads to oxidative stress mediated by succinicGSH, a covalent adduct between fumarate and glutathione. Furthermore, chronic succination of GSH causes persistent oxidative stress and cellular senescence. In these circumstances, benign renal cysts are only transformed into hyperplastic lesions if a key mediator of senescence, p21, is also ablated suggesting that fumarate-induced senescence needs to be bypassed for initiation of renal cancers.

Institute of Cancer Sciences

Ichim G, Lopez J, Ahmed SU, Muthalagu N, Giampazolias E, Delgado ME, Haller M, Riley JS, Mason SM, Athineos D, Parsons MJ, van de Kooij B, Bouchier-Hayes L, Chalmers AJ, Rooswinkel RW, Oberst A, Blyth K, Rehm M, Murphy DJ, Tait SW.
Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. ***Mol Cell*** 2015; 57: 860-72

It is now well established that mitochondrial outer membrane permeabilization (MOMP) in cells leads to release of cytochrome c, caspase activation and apoptosis. In this paper, however, the authors use a new imaging technique to show that MOMP can also occur in a limited number of mitochondria within a cell, triggering DNA damage rather than cell death. Furthermore, this so-called minority MOMP leads to genomic instability, transformation and tumourigenesis. These pro-oncogenic effects are significant as they suggest that in some instances engaging apoptosis might have a potential downside.

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 to 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 that regulate the essential metabolic requirements of cancer cells.

Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis

The tricarboxylic acid (TCA) cycle is a key source for mitochondrial NADH and the core metabolic route for production of many biosynthetic precursors. Despite their pivotal metabolic role, oncogenic mutations in three TCA cycle-related enzymes, succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH) have been identified. While these discoveries implicated a causal link between altered metabolism and neoplastic transformation, they left the question of how these cancer cells fulfil bioenergetic and anabolic demands largely unresolved. SDH is a hetero-tetrameric, nuclear-encoded protein complex responsible for oxidation of succinate to fumarate in the TCA cycle and for feeding electrons into the mitochondrial respiratory chain for ATP production. Inactivating mutations in the human genes for any of the SDH subunits, or the SDH complex assembly factor (SDHAF2), are associated with susceptibility to develop neuroendrocrine neoplasms, gastrointestinal stromal tumours and renal cell carcinoma. We and others have previously demonstrated that the loss of SDH causes succinate accumulation in cells, which activates hypoxia-inducible factors at normal oxygen tension and inhibits α-ketoglutarate-dependent histone and DNA demethylases, thereby establishing, respectively, a pseudohypoxic and hypermethylator phenotype in tumours.

Although SDH is the first discovered TCA cycle enzyme with tumour suppressor properties, the molecular mechanisms that enable the survival and growth of SDH defective cells remain largely unexplored. We generated Sdhb-ablated, immortalised kidney mouse cells and by unsupervised metabolomic screenings and isotope tracing approaches, we identified metabolic pathways essential to support their proliferation. We found that SDHB loss is sufficient to ensure a complete block of the TCA cycle and to drive Warburg-like bioenergetic features of aerobic glycolysis in proliferating cells. We demonstrated that ablation of SDH activity commits cells to consume extracellular pyruvate needed to sustain maximal glycolytic flux and support the diversion of glucose-derived carbons into aspartate biosynthesis via pyruvate carboxylase activity (Fig. 1). This study unveiled a metabolic vulnerability for potential treatment of SDH-associated neoplasms (Cardaci et al, 2015).

Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma

Glutamine and glutamate constitute a metabolic hub in cellular physiology. An increased demand for glutamine by transformed cells has been recognised for almost a century and has been linked to its role as an abundant circulating respiratory fuel. Notably, glutamine carbons can support anabolism through entering the TCA cycle via glutaminolysis. In certain cancer models, the inhibition of glutaminase, which deaminates glutamine to glutamate, reduces proliferation and tumourigenicity. Glutamine addiction has been proposed as a mark of glioblastoma, the most aggressive glioma. Using isotope tracing (^{13}C- and ^{15}N-labelled glutamine) *in vitro* and *in vivo*, we dissected the differential metabolic roles of glutamine-derived carbon and nitrogen atoms in sustaining anabolism and growth in six human glioblastoma cell lines, primary glioblastoma stem-like cells and normal astrocytes. Additionally, glutamine-related metabolism was investigated *in vivo* utilising isotope tracing in both primary orthotopic murine xenografts and glioblastoma patients. These studies led to the discovery of a metabolic crosstalk between glioblastoma cells and normal astrocytes in the brain (Fig. 2).

In contrast to the current view that in cancer cells that undergo aerobic glycolysis, accelerated anabolism is sustained by glutamine-derived carbons, which replenish the TCA cycle (anaplerosis), we have demonstrated that in glioblastoma cells, almost half of the glutamine-derived carbon is secreted as glutamate and does not enter the TCA cycle. Furthermore, the inhibition of glutaminolysis did not block proliferation of glioblastoma cells. Instead, the conversion of glutamate to glutamine by glutamine synthetase (cataplerosis) confers glutamine prototrophy and fuels de novo purine biosynthesis in glutamine-deprived cells. In both orthotopic glioblastoma mouse models and in patients, ^{13}C-glucose tracing showed that glutamine synthetase produces glutamine from TCA cycle-derived carbons. Finally, while glutamine is only marginally supplied by the circulation to the brain, the glutamine required for the growth of glioblastoma tumours is either autonomously synthesised by glutamine synthetase-positive glioma stem cells or supplied locally by astrocytes (Tardito et al, 2015).

Publications listed on page 85

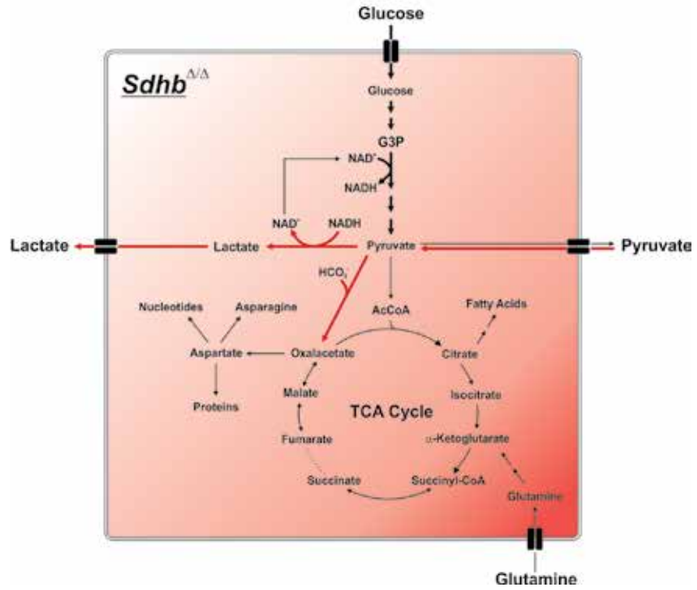
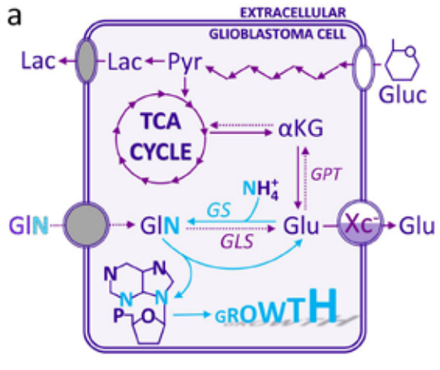


Figure 1.
Schematic representation of metabolic rewiring observed in Sdhb-null cells. Lack of SDH activity commits cells to consume extracellular pyruvate, which sustains Warburg-like bioenergetic features. Moreover, pyruvate carboxylation diverts glucose-derived carbons into aspartate biosynthesis, thus sustaining cell growth of SDH-deficient cells. The size of arrows is proportional to the relative contribution/rate of the corresponding metabolic pathway. Red arrows indicate metabolic pathways required to support proliferation of Sdhb-null cells.



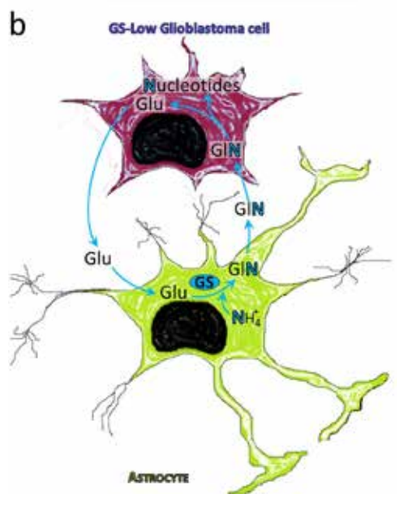


Figure 2.
Glutamine metabolism in glioblastoma. (a) Glutamine-restricted glioblastoma cells utilise glucose-derived carbons to produce alpha-ketoglutarate (solid arrows). This keto acid is transaminated to glutamate, mostly by the glutamate pyruvate transaminases (GPT). Glutamate is then either secreted by the Xc^- antiporter or converted to glutamine by glutamine synthetase (GS). GS supplies glutamine to nitrogen-demanding catabolic pathways essential for growth, such as nucleotides biosynthesis. (b) A proposed model for a novel tumour-glia interaction. Glioblastoma cells expressing low levels of GS rely on the glutamine produced and released from GS-positive astrocytes to synthesise nucleotides for growth under glutamine-restricted conditions.

UBIQUITIN SIGNALLING

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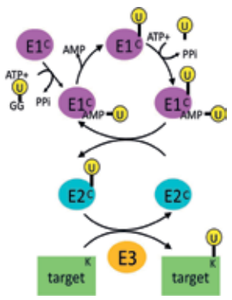
Post-translational modification of ubiquitin (Ub) by Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3) regulates a range of cellular processes, and deregulation of this pathway is often associated with human pathogenesis, including cancer.

Our group uses X-ray crystallography and biochemistry to study the Ub pathway enzymes to understand their regulation, mechanistic functions and mutation-induced deregulation. We anticipate that the knowledge gained from these studies will assist in the development of selective therapeutic targets within the Ub pathway.

Ubiquitin conjugation cascade

Covalent attachment of Ub involves the enzymes E1, E2 and E3 (Fig. 1). E1 initiates the cascade by adenylating Ub's C-terminus, followed by the formation of a covalent thioester intermediate with Ub. E1 then recruits E2 and transfers the thioesterified Ub to E2's catalytic cysteine. E3 plays a pivotal role in determining substrate fate, and 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. Our group is interested in understanding the regulation and mechanistic functions of RING E3s, particularly those linked to cancer.

Figure 1.
Enzymatic cascade of Ub modifications.

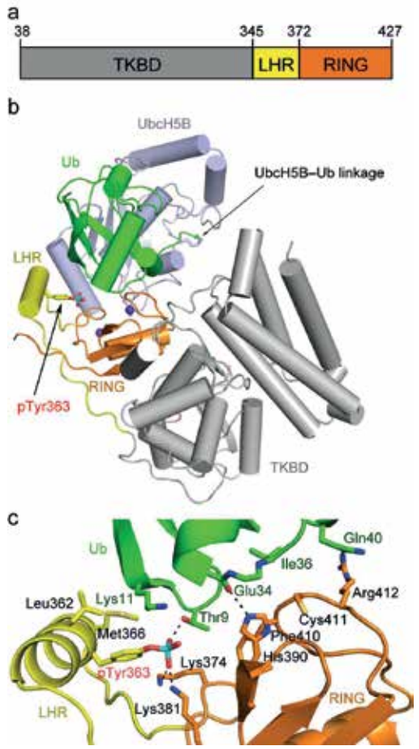


Cbl proteins and receptor tyrosine kinase signalling

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) are RING E3s that negatively regulate RTKs, tyrosine kinases and other proteins by promoting their ubiquitination and subsequent degradation. Cbls also function as adaptor proteins. 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. 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, 2010). However, it remains elusive how Cbls are regulated and how these mutations contribute to oncogenicity.

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, 2000) 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. It activates c-Cbl ligase activity by inducing dramatic LHR conformational changes that (1) enhance overall E2 binding affinity by

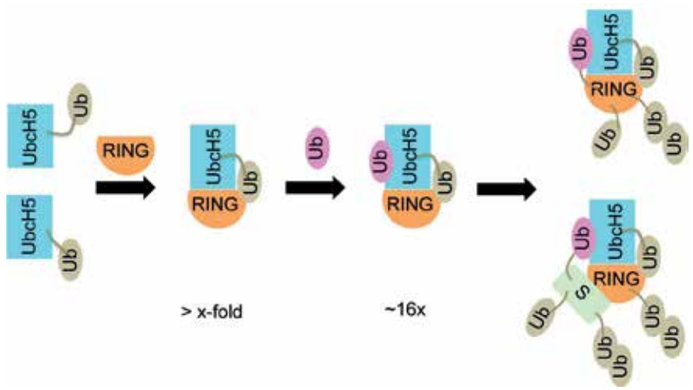
Figure 2.
Phosphorylated LHR tyrosine participates in activation of E2~Ub. (a) The conserved Cbl's N-terminal domain containing TKBD, LHR and RING domain. (b) Structure of Tyr363 phosphorylated Cbl-b bound to an E2~Ub and a TKBD substrate peptide. (c) Close-up view of pTyr363-Ub and RING-Ub interactions.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



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. Together these results demonstrate how Tyr371 phosphorylation could transiently switch on Cbl's ligase activity to attenuate RTK signalling (Dou et al, 2012).

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 side chain (Fig. 2c). These Ub interactions alone enhance Cbl's catalytic efficiency by ~200-fold, explaining the massive rate enhancement upon phosphorylation (Dou et al, 2013).

Figure 3.
Allosteric activation of RING E3-UbcH5~Ub complex by Ub^B. RING E3 binding locks UbcH5~Ub into a closed active conformation for catalysis. Binding of Ub^B further enhances the catalytic efficiency of RING E3-UbcH5~Ub complex. When RING E3 or substrate is ubiquitinated, the ubiquitin moiety can function as Ub^B *in cis* to stimulate Ub transfer.



c-Cbl's Ty371 is one of the mutational hot spots in patients with myeloid neoplasms. Our results explain why Tyr371 mutants cannot adopt an 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

We have determined two crystal structures of RING E3 bound to E2 UbcH5B covalently linked to Ub at its active site revealing how RING E3 locks E2~Ub in a closed conformation such that the E2~Ub thioester bond is optimally oriented for catalysis (Dou et al, 2012, Dou et al, 2013). More recently, we found that Ub binding at the 'backside' of UbcH5B (Ub^B) can directly stimulate the activity of the RING E3-UbcH5B~Ub complex (Buetow et al, 2015). We showed that the presence of Ub^B enhances RING E3's binding affinity for UbcH5B~Ub by 10-12-fold and improves the catalytic efficiency of Ub transfer by 16-fold. To elucidate the molecular mechanism, we determined crystal structures of RING E3-UbcH5B~Ub complex alone and in the presence of Ub^B. Structural comparison revealed that Ub^B packs against UbcH5B's N-terminal helix and α1β1 loop, and alters their conformations such that they form more favourable interactions with the RING domain and donor Ub. Remarkably, formation of the RING E3-UbcH5B~Ub complex enhances Ub^B affinity and when Ub is conjugated to E3 or substrate, the conjugated Ub can serve as Ub^B *in cis* to stimulate Ub transfer. The *in cis* arrangement would increase the local concentration of Ub^B, explaining how low cellular Ub concentration could stimulate the RING E3-UbcH5B~Ub complex. Our work demonstrated that UbB promotes RING E3-catalysed processive poly-Ub chain formation by enhancing the overall catalytic efficiency of the RING E3-UbcH5~Ub complex (Fig. 3). This leads to fast initial Ub transfer and subsequent poly-Ub chain formation. The rate of Ub transfer becomes faster when the reaction shifts from *in trans* to *in cis* (Fig. 3).

Publications listed on page 86

PROSTATE CANCER BIOLOGY

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Prostate cancer remains a major global health issue, resulting in significant morbidities among prostate cancer sufferers. It is also the second commonest cause of premature cancer-related death in men worldwide.

Our research objective is to identity, characterise and validate key aberrant cellular signalling events (including androgen receptor, Sprouty2 and ERK5) to inform and facilitate ongoing efforts in developing novel therapies. We believe that the use of highly novel preclinical models within our laboratory will enable us to fast track the development of new treatment agents/strategies for prostate cancer through cross-disciplinary collaborations. We are particularly encouraged by the opportunity to apply what we have learned in the laboratory to design the SPECTRE clinical trial to treat patients with treatment (hormone) resistant prostate cancer.

Exploiting laboratory findings to design a clinical study

We found that PTEN inactivation cooperates with Sprouty2 (SPRY2) deficiency to bypass key tumour suppressor checkpoints in driving prostate carcinogenesis (Patel et al, 2013). We further discovered that such tumours are prone to develop treatment resistant disease, particularly castration resistant prostate cancer. We studied an extensive range of *in vitro* and *in vivo* model systems developed within our group (including a castration resistant tumour orthograft model derived from prostate cancer cell lines previously isolated and propagated from human and genetically modified mice).

As expected, following surgical castration in the orthograft model, serum testosterone levels were profoundly suppressed. Despite this, the intra-tumoural testosterone levels in castration resistant prostate cancer demonstrated remarkably sustained testosterone levels. Furthermore, the expression of key enzymes for androgen biosynthesis were significantly elevated in the CWR22 SPRY2 knockdown orthografts, signifying *de novo* androgen synthesis upon castration.

Detailed mechanistic analysis points to an important role for cholesterol in castration resistant prostate cancer. We therefore carried out a clinical study (trial) in mice using a statin to block the contribution of cholesterol in 'fuelling' androgen biosynthesis for the development of castration resistant prostate cancer. It was pleasing to observe a dramatic tumour response with castration resistant prostate cancer failing to develop in the presence of combined simvastatin and androgen deprivation therapy.

Based on this promising preclinical data, we have now secured funding for a phase 2 clinical study with collaborators Rob Jones, Jim Paul (West of Scotland Beatson Cancer Centre), Christian Delles and Jason Gill (Institute of Cardiovascular & Biomedical Science, University of Glasgow); Combined Suppression of Cholesterol Bioavailability and Androgen Deprivation Therapy to Treat Castration Resistant Prostate Cancer (SPECTRE, Fig.1). We will formally test the potential value of adding a statin to androgen deprivation therapy in patients who have shown the earliest sign of castration resistant prostate cancer, i.e. rising serum levels of prostate specific antigen following previous hormonal therapy. This timely, and hopefully impactful study, is due to be launched and start to recruit in 2016.

Novel preclinical model identifies *Pparg* activation as a key interacting event accelerating *Pten*-mediated prostate tumourigenesis and resulting in lethal metastatic disease

This work was done in collaboration with 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'

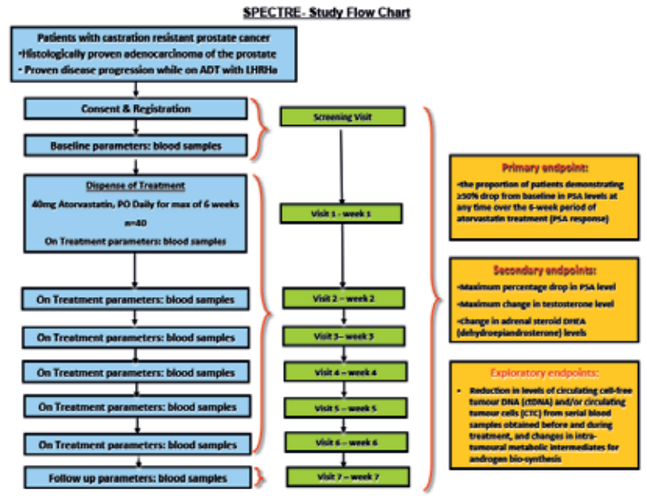

Figure 1
Flowchart for SPECTRE study.

are required (Ahmad et al, 2011). The experimental cohort *SB:Pten*^{Null} (*Pb-Cre4:Pten*$^{fl/fl}$*T2Onc3/+Uren/+*) was generated, along with the relevant controls. Tumours isolated were then analysed to detect unique 'barcodes' (or common insertion sites) that marked potential genes of interest. Overall, comparing to the controls, *SB:Pten*^{Null} mice exhibited accelerated prostate tumourigenesis with larger tumours.

Among the common insertion sites, we were interested to note that these were commonly found to involve the *Pparg* gene, a critical regulator of lipid metabolism. Furthermore, we found that tumours with *Pparg* involvement were larger than those from non-*Pparg SB:Pten*^{Null} mice. Importantly, *Pparg SB:Pten*^{Null} mice also often had metastases in their lymph nodes and lungs at the study clinical endpoint. These findings argue a very important functional interaction between *Pten* and *Pparg* in driving lethal aggressive metastatic disease.

We carried out *in vitro* experiments to validate the role of PPARG in invasive disease by manipulating its expression and function by means of siRNA-mediated gene silencing and small molecule inhibitor (GW9662), respectively. In order to test the value of *in vivo* PPARG suppression as a potential therapeutic strategy, we applied the

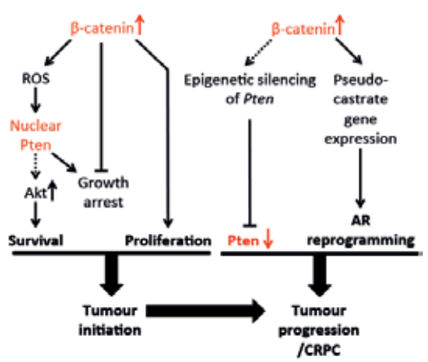

Figure 2
Schematic representation of beta-catenin driven prostate carcinogenesis.

mouse orthograft prostate cancer model and treated mice with the PPARG inhibitor or vehicle control. GW9662 treatment resulted in reduced tumour proliferation (Ki-67 staining) and suppressed fatty acid synthase expression, in keeping with PPARG driving invasive disease, at least in part, through altered lipid metabolism. Importantly, there was a substantial reduction in the metastatic burden within the lymph nodes studied. Therefore, we are now considering the optimal strategy for applying these findings to design targeted therapy for prostate cancer with high risk of metastatic progression.

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

The activation of Wnt/beta-catenin signalling is a common event in patients with the poorest prognosis, and is frequently associated with loss of PTEN and activation of the PI3K/Akt signalling pathway. However, the molecular basis for the significant impact of these aberrations in prostate cancer remains unclear.

By using preclinical transgenic *in vivo* models, we have demonstrated that beta-catenin is a potent proto-oncogene that drives prostate cancer tumourigenesis. Concurrent heterozygous loss of *Pten* exacerbates beta-catenin driven tumour progression and decreases host survival, while tumours are most aggressive when *Pten* is deleted. Our study has uncovered mechanisms through which beta-catenin modulates Pten to progress tumourigenesis (Fig. 2); beta-catenin/ROS-mediated modulation of Pten localisation, and epigenetic silencing of *Pten* through deranged miRNA expression and *Pten* haploinsufficiency.

Furthermore, by examining novel *in vivo* models of beta-catenin driven castration resistant prostate cancer, we have indicated that beta-catenin may promote treatment resistance through androgen receptor (AR) reprogramming. We propose an AR-independent mechanism of castration resistance is active in beta-catenin driven castration resistant prostate cancer, mediated through significant upregulation of canonical and non-canonical Wnt pathway components, which may be effectively targeted by Wnt inhibition.

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 87

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.

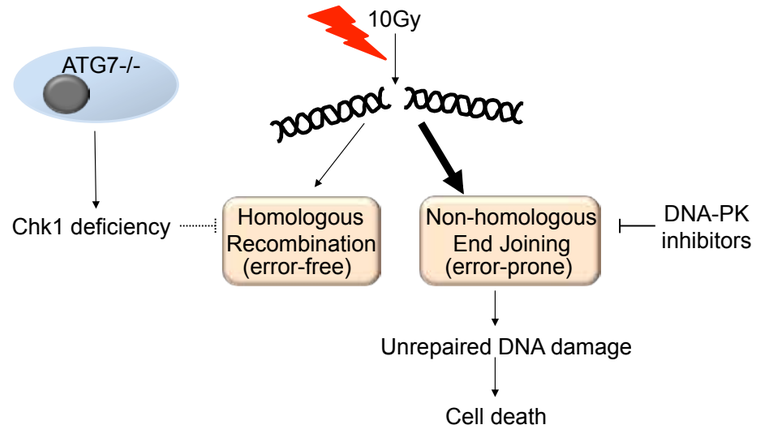
Autophagy and DNA repair

There are multiple mechanisms within cells that determine viability. Our laboratory is particularly interested in a process termed macroautophagy (or more simply, autophagy) that has been shown to both promote and repress cell death depending on the context. Autophagy is a membrane-trafficking process that encapsulates and delivers cargoes to lysosomes for degradation. Under basal conditions, it serves to remove damaged proteins and organelles thereby acting as a mechanism to preserve cytoplasmic integrity. Following delivery to lysosomes, the cargoes contained within autophagosomes are broken down into constituent parts such as amino acids and lipids, which are then recycled into biosynthetic pathways. In some situations, for example upon nutrient deprivation, breakdown products can be further catabolised for the generation of energy for a limited period of time until external sources of nutrients are regained. Due its fundamental role in cellular homeostasis, autophagy has been shown to have important roles during tumour development and in response to cancer therapy.

Although autophagy is a cytoplasmic process, reports have shown that autophagy-deficient cells accumulate DNA damage. Since preservation of genomic integrity is a key factor in protecting us against cancer, we were intrigued by these reports and decided to explore the mechanism underlying this effect. Initially, it might be considered that autophagy-deficient cells have diminished protein and organelle fidelity, which would lead to increased levels of reactive oxygen species and subsequently DNA damage. While this is highly plausible, we considered it may also be possible that due to the natural incidence of DNA damage, autophagy-deficient cells accumulate damage, at least in part, due to a defect in DNA repair. To explore this possibility, we examined the impact of deleting essential autophagy genes on the efficiency of two important mechanisms of DNA repair, homologous recombination (HR) and non-homologous end joining (NHEJ). This revealed that while loss of autophagy has no impact on NHEJ, the ability to repair DNA double-strand breaks by HR was greatly impaired. Importantly, since HR is error-free and NHEJ is error-prone, the deficiency in HR causes by loss of autophagy could well be a major contributing factor leading to the accumulation of DNA damage in autophagy-deficient cells.

To explore the mechanism underlying this effect, we analysed a number of components of the signalling pathways leading to HR. This strikingly revealed that in the absence of autophagy there was an apparent deficiency in the ability to activate the checkpoint kinase Chk1, an event critical for HR. Over time, however, not only was there a deficiency in the activating phosphorylation of Chk1, but total Chk1 levels also decreased in autophagy-deficient cells. Previous studies had shown that the phosphorylation of Chk1 by the upstream kinase ATR not only leads to activation of Chk1 but also marks the protein for degradation by the proteasome. We therefore examined ATR levels and activation in the absence of autophagy, but we did not find any differences when compared to wild type cells. Instead, we found that loss of autophagy led to hyperactivation of the proteasome such that the half-life of phosphorylated Chk1 is diminished in the absence of autophagy.

Figure 1
Loss of autophagy impairs HR, which uncovers a synthetic lethal strategy to kill autophagy-deficient cells. Autophagy-deficient cells have diminished levels of Chk1. This impairs HR and leads to a hyper-dependency on NHEJ for DNA repair. Inhibition of NHEJ (using DNA-PK inhibitors) results in persistence of DNA damage and cell death in the absence of autophagy.

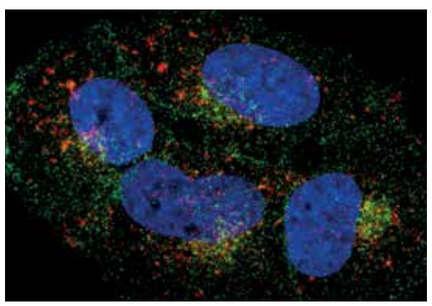


HR and NHEJ are the two principal mechanisms for the repair of DNA double-strand breaks. As a result, we hypothesised that because autophagy-deficient cells have diminished HR, they should be hyper-reliant on NHEJ for repair of genomic damage. Theoretically, this would set up a synthetic lethal situation in which inhibition of NHEJ in autophagy-deficient cells would result in persistence of DNA damage and ultimately programmed cell death (Figure 1). To test this, we treated cells with an inhibitor of the catalytic subunit of the DNA-dependent protein kinase (DNA-PK), which is required for NHEJ. In this context, induction of DNA double-strand breaks by treatment with a chemotherapeutic drug or exposure to irradiation resulted in unresolved DNA damage and rapid cell death in cells lacking autophagy. Taken together, our work in the area has linked two important areas of biology relevant to cancer – autophagy and DNA repair – and has uncovered a novel strategy to kill autophagy-deficient cells.

DRAM-3, a new modulator of autophagy

In our quest to understand the role of autophagy in cancer, we are actively searching for new autophagy regulators that may be perturbed in forms of the disease. Approximately 10 years ago, we identified the damage-regulated autophagy modulator (DRAM-1) as a molecular link between autophagy and the tumour suppressor p53. Subsequently, we realised that DRAM-1 belongs to a previously undescribed protein family, with five family members in humans encoded by five separate genes. During this last year, we published our initial characterisation of one of these genes, which we have named DRAM-3 for DRAM-related/associated member 3.

Figure 2
The autophagy regulator DRAM-3 partially localises to lysosomes. Myc-tagged DRAM-3 was transfected into Saos-2 cells. Cells were stained with anti-Myc (green), anti-LAMP2 (as a marker of lysosomes, red) and DAPI to stain DNA (blue).



Analysis of DRAM-3 expression revealed that it is expressed to varying degrees in a variety of human tissues and tumour cell lines. What is notable is that DRAM-3, unlike DRAM-1, is not induced by p53 or chemotherapeutic agents that cause DNA damage. In parallel with DRAM-1, however, we found that DRAM-3 can be found in close proximity to endosomes and lysosomes (Fig. 2), indicating that may play a role in the modulation of autophagy. Indeed, we found that overexpression of DRAM-3 enhanced autophagic flux and reciprocally CRISPR-mediated deletion of DRAM-3 had the converse effect.

As autophagy can have a major impact on cell viability, we finally examined whether DRAM-3 can affect cell death/survival in response to various forms of cellular stress. This revealed that expression of DRAM-3 can improve cell survival in response to glucose deprivation. Interestingly, however, we found that this effect was independent of autophagy indicating that the effects of DRAM-3 on autophagy and cell survival are likely mediated by separate pathways and further studies are undoubtedly required to shed more light on the functions of this new protein.

Publications listed on page 90

MATHEMATICAL MODELS OF METABOLISM

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Metabolism is essential for life and its alteration is implicated in multiple human diseases. The transformation of a normal cell to a cancerous one requires metabolic changes to fuel the high metabolic demands of the cancer cell, including its proliferation and 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 will therefore be selected for during cancer development. First, we aim to uncover the metabolic objectives and constraints upon which natural selection is acting. Second, we aim to determine which known (and yet to be discovered) molecular alterations are driving the deterministic or stochastic occurrence of optimal metabolic modes.

Quantification of one-carbon metabolic fluxes

In the past year, our research has focused on approaches to quantify metabolic fluxes in cancer cells cultured *in vitro*. We have directed our efforts here because systematic, quantitative methodologies are needed to understand the heterogeneity of cell metabolism across cell types during normal physiology, disease and treatment. Metabolic flux analysis (MFA) can be used to infer steady state fluxes but cannot be applied to transient dynamics. Kinetic flux profiling (KFP) can be used in the context of transient dynamics, and is the current gold standard. However, KFP requires measurements at several time points, limiting its use in high-throughput applications.

We proposed transient MFA (tMFA) as a cost effective methodology to quantify metabolic fluxes using metabolomics and isotope tracing (Fig. 1). tMFA exploits the timescale separation between the dynamics of different metabolites to obtain mathematical equations relating metabolic fluxes to metabolite concentrations and isotope fractions. Using tMFA, we investigated the heterogeneity of folate metabolism and the response to the anti-folate methotrexate in breast cancer cells. Our analysis indicates that methotrexate not only inhibits purine synthesis but also induces an increase in the AMP/ATP ratio, activation of AMP kinase (AMPK), and inhibition of protein and glutathione synthesis. We also find that in some cancer cells, the generation of one-carbon units from serine exceeds the biosynthetic demand.

This work validated tMFA as a cost effective methodology to investigate cell metabolism. Using tMFA, we have shown that the effects of treatment with methotrexate extend beyond inhibition of purine synthesis to other core metabolic pathways. More importantly, we have provided quantitative evidence that one-carbon metabolism in cancer cells is operating beyond its requirement to supply one-carbon units for biosynthesis. This observation resonates with our previous work indicating that one-carbon metabolism may also contribute to energy and redox balance in cancer cells.

Publications listed on page 93

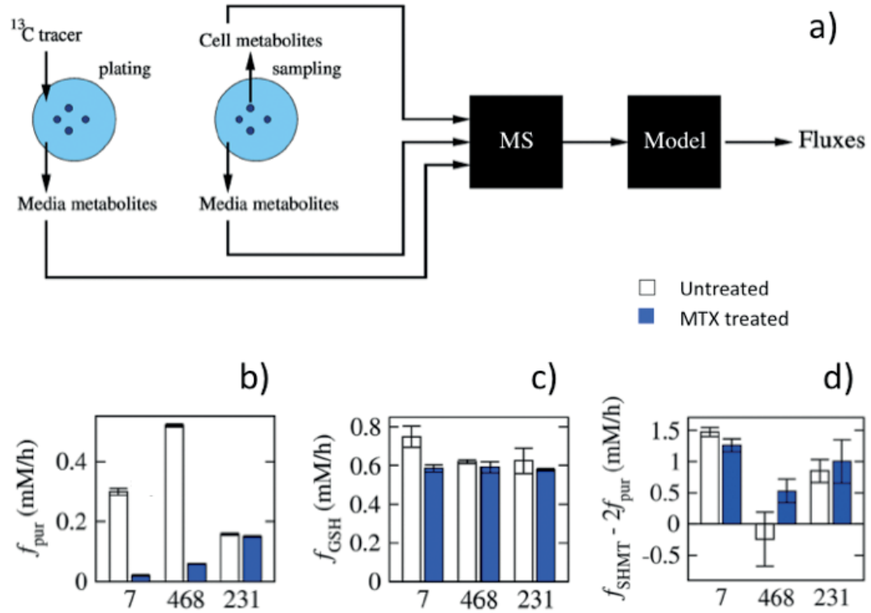

Figure 1
a) Experimental protocol implementing transient metabolic flux analysis (tMFA). Media samples are taken at plating and sampling time points to estimate exchange rates between the media and cells. Cell samples are taken at sampling time points to quantify intracellular metabolite concentrations and isotope fractions. The quantification of media and cell metabolite concentrations and isotope fractions is done using mass spectrometry (MS). The tMFA equations are then used to infer the metabolic fluxes from these measurements. b)-c) Estimated rates of purine (b) and glutathione (c) synthesis from [U-^{13}C]-L-serine tracing experiments, in breast cancer cell lines MCF7 (7), MDA-MB-468 (468) and MDA-MB-231 (231), untreated or treated with methotrexate (MTX). As expected, MTX inhibits purine synthesis in the MTX sensitive cell lines MCF7 and MDA-MB-468. d) Using tMFA, we estimated an excess of one-carbon unit production by serine hydromethyltransferase (SHMT) relative to the one-carbon unit consumption by purine synthesis.

TUMOUR SUPPRESSION

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This year, our studies have focused on the function of the p53-induced protein TIGAR and its link to the regulation of reactive oxygen species in cancer cells. We have also shown that p53 function can be regulated by PHD1 and revealed more about the role of p53 in helping cells adapt to metabolic stress.

The contribution of reactive oxygen species (ROS) to the development of cancer is an interesting but highly complex topic. ROS are important in many cellular signalling pathways and can play a key role in promoting cell proliferation. However, ROS can also be damaging to DNA, proteins and lipids, leading to the accumulation of genomic damage. It has therefore been suggested that limitation of ROS could be effective in preventing and treating cancers, and there has been much publicity around the potential health benefits of a diet rich in antioxidants. Nevertheless, clinical trials of antioxidants for cancer treatment have not shown consistent evidence of a beneficial effect, and indeed some studies have even shown that taking supplements to boost antioxidant activity can result in enhanced tumour risk. Various studies have shown that the activation of oncogenes and aberrant behaviour of cancer cells can increase intracellular ROS levels and enhance oxidative stress to a point that could be toxic. Most cancers therefore enhance their antioxidant defences to counterbalance this increase in ROS. While this allows cancer cell survival, the resultant tumours have a much higher rate of ROS production than normal cells and are much more dependent on the ROS scavenging pathways. In this model, treatments that increase ROS may be selectively toxic to tumour cells, sparing normal cells that are generally under much lower levels of oxidative stress. Indeed, many chemotherapeutic drugs kill tumour cells by increasing ROS.

Given the complexity of the response to ROS signalling, it is not surprising that predicting the effect of ROS modulation is difficult. To understand how ROS might be controlled in cancer cells, and the consequences of interfering with this regulation, we have focused over the past year on the analysis of the function of TIGAR, a protein we initially described several years ago. TIGAR can control glucose metabolism and helps to maintain NADPH levels to regenerate oxidised glutathione (GSH) – a key intracellular antioxidant. Loss of TIGAR expression leads to increased ROS and although this is not obviously toxic under normal growth conditions, our previous work showed that loss of TIGAR leads to a defect in proliferation and survival in response to stress. In the mouse intestine, loss of TIGAR can decrease the proliferation that accompanies tissue regeneration after genotoxic stress and limit the hyperproliferation seen in Wnt-driven adenoma, through the failure to limit ROS.

Our original work showed that TIGAR is a p53 target gene in human cells, and that activation of p53 resulted in enhanced expression of TIGAR. However, we also noted that enhanced TIGAR expression is also seen in many human cancer cell lines that lack wild type p53. We have now shown that human TIGAR expression can be induced by p53 and also by the p53 family members TAp63 and TAp73. However, mouse TIGAR was much less responsive to the p53 family members and basal levels of TIGAR expression did not depend on p53 or TAp73 expression in most mouse tissues in vivo. Although mouse TIGAR expression was clearly induced in the intestines of mice following DNA damaging stress such as ionising radiation, this was also not dependent on p53 or TAp73. The strong p53 binding site that is present in the transcriptional regulation region of human TIGAR is lacking in the mouse TIGAR promoter, and we were unable to detect p53 binding to the mouse TIGAR promoter after the induction of a p53 response. Nevertheless, TIGAR expression was induced in the mouse in response to stress. We therefore concluded that mouse (and human) TIGAR must be regulated through mechanisms that are independent of p53.

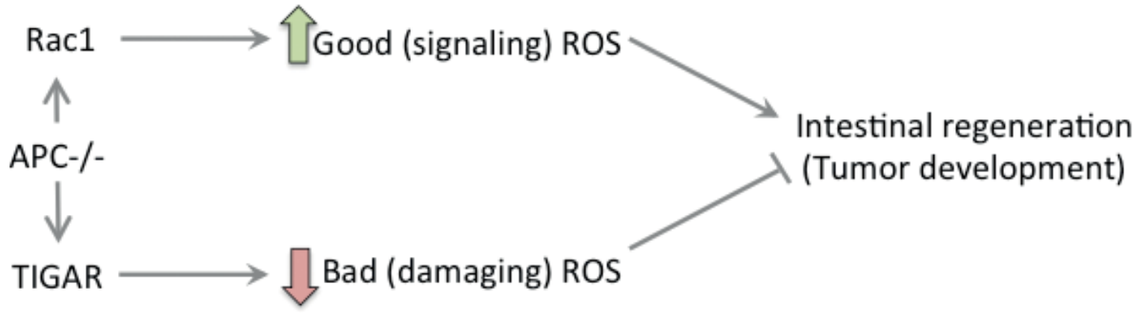

Figure 1
APC loss leads to the activation of both Rac1 and TIGAR, which have opposing effects on ROS regulation. Rac1 promotes the production of signalling ROS, while TIGAR limits the accumulation of damaging ROS. Although these responses elicit different effects on ROS, they cooperate to fully support the hyperproliferation seen following APC deletion.

To gain further insight into the control of TIGAR expression, we turned to the mouse intestine model where we can detect clear activation of TIGAR in response to ionising radiation or the activation of Wnt signalling in response to APC deletion. A key transcriptional target of beta-catenin/TCF is Myc, which has been shown to mediate many of the cellular responses to Wnt signalling. We found that TIGAR was not induced in APC/Myc deleted crypts, indicating that Myc was responsible for the induction of TIGAR. Furthermore, tumours driven by deregulated Myc showed substantial upregulation of TIGAR. Finally, we showed that the ability of Myc to promote ROS was important for TIGAR activation, demonstrating that the ROS limiting activities of TIGAR are induced by increased ROS – a feedback control that functions to limit oxidative stress. Using this system we tested the effects of ROS from different sources on the proliferation of intestinal cells *in vitro* and *in vivo*. Following APC loss, Myc has also been shown to upregulate RAC1, a component of the NOX signalling complex that uses NADPH to signal proliferation. This activity of RAC1 leads to the increase of ROS that signals proliferation, and the defect in ROS induction following RAC1 deletion impairs Wnt-dependent intestinal proliferation. This raises a paradox in that both decreased or increased ROS in the intestine can lead to a decrease in proliferation. We have therefore exploited the observation that the activation of Wnt results in the control of both signalling ROS and deleterious ROS, using this opportunity to modulate the endogenous amount of these different types of ROS by manipulating these two pathways in a three-dimensional organoid culture system as well as genetically *in vivo*. In this way we were able to demonstrate that differentially modulating the different types of ROS can affect the optimal growth response (Fig. 1).

Our analysis of the regulation of the tumour suppressor protein p53 continued in collaboration with Massimiliano Mazzone (VIB), in a study showing that p53 function can be regulated by the prolyl-hydroxylase domain protein PHD1. This work indicated that p53 is hydroxylated, and that this modification leads to an increased interaction with the p38α kinase. This interaction then allows p53 to help support nucleotide excision repair, an activity of p53 that is protective under conditions of DNA damage. This work extends our observations that p53 can help to support and protect cells from various types of stress, complementing our previous studies that demonstrated a role for p53 in helping in the adaptation to serine starvation. Our more recent work is revealing a general role for p53 in helping cells to survive various types of metabolic stress, including the depletion of nutrients that may be limiting in cancers *in vivo*. We are also developing models in which to test how dietary limitation of nutrients such as serine can affect normal health and the development of cancers, with preliminary evidence showing an encouraging effect of a serine-free diet in several tumour systems. It is clear that the mechanisms through which p53 functions to support cell survival can differ depending on the type of stress the cell encounters, but generally the concept that p53 can function to allow cell survival is an interesting and important counterpoint to the generally accepted canonical p53 functions in promoting cell death or senescence. Protection from the accumulation of damage can clearly function as a tumour suppressor mechanism, but the potential role of p53 in survival may have significant impact on the response of cancers retaining wild type p53 to various forms of therapy. Conversely, understanding these functions of p53 may help us to identify vulnerabilities in cancer cells lacking wild type p53, and point the way to the development of new therapeutic combinations that specifically inhibit tumour but not normal cell growth and survival.

Publications listed on page 94

REGULATION OF CANCER CELL INVASION AND METASTASIS

CANCER RESEARCH UK BEATSON INSTITUTE

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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 - Tumour Microenvironment and Proteomics

TUMOUR CELL MIGRATION

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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 work focuses on development of imaging approaches to study the cellular and molecular dynamics of metastasis *in vitro* and *in vivo*. We aim to develop mechanistic readouts of cell migration and apply them to mouse cancer models, including pancreatic ductal adenocarcinoma (PDAC), melanoma and breast cancer. Recent work has focused on the use of FRAP and FLIM-FRET to study E-cadherin dynamics and Rac signalling during pancreatic cancer disease progression and in response to therapy.

Understanding E-cadherin dynamics

We previously published the first use of FRAP to study the tumour suppressor and cell adhesion molecule E-cadherin in mouse tumours. This work demonstrated the power of FRAP to investigate cell adhesion at the molecular level *in vivo*, but left open many questions about the molecular interactions that define the immobile fraction and determine the recovery rate of the mobile fraction. We therefore undertook a systematic dissection of the contribution of cis, trans, and actin interactions to the mobility of E-cadherin at cell-cell junctions, using mutant analysis, chemical crosslinking, co-culturing of expression level variants, and super-resolution microscopy. We found that the mobile and immobile fractions identified by FRAP each had adhesion-specific and non-specific components. Interestingly, inclusion of E-cadherin monomers into either adhesion-specific fraction required all three interactions, cis, trans and actin, demonstrating a cooperative effect between them. We also found that intracellular tail-mediated interactions slowed the diffusion of cadherin, whereas extracellular interactions did not (Fig. 1A). These data challenge the 'outside in' mechanism of cell junction formation, in which extracellular interactions precede intracellular ones, and allowed us to identify four distinct populations of E-cadherin (Fig. 1B) based on their differential inclusion into adhesive structures, and FRAP recovery rates.

E-cadherin is mobilised by mutant p53

Subcutaneous tumour models are useful for studying certain aspects of cancer but do not recapitulate the full complexity of the disease in its native tissue microenvironment. Having established that FRAP could be used to study the molecular dynamics of cell adhesion in subcutaneous tumours, we were keen to use this method in conjunction with genetically engineered models of cancer, such as the KPC model of PDAC. In this model pancreatic expression of Cre recombinase activates expression of $KRas^{G12D}$, which drives tumour initiation, and $p53^{R172H}$, which drives metastasis. In conjunction with the Transgenic Technology team, we therefore made a mouse that would express GFP-labelled E-cadherin (eCad-GFP) from the Rosa-26 locus under control of Cre recombinase. This mouse can selectively express eCad-GFP in a wide variety of tissues (Fig. 1C). Crossing the eCad-GFP mouse with various pancreatic mutants, allowed us to establish that the metastatic programme controlled by mutant p53 involves mobilisation of E-cadherin within pancreatic ductal cell-cell junctions (Fig. 1D). The reduction in the adhesive, immobile fraction mediated by $p53^{R172H}$ was reversed by inhibition of Src using Dasatinib, which recently completed clinical trial in Glasgow for anti-metastatic treatment of pancreatic cancer patients following primary tumour resection. These data pave the way for the use of E-cadherin FRAP to study cell adhesion dynamics in a wide variety of metastatic mouse cancer models.

Monitoring Rac activity in metastatic cancer

Rac GTPase is a master regulator of actin polymerisation, which is upregulated in many metastatic cancers. We have developed the use of the Rac-Raichu biosensor (Fig. 2A) to study Rac activity in mouse cancer models. This biosensor changes its conformation in response to the GTPase activity cycle, leading to fluorescence resonance energy transfer (FRET) that can be read out using fluorescence lifetime imaging (FLIM). Expression of the biosensor is under the control of Cre recombinase, meaning that it can be expressed selectively in any tissue, in combination with any cancer model. A variety of Rac inhibitors are under development for use in cancer therapy. It remains challenging to assess when and where such experimental drugs reach their targets in living tumours. To address this issue, we have used the highly metastatic PyMT model of breast cancer to investigate the efficacy of targeting Rac in primary tumours using the Rac inhibitor NSC 23766. The use of optical imaging windows enabled visualisation of Rac inhibition in response to IP injection of NSC 23766 in the same mice over time. Our data showed that breast cancer cells responded to treatment within 50 minutes of injection of the drug, and that significant Rac inhibition could still be detected after 18 hours (Fig. 2C). In collaborative project with Henning Walczak (University College London), we used the Rac biosensor to study the role of the mouse TRAIL receptor (mTRAIL-R), which had been identified as a therapeutic candidate to promote cancer cell apoptosis. We have previously shown that Rac is active in the KPC model of pancreatic cancer. Surprisingly, knockdown of mTRAIL-R by shRNA in subcutaneous PDAC tumours led to a reduction in Rac activity (Fig. 2D). Blockade of mTRAIL-R using antibody treatment similarly reduced Rac activity. Our data thus support a role for mTRAIL-R in activation of Rac signalling, and therefore promotion of survival and metastasis.

Publications listed on page 82

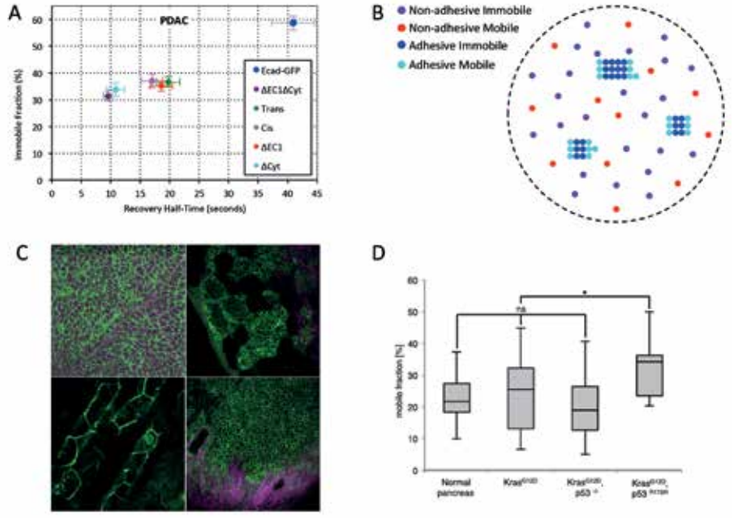

Figure 1
(A) FRAP was used to characterise the effects of mutations (trans and cis) and domain deletions (ΔCyt, ΔEC1 and ΔEC1ΔCyt) on E-cadherin dynamics. (B) Schematic diagram showing distribution of four E-cadherin populations within the ROI of a FRAP experiment. Non-adhesive, immobile monomers (purple) are trapped through non-specific interaction with the cortical cytoskeleton. Non-adhesive, mobile monomers (red) are able to move but do not bind to complexes. Adhesive immobile monomers (blue) remain stationary, possibly because they are trapped within cis-strands. Adhesive, mobile monomers (cyan) are in dynamic equilibrium with stationary complexes and alternate between transient binding and diffusion. (C) The eCad-GFP mouse was crossed with mice expressing tissue-specific forms of Cre recombinase to drive E-cadherin expression in different tissues. Clockwise from upper left: pancreas, breast, kidney and salivary gland. (D) The immobile fraction of E-cadherin in pancreatic epithelial cell-cell junctions is not affected by tissue-specific expression of $KRas^{G12D}$ alone or in combination with deletion of wild type p53. However, expression of $p53^{R172H}$ mobilises E-cadherin, reducing its involvement in cell-cell adhesion.

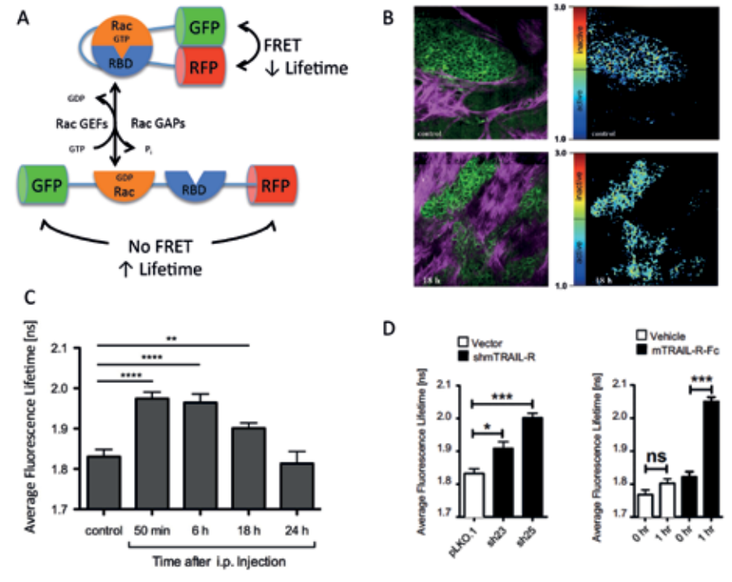

Figure 2
(A) Schematic diagram showing the conformational change of the Rac-Raichu biosensor upon activation of Rac to the GTP-bound state. (B) Images of the Rac-Raichu biosensor expressed in mouse PyMT breast tumours: left panels show fluorescence intensity (green) and collagen SHG (magenta), right panels show Rac activity as a function of fluorescence lifetime in control mice (top) and after 18 hours of Rac inhibitor injection (bottom). (C) Quantification of Rac inhibition in murine breast tumours over time following a single injection of NSC 23766. (D) Knockdown of mTRAIL-R in subcutaneous PDAC tumours using two different shRNAs resulted in increased fluorescence lifetime of the Rac-Raichu biosensor, indicating reduced Rac activity. Inhibition of mTRAIL-R following antibody injection also inhibited Rac activity.

TRANSLATIONAL CANCER THERAPEUTICS

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Our group is developing novel laboratory models that allow us to understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours.

We aim 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.

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

Inhibition of metastases - targeting the LOX/hypoxia axis

To elucidate the key components that contribute to poor prognosis in patients with resected PDAC, we performed a gene signature analysis on the transcriptome of 73 PDACs in collaboration with Owen Sansom and Andrew Biankin (University of Glasgow). From this, a single signature was identified that, following cross-validation, could predict survival in the discovery cohort. This signature could be split into two gene sets: a hazardous set of 321 transcripts, for which increased expression was associated with poor prognosis, and a protective set of 238 transcripts, for which increased expression was beneficial. By testing the hazardous and protective sets for overlap against the MSigDB gene set database, we observed that the hazardous subset showed significant overlap with numerous hypoxia signatures, and the protective subset was linked to lymphocyte and antigen presentation signatures.

Lysyl oxidase (LOX) is a copper dependent enzyme that crosslinks collagen and elastins to drive tissue stiffness. High expression of LOX and of the hypoxia signatures correlated with each other and with poor prognosis. LOX is associated with regulation of collagen crosslinking, and we showed that collagen crosslinking is significantly associated with reduced survival (P=0.033), and with increased tumour stage, lymph node involvement, and vascular invasion in a human pancreatic tissue microarray.

We observed gene expression changes between metastatic and non-metastatic murine PDAC tumours by microarray analysis. There was a clear increase in expression of LOX and LOX family members in cell lines (KPC, and cells derived from *Pdx1-Cre Kras*$^{G12D/+}$ *p53*$^{flox/+}$ [KPflC] tumours) carrying mutant but not loss-of-function p53. Loss of LOX expression (siRNA knockdown experiments) significantly impaired KPC cell migration, and stable knockdown of LOX in KPC cells using shRNA significantly reduced invasion of mutant p53 cells. Conversely, overexpression of LOX in p53 loss-of-function tumour cells promoted invasion of these cells.

The combination of a LOX blocking antibody and gemcitabine significantly slowed tumourigenesis with a median survival of 226 days, compared with 153 days with either gemcitabine or gemcitabine + isotype control antibody in the KPC model. A significant reduction in metastasis formation was observed with LOX + gemcitabine treatment, while a complete absence of metastasis was observed with LOX blocking antibody. In contrast, metastasis developed in 80% of cases in the KPC model following treatment with gemcitabine alone or gemcitabine + isotype control antibody (collaboration with Owen Sansom, Richard Marais [CRUK Manchester Institute] and Janine Erler [University of Copenhagen]).

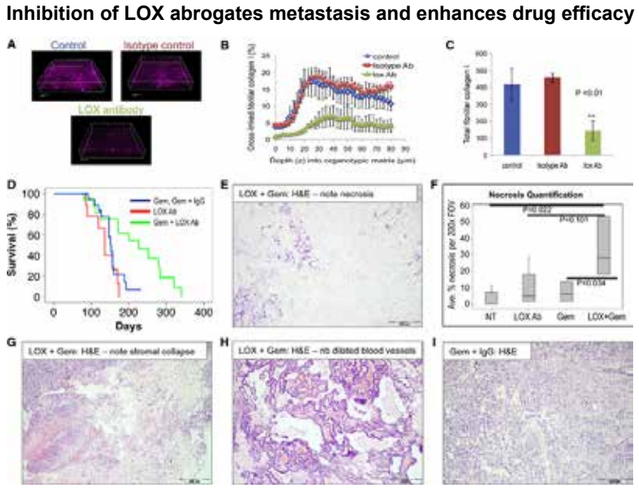

Figure 1
A) Representative fluorescence images showing the SHG signal from crosslinked collagen in untreated, LOX antibody-treated or isotype control-treated organotypic cultures of primary fibroblasts and rat tail fibrillar collagen. B) Chart showing the percentage of crosslinked collagen by depth, based on SHG signal, in organotypic matrices treated as indicated. C) Bar chart showing total crosslinked collagen in organotypic matrices treated as indicated. Data are shown as total pixels ± SEM. D) Kaplan–Meier (non-parametric) survival curve showing significantly extended tumour-free survival (KPC) treated with LOX antibody + gemcitabine (green line, n = 17), compared with gemcitabine ± isotype control antibody-treated (blue line, n = 22, P = 0.014) or LOX antibody alone (red line, n = 9). E) H&E stained section of PDAC harvested from LOX + gemcitabine-treated KPC models. Note necrosis. F) Boxplot showing quantification of necrosis in H&E sections of PDACs. G–H) H&E stained sections of PDAC harvested from LOX + gemcitabine-treated KPC models. Note stromal collapse (G) and marked dilation of blood vessels (H). I) Representative H&E image showing a section of PDAC harvested from a control-treated KPC.

Tumours treated with LOX blocking antibody + gemcitabine had significantly less crosslinked collagen (SHG). We proposed that the effects on the stroma result in the loss of key microenvironment-mediated pro-survival signals that would normally drive resistance to therapy. We predicted that if tumours already have significant collagen crosslinking, i.e. at late stage disease, then LOX inhibition would have a more marginal effect. Treatment of KPC murine models with palpable tumours with LOX blocking antibody and gemcitabine did not have a significant effect on survival. We also did not observe alterations of gemcitabine uptake into the tumour or changes in collagen levels. We therefore hypothesise that anti-LOX therapies work by modulating collagen crosslinking, and that evaluation of LOX inhibitors in human clinical trials should be explored in early stage rather than advanced disease.

Local control of inoperable disease

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. We have initiated a phase 1/2 study of the PARP inhibitor, olaparib, in combination with chemo-radiation in locally advanced pancreatic cancer in collaboration with Belfast, Leicester, and Guy's and St Thomas' ECMCs. In the initial phase 1 part of the study, we will determine the optimal dose of olaparib when used in combination with fluoro-pyrimidine-based chemo-radiation therapy in patients with locally advanced inoperable disease. We will then evaluate the recommended doses of this regimen in a cohort of patients with 'borderline' operable disease. The ultimate aim is to improve objective response in the primary tumours to increase the number of patients who are accessible to potentially curative surgery.

Exploiting tumour biology for therapy 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.

Using these models, we are investigating key pathways downstream of mutant Kras in PDAC, particularly the mTOR pathway. We have previously shown that KC Pten murine models are dependent on S6K signalling downstream of mTORC1, and blocking mTORC1 signalling with rapamycin extends survival even in late stage disease. In contrast, KPC tumours are resistant to treatment with rapamycin, and thus less dependent on mTORC1 signalling. We are now exploring which downstream targets of mTORC2 are important for tumour cell survival and proliferation, and we will identify in which genetic backgrounds target inhibition will have most efficacy, and preclinically test inhibitors and combinatorial approaches.

We are also targeting myeloid cells to improve immunotherapy in PDAC. One dominant player that can contribute to resistance to immunotherapy in PDAC is the presence of a suppressive immune microenvironment. Key drivers of this immune-suppressive microenvironment include tumour-associated macrophages, monocyte myeloid-derived suppressor cells and granulocyte MDSCs. These leucocytes can also promote tumour cell proliferation, confer resistance to cytotoxic stress and facilitate metastatic dissemination. We hypothesise that the efficacy of immunotherapy in PDAC could be improved by overcoming this immune suppression and allowing activated T cells into the tumour, and this is currently being explored.

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) 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.

Publications listed on page 84

CELL MIGRATION AND CHEMOTAXIS

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Crawling movement is a fundamental behaviour of most eukaryotic cells. Indeed, most evidence suggests that the first eukaryotic cells used amoeboid crawling movement. However, in multicellular organisms migration must be suppressed, so the architecture of tissues and organs can be maintained. In most early-stage cancers, cell migration remains repressed. However, when tumours become metastatic, its suppression may be lost - 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 turns cells movement on and off, or what steers them when they move.

Our group aims to bring together multiple tools, from different academic disciplines, to improve understanding of how cell migration is controlled. We currently use *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 how cells are steered by external signals, a process known as chemotaxis, which is increasingly seen as a fundamental cause of cancer metastasis. Recently, our focus has shifted towards a related process in which cells steer themselves by manipulating external signals. The second is the mechanics that drive cell migration.

Most mammalian cells use pseudopods made of polymerised actin to power migration. Our current research focuses on the proteins and pathways that control these pseudopods. We use three approaches. For genetic studies we use *Dictyostelium*, taking advantage of its ease of manipulation, and prominent cell movement and chemotaxis. To apply our knowledge to cancer, we use a range of melanoma cells cultured from tumours with different degrees of metastasis, and actual tumours from mouse models and, when possible, from fresh patient tissue. We also develop computational models of single cells in collaboration with the Mathematics Department, University of Strathclyde, and of populations of moving cells with the School of Mathematics & Statistics, 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 actin and the Arp2/3 complex

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. WASP's behaviour is slightly anomalous - there is a high degree of consensus among cell biologists about how it is controlled, but the standard view does a poor job of explaining the observed behaviour. We are working towards a more detailed and consistent narrative.

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 melanoma cells' 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 Universities of Strathclyde and 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.

Publications listed on page 86

STRUCTURAL BIOLOGY OF CILIA

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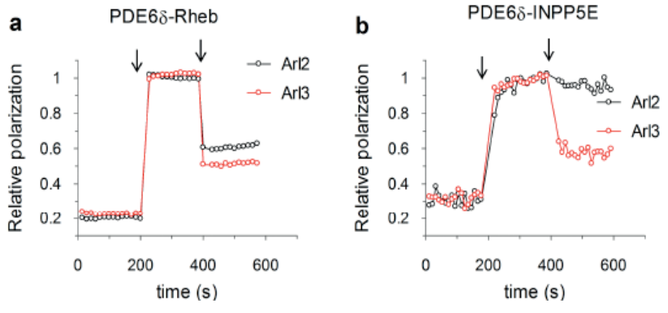
The assembly of cilia is tightly synchronised with the cell cycle and coordinates signalling pathways such as the Wnt and Hedgehog pathways, both of which are therapeutic targets for cancer.

Primary cilia are microtubule-based sensory organelles that emerge from centrioles and can be found in almost all human cell types. Defects in their structure or function result in a copious spectrum of diseases collectively called ciliopathies. Cilia are involved in regulating several signalling pathways, including Hedgehog, Wnt and Notch pathways. They communicate with the external environment by receiving external signals and stimuli, which are then transmitted into the cell via receptors and signalling proteins concentrated within the cilia.

Unlike most organelles, the cilia are not fully enclosed within membranes and are open to the cell body. Nevertheless, although the ciliary membrane appears to be an extension of the plasma membrane, the ciliary content is distinct from that of the plasma membrane and the cell body. This distinct composition is achieved partly through a diffusion barrier, found at the base of cilia, where the entry and exit of ciliary components is regulated. Proteins destined for cilia can be divided into four major groups; integral membrane proteins, small soluble proteins, large soluble proteins and membrane-associated proteins.

Figure 1.
INPP5E and Rheb release from PDE6δ by Arl2•GppNHp and Arl3•GppNHp. (a) Fluorescence polarisation measurements of 0.5 µM FITC-labelled Rheb peptide followed by addition of 0.5 µM PDE6δ and 5 µM Arl2•GppNHp or Arl3•GppNHp (arrow). (b) Fluorescence polarisation measurements of 0.2 µM TAMRA-labelled INPP5E peptide followed by the addition of 0.2 µM PDE6δ (arrow) and 5 µM Arl2•GppNHp or Arl3•GppNHp (arrow).



Among the membrane-associated proteins are the lipid-modified 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. Furthermore, the solubilising function of GSFs is regulated by the small G proteins Arl2 and Arl3 in a GTP-dependent manner.

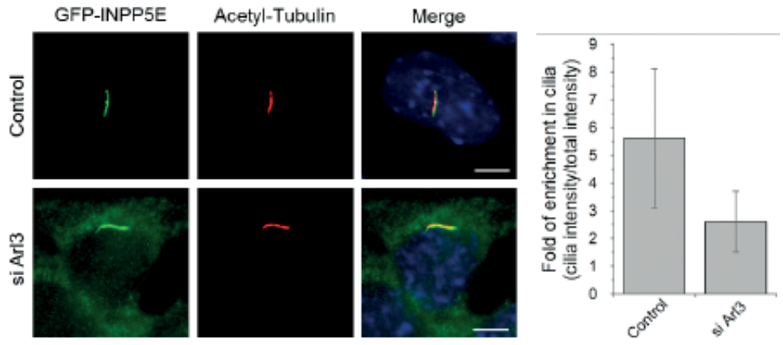
Our group aims to unravel the regulation of ciliary access, for membrane-associated, integral membrane and large soluble proteins, by using an interdisciplinary approach where we combine structural biology, biochemistry and cell biology.

Shuttling of lipid-modified signalling proteins into cilia

Several lipid-modified proteins (myristoylated and farnesylated) traffic to, and are retained in, the cilium. This involves the binding of lipid binding proteins, UNC119 and PDE6D, to the lipid-modified cargo (Ismail et al, 2012, Ismail et al, 2011, Wright et al, 2011, Zhang et al, 2004). However, it has been extensively documented that non-ciliary, farnesylated proteins such as Ras, as well as Rheb, require PDE6D for their proper localisation at the plasma membrane or internal membranes.

This begs a question about the mechanism underlying PDE6δ-mediated sorting of farnesylated cargo between the cilium and other cellular compartments. In a recent study,

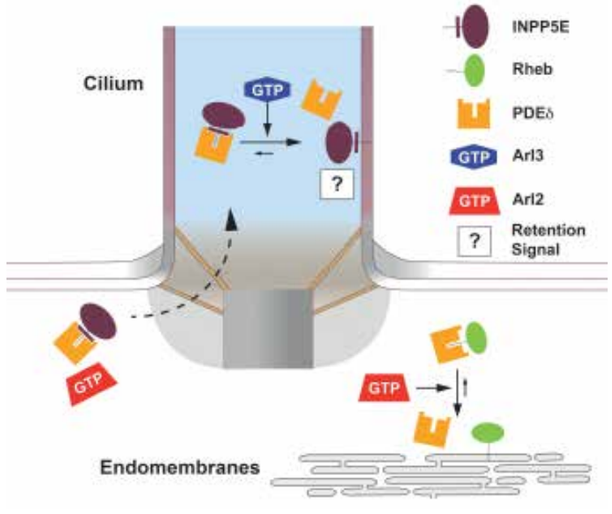
Figure 2.
INPP5E ciliary enrichment is dependent on Arl3. (a) Localisation of INPP5E (green) in IMCD3 cells that were stably transfected with the LAP-tagged protein followed by the transfection with either negative control siRNA or siRNA directed against *Arl3*. White bar indicates 5 µm. (b) Bar chart showing ratio of GFP intensity in cilia compared to the total GFP intensity, indicating the enrichment of GFP-tagged protein in cilia. Data have been collected for 90 cells treated with control siRNA and 82 cells treated with siRNA against *Arl3*. Analysis was performed using CellProfiler.



we investigated the molecular basis of farnesylated cargo sorting using ciliary INPP5E and non-ciliary Rheb as examples. We show a 100-fold difference in the binding affinity of farnesylated cargo with PDE6δ, and demonstrate that the specific release of high affinity cargo by activated Arl3•GTP determines cargo sorting into cilia, while low affinity cargo can be released by both Arl3•GTP and Arl2•GTP, and stays outside the cilium. Moreover, we show by structural, biochemical and cell biological approaches, how and why the binding affinity is dependent on the residues at the 1 and 3 positions preceding the farnesylated cysteine, and that sorting of farnesylated cargo can be manipulated by changing the affinity to PDE6δ (Fig.s 1-3, see Fansa et al. Nat Commun 7; 11366, 2016).

Publications listed on page 86

Figure 3.
Model of PDE6δ-mediated sorting of farnesylated cargo. High affinity cargo such as INPP5E can be specifically released from PDE6δ by Arl3•GTP in the cilium, but not by Arl2•GTP in the cytosol. In contrast, low affinity cargo such as Rheb can be released by Arl2•GTP. As a consequence, PDE6δ-free INPP5E can be specifically retained and thus be enriched in the ciliary compartment, while PDE6δ-free Rheb is retained at endomembranes and stays outside the cilia.



MIGRATION, INVASION AND METASTASIS

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Cancer metastasis is a huge challenge for cancer research, as it is often the main barrier to effective treatment. 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. Additionally, it plays a key role in how a cell interacts with and remodels the extracellular environment. The extracellular matrix contributes to the development and homeostasis of organs and tissues, and in tumours matrix and stroma provide key support for growth, invasion and metastasis. We aim to understand how various actin regulators control interaction with matrix and how tumours subvert both the actin cytoskeleton and the surrounding tumour stroma to gain advantages.

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 (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 functions. 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 production of actin networks on endocytic vesicles to regulate cell signalling and motility in three-dimensional matrix. Postdoc Iben Veland is funded by the Danish Research Council to investigate the role of WASH proteins and associated adapter proteins in invasive migration, and delivery of matrix metalloproteases and receptors into invasive pseudopods. 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 determine their role in migration and invasion.

Role of actin regulatory proteins in colorectal and pancreatic cancers

N-WASP is established as a key driver of formation of invadopodia and of cancer cell invasion in vitro, but much less is known about its potential role in vivo. This year, MRC-funded clinical research fellow Hayley Morris continued her study of N-WASP in colorectal cancer (in collaboration with Owen Sansom). She finds a role for N-WASP in the early conversion between polyps and carcinoma. Postdoc Amelie Juin is studying N-WASP in pancreatic ductal adenocarcinoma (PDAC). High levels of N-WASP have recently been correlated with poor outcome in human patients with PDAC (Guo et al, 2014) pointing to N-WASP as a potentially interesting new target for this cancer. 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 impact of N-WASP on invasion and metastasis and are working to discover the mechanisms by which the actin cytoskeleton confers invasiveness and metastatic capability to pancreatic cancer cells.

Student Loic Fort and Pancreatic Cancer Research Fund postdoc Nikki Paul are studying how fascin upregulation affects the initiation of pancreatic cancer from the precursor lesions (pancreatic intraepithelial neoplasia). Nikki leads our ongoing efforts to develop and test fascin-1 inhibitor compounds together with Martin Drysdale's Drug Discovery team. We continue to explore the mechanisms by which fascin contributes to invasiveness and metastatic spread of pancreatic cancer.

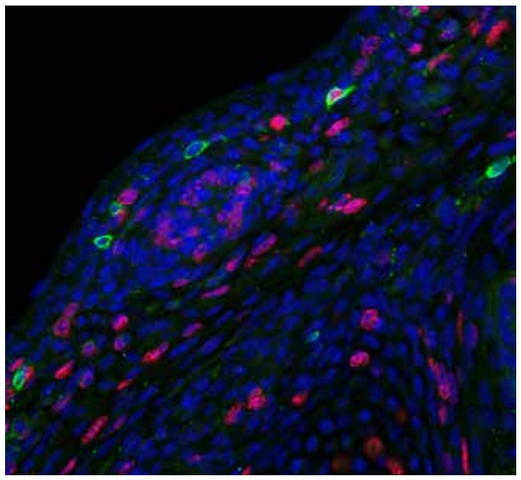

Figure 1
Melanoblasts in mouse embryo skin are marked with dopachrome tautomerase (DCT, green) and BrDu (red) to indicate proliferation. Nuclei are shown in blue (DAPI), revealing also surrounding keratinocytes in the developing skin. Photo credit: Emma Woodham

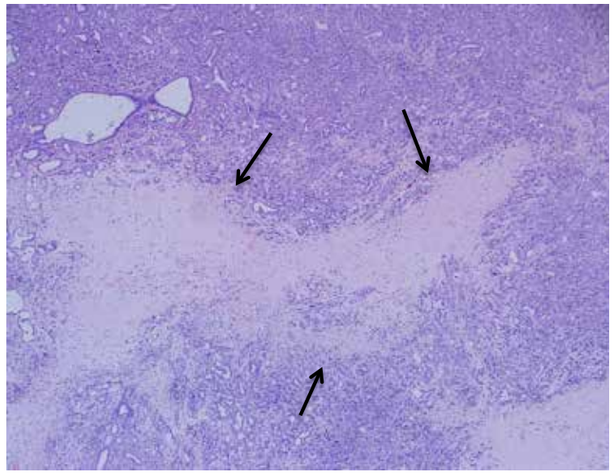

Figure 2
H&E staining of a mouse PDAC tumour showing regions Of dense desmoplastic stroma (pink staining, black arrows). Photo Credit: Amelie Juin

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, 2011). We continue to investigate the roles of RhoA and Cdc42 in melanoblasts (Fig. 1) with PhD student Emma Woodham and postdoc Ben Tyrrell, together with Cord Brakebusch (BRIC, University of Copenhagen). Emma has found a major role for Cdc42 in melanoblast migration and proliferation and is currently working with primary cultured melanocytes to unravel the molecular mechanisms. Postdoc Karthic Swaminathan studies the role of the major actin nucleation promoting complex Scar/WAVE in melanoblast migration and melanoma metastasis. He and student Loic Fort are also setting up a model to study melanoma metastasis (in collaboration with Jim Bear, University of North Carolina) where we will use intravital imaging to view events in early tumours and track cells escaping from the tumours.

Role of extracellular matrix in migration and invasion of tumours

PDACs contain a dense fibrous stroma rich in collagen, fibronectin and other components (Fig. 2). This is thought to serve both as a barrier to chemotherapeutic treatment and an inducer of more aggressive behaviour of the tumour cells. We have a new CRUK Glasgow Centre-funded PhD student, Vassilis Papalazorou, co-supervised by Manuel Salmeron-Sanchez (Engineering Department, University Glasgow), to study how matrix stiffness and composition affects tumour migration and invasion. Together with postdoc Amelie Juin, we are studying the mechanisms of matrix remodelling by cancer associated fibroblasts as well as the effects of this matrix on cancer cells using engineered matrix with defined stiffness and composition. Our goal is to better understand the crucial properties of desmoplastic stroma so that PDAC treatments can be improved in the future.

Publications listed on page 88

INTEGRIN CELL BIOLOGY

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We are interested in the mechanism through which integrins and other adhesion receptors control the metastatic cascade. Over the last few years we have focused on the endocytosis and recycling of integrins and how the molecular machinery responsible for these intracellular trafficking events drives cancer cell migration and invasion. To do this we have developed genetically engineered models of cancer in which particular components of the endocytic and recycling machinery have been disrupted. Furthermore, we have discovered how an oncogenic mutation in one cell can, via the release of exosomes into the circulation, influence endosomal behaviour in other cells, indicating that the control of integrin trafficking in cancer is not cell autonomous. More recently, we have studied the relationship between energy metabolism and cell migration and invasion, and have found that the excitatory amino acid, glutamine is responsible for linking glutamine metabolism to breast cancer metastasis. We will continue to assemble a detailed molecular picture of integrin recycling and the relationship between energy metabolism and integrin function and determine how this contributes to metastasis.

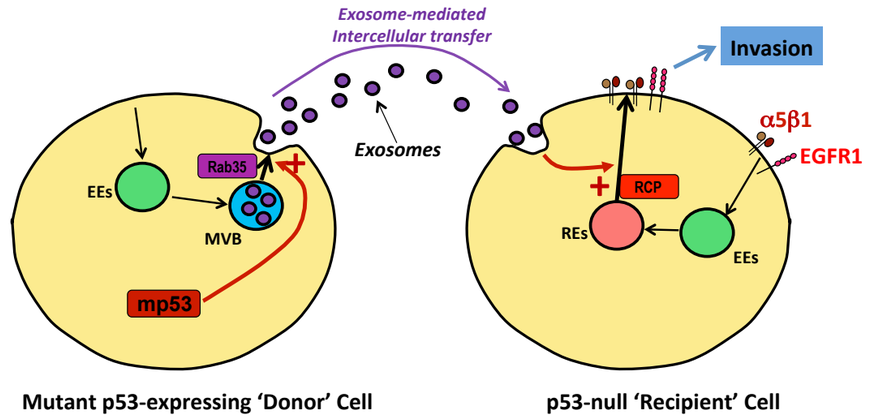
A new role for exosomes in transferring the mutant p53-driven invasive phenotype between cells

We have previously shown that mutants of the p53 tumour suppressor 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 to 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 procedes via a mechanism in which mutant p53 drives Rab35-dependent production of exosomes. These exosomes are then released from mutant p53-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.

Glutamine metabolism drives breast cancer invasion by providing a source of extracellular glutamate to activate the GRM3 metabotropic glutamate receptor

Glutamine metabolism is well established to contribute to cancer cell growth and proliferation by providing a source of

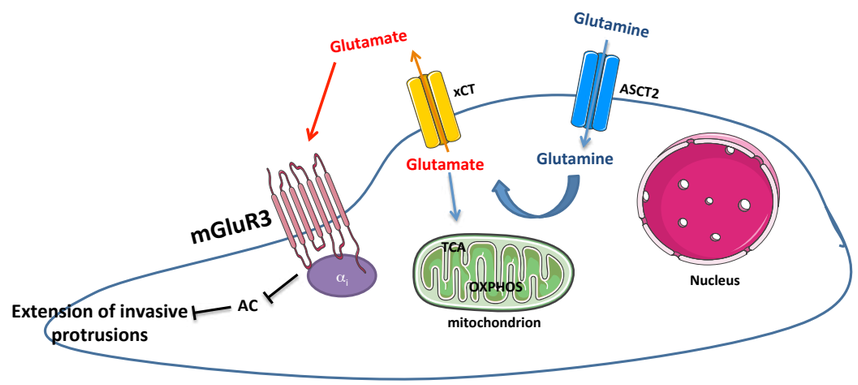
Figure 1
Intercellular transfer of mutant p53's invasive phenotype is mediated by exosomes. The 'donor' cell depicted on the left expresses mutant p53, whereas the 'recipient' cell on the right is p53-null. In the donor cell, mutant p53 activates a Rab35-dependent process that leads to exosome release. 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.



α-ketoglutarate to fuel the Krebs cycle, and nitrogen equivalents for anabolic reactions. However, despite accumulating evidence that glutamine metabolism may also contribute to metastasis, the cellular mechanisms underlying this remain poorly defined. We have generated a number of highly invasive primary cell lines from the polyoma middle-T genetically engineered mouse model of breast cancer (MMTV-PyMT cells). We find that withdrawal of glutamine from these cells reduces not only their growth and proliferation, but also their ability to invade into 'stroma-like' preparations of fibroblast-derived extracellular matrix. The addition of glutamate, a product of glutamine metabolism, to glutamine-starved MMTV-PyMT cells is sufficient to restore invasiveness (but not cell growth or proliferation). Our metabolomic analyses indicate that MMTV-PyMT cells actively secrete glutamate into the extracellular milieu via the xCT glutamate-cystine antiporter. We have pursued these findings by investigating the role played by plasma membrane receptors for glutamate in cell migration and invasion. Indeed, we provide evidence that glutamate generated within the cell by deamidation of glutamine leaves the cell via the xCT antiporter to bind to and activate the GRM3 metabotropic glutamate receptor at the cell surface. This, in turn, suppresses adenylate cyclase activity to prevent protein kinase A activation, and to drive an invasive programme. Indeed, specific inhibition or siRNA of GRM3 is sufficient to oppose breast cancer cell invasiveness without compromising cell growth or proliferation, and pharmacological activation of GRM3 drives invasiveness without increasing proliferation. Our results provide the first mechanistic link between glutamine metabolism and invasion, and shed light on the emerging relationship between glutamine metabolism and invasiveness observed in human tumours.

Publications listed on page 89

Figure 2
Glutamine metabolism influences breast cancer invasion by providing a source of extracellular glutamate. Glutamine is taken up by breast cancer cells via the ASCT2 transporter. In the cytosol, glutamine is converted to glutamate. A proportion of cytoplasmic glutamate is exported from the cell through the xCT glutamate-cystine exchanger. The concentration of extracellular glutamate increases, and this leads to activation of the metabotropic glutamate receptor mGluR3, which drives invasion and metastasis by suppressing the activity of adenylate cyclase (AC).



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.

ROCK signalling in pancreatic cancer

Pancreatic cancer is one of the deadliest forms of the disease that, despite intensive research worldwide, has not seen the improvements in patient survival observed in many other cancer types. The most common form of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), which is characterised by a dense stroma due to extensive proliferation of myofibroblasts and increased production of extracellular matrix (ECM) molecules, particularly collagens. At the time of diagnosis, the majority of patients have surgically unresectable tumours due to extensive local invasion and metastasis, such that chemotherapy is the only viable treatment option. Studies have shown that the dense stroma serves as a physical barrier that impairs drug uptake. Consequently, efforts have been undertaken to deplete the stromal component to enhance drug uptake. Although acute depletion of tumour-associated stromal tissue did indeed increase drug uptake, it was found that depletion of myofibroblasts led to more invasive, undifferentiated tumours with shorter survival times in mice. These observations suggested that some components of the tumour microenvironment act to restrain PDAC tumour growth.

Previous research identified ROCK1 as being frequently amplified in a subset of pancreatic cancer patients. Using well-validated antibodies, we determined that ROCK2 expression progressively increased with tumour grade, consistent with a role for ROCK signalling in pancreatic cancer. To determine the mechanism by which ROCK might promote tumour growth and progression, we expressed conditionally-activated ROCK-estrogen receptor fusion proteins in mouse PDAC cell lines. By treating cells with estrogen-analogues to activate ROCK signalling, we determined that ROCK activation promotes extracellular matrix degradation that enables increased invasive tumour growth. Conversely, ROCK inhibition reduced tumour cell invasion and proliferation. Taken together, the role of ROCK activation in pancreatic cancer progression appears to be to promote degradation of the extracellular matrix, thereby relieving the restraints imposed by the dense stromal microenvironment on tumour growth. An implication of these studies is that ROCK inhibition would be beneficial as an anticancer therapeutic strategy, a possibility we are currently testing in preclinical models.

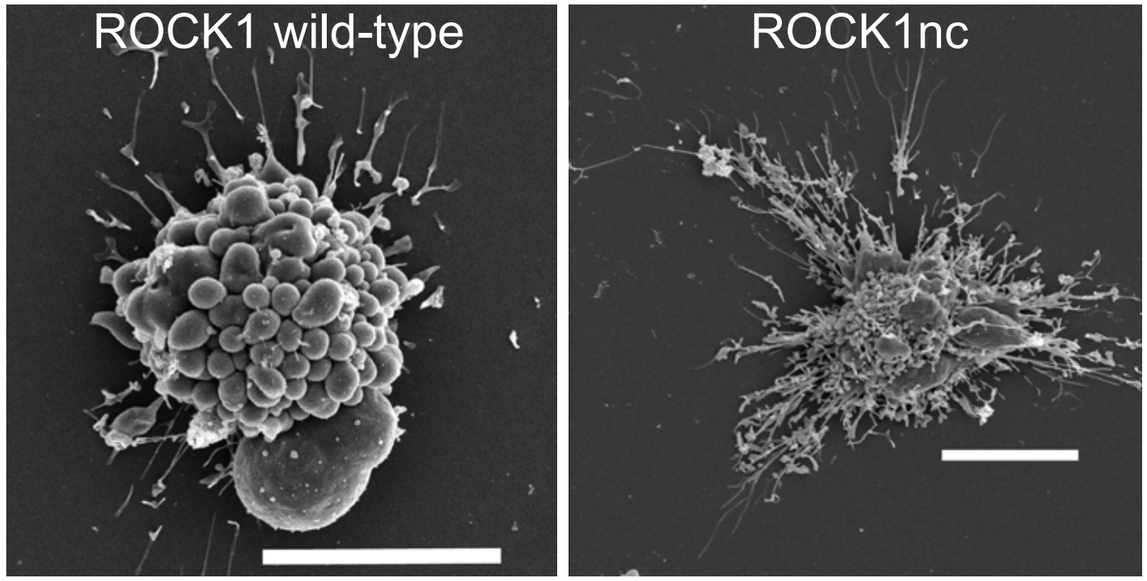

Figure 1
Mouse embryo fibroblasts from mice expressing wild type ROCK1 (left panels) or the caspase-resistant mutant form (ROCK1nc) were induced to die via apoptosis, then imaged by scanning electron microscopy. Scale bars indicate 10 μm.

Caspase cleavage of ROCK1 in tissue homeostasis and cancer

The importance of apoptosis in tissue homeostasis is well documented, and the ability to evade apoptosis has been widely accepted as one of the classical hallmarks of cancer. Although the morphological changes observed in cells undergoing apoptosis (e.g. contraction, blebbing, apoptotic body formation) were identified over 40 years ago, the biological purpose and importance of these dramatic events have not been determined. We previously showed that during apoptosis, caspase cleavage and consequent activation of ROCK1 drives the actin-myosin contractile force generation that is responsible for the observed morphological changes and consequent destruction of nuclear integrity. Although received wisdom is that apoptotic membrane blebbing is important for the recognition and clearance of cell corpses, there has been no model system that allowed this concept to be experimentally tested in vivo. Given that we had identified the caspase cleavage site on ROCK1, we hypothesised that mutating this caspase site ending at D1113 and generating a mouse that expressed a non-cleavable form of ROCK1 (ROCK1nc) would alter apoptotic morphological responses.

We found that when apoptosis was induced in mouse embryo fibroblasts (MEFs), cell death in mutant ROCK1nc MEFs was no different from wild type MEFs, but apoptotic morphologies were altered such that unlike the typical morphologies of cell contraction and membrane blebbing (Fig. 1, left), ROCK1nc MEFs were less contracted and disordered in appearance. Thus, ROCK1nc mice are the first genetically modified model that will enable us to answer fundamental questions about the role of apoptotic membrane blebbing in tissue homeostasis and tumour development.

Publications listed on page 89

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 (c. 75%) whilst activating mutations in beta-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 beta-catenin within the murine small and large intestine. Compared to Apc loss, beta-catenin activation took longer to lead to accumulation of beta-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 beta-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, we discovered that there were many more beta-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 beta-catenin mutation, led to a rapid re-localisation of beta-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 beta-catenin activating mutations all having low levels of E-cadherin (Huels et al, 2015).

Elucidating the cell of origin for colorectal cancer

Understanding these differences between *APC* and beta-catenin mutations in terms of signalling provided mechanistic insights but one question that still puzzled us was that if an activating mutation of beta-catenin was within an intestinal stem cell, then this would be long lived and therefore cells should have ample time to accumulate beta-catenin and transform the intestine. For many years the rapid turnover of the intestine (4-6 days) had suggested that the intestinal stem cell was the most likely cell of origin and our work had shown if we targeted APC loss to stem cells, mice would rapidly develop cancer. Non-stem cells could be transformed, but with much less efficiency. To examine this further we modelled the likelihood of cancer comparing a single activating mutation of beta-catenin versus bi-allelic *APC* mutation. We took into account, the likelihood of the mutation, the requirement for two *APC* mutations and the fact that it took much longer for a b-catenin mutation to produce a phenotype. Using these parameters the model predicted that if the stem cell was the cell of origin one would expect b-catenin mutations, but if you include addition transit amplifying (TA) cells then bi-allelic *APC* mutations were much more likely. Interestingly, the human colon has many more TA cells than the mouse, which may explain why an *Apc* mutation leads to small intestinal tumours in the mouse and colonic tumours in man.

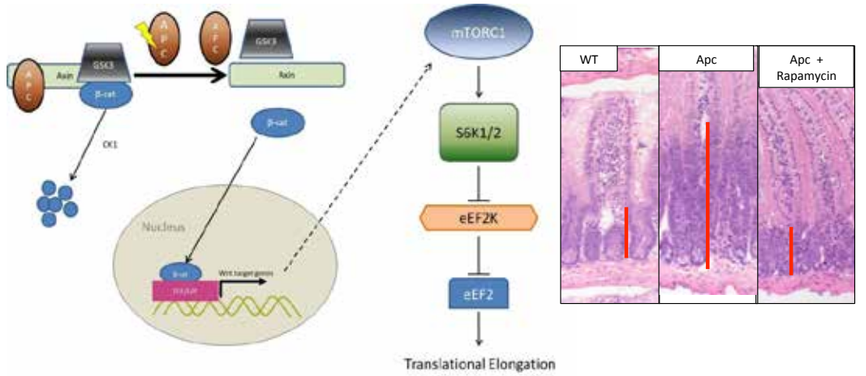

Figure 1
E-cadherin-beta-catenin complexes are increased in the colon compared to the small intestine. A) Complexes of beta-catenin-E-cadherin (in red), blue stain is DAPI nuclei in small intestine and colon. B) Quantification of complexes in small intestine versus colon (each colour spot represents a different sample). C) beta-catenin immunohistochemistry showing five days post Cre induction, no nuclear beta-catenin in AhCreER Catnblox(ex3)/+ intestines apart from the base of the crypt (arrowed). When a single copy of E-cadherin is deleted there is now increased nuclear beta-catenin and much larger intestinal crypts.

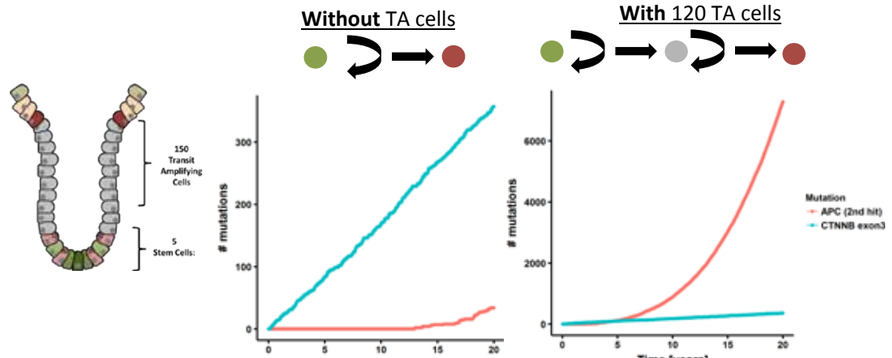

Figure 2
Simulation suggests that transit amplifying (TA) cells can act as the cell of origin in colon cancer. Left hand panel, if just the stem cell is considered then beta-catenin mutations are more likely to occur (blue line) rather than two APC mutations (red line). If the TA cells are included then two APC mutations accumulate.

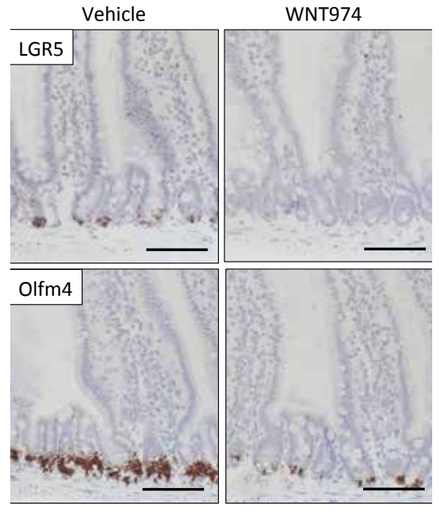

Figure 3
WNT974 treatment reduces intestinal stem cell markers. Porcupine inhibition reduces the expression of the stem cell markers Lgr5 and Olfm4 using RNASCOPE in-situ hybridisation (performed by Colin Nixon and his group).

Inhibiting Wnt signalling *in vivo*

Given the strong link between deregulated Wnt signalling and colon cancer, we have tested the impact of Wnt inhibition on the normal intestine using a clinically relevant Wnt inhibitor, WNT974. This inhibitor blocks the protein porcupine, which is required for Wnt ligand secretion, and is well tolerated in mouse and man. Within the intestine of treated mice we found that there was a marked reduction in Lgr5+ intestinal stem cells. This caused intestinal crypts to be functionally monoclonal (i.e. with only one stem cell) rather than polyclonal (with up to 8-10 stem cells). The consequence of this was that there was reduced stem cell competition, and that if mutations occurred they rapidly repopulated the entire crypt. Therefore, this suggests that one of the key evolutionary reasons for high levels of Wnt signalling in the intestinal crypt is to drive stem cell competition and prevent accumulation of deleterious or cancer causing mutations.

Publications listed on page 90

DROSOPHILA APPROACHES TO CANCER

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We use the fruit fly *Drosophila* to understand fundamental aspects of cancer biology. We previously identified an inflammatory response of the innate immune system to the appearance of tumours, comparable to that observed in cancer patients. Importantly, fly genetic tools allowed us to define, for the first time, the contexts dictating 'good' versus 'bad' inflammation. We next dissected the systemic crosstalk between tumours and peripheral energy stores such as adipose tissue. Remarkably, our data demonstrated that adipocytes can sense tumours at a distance and react by activating proto-inflammatory pathways that act as tissue non-autonomous tumour suppressors. We have also characterised the mechanism by which enteroendocrine cells act as tumour suppressors via Bursicon/LGR2 signalling, to restrict intestinal stem cell proliferation.

Mechanisms of distant tumour suppression

We aimed to identify the mechanism by which adipocytes can affect tumour cell death at a distance. To achieve this, we obtained the transcriptomic profiles from adipose tissue in control tumour-bearing animals and from those lacking the immune response to tumours, obtained by mutation of Eiger/TNF. We found an upregulation in several enzymes involved in lipid catabolism, which extends our previous results indicating a cachexia-like wasting in the adipocytes of tumour-bearing animals. Interestingly, there was also an upregulation of several immune pathways in these adipocytes, cells known to constitute the humoral arm of the fly immune system. These genes included markers of the Toll signalling pathway, which further confirmed our previous results demonstrating a role for Toll in the remote control of tumour cell apoptosis. This array of genes included immune-modulated peptides of unknown function and antimicrobial peptides (AMPs), which are known to mediate the immune response to invading microbial pathogens.

We initiated a functional screen to identify which of these upregulated factors was mediating the effects of Toll signalling in the fat body. When mutated, the AMP Drosomycin, known for its antifungal activity, did not affect the size of the epithelial tumours or their levels of apoptosis. In contrast, mutation of the AMP Defensin, known for its activity against Gram-positive bacteria, resulted in larger tumours with reduced levels of apoptosis (Fig. 1). This result indicates that Defensin mediates, at least in part, the remote anti-tumour effects of Toll pathway activation in adipocytes. This model will allow the characterisation of the conserved AMP Defensin as an anti-tumour molecule, and will inform as to the potential use of AMPs as therapies in several cancer types.

Enteroendocrine control of systemic metabolism

The neuropeptide Bursicon is produced by enteroendocrine cells and acts as a tumour suppressor in the *Drosophila* midgut via its receptor dLGR2, which is expressed in visceral muscle. This paracrine signalling results in

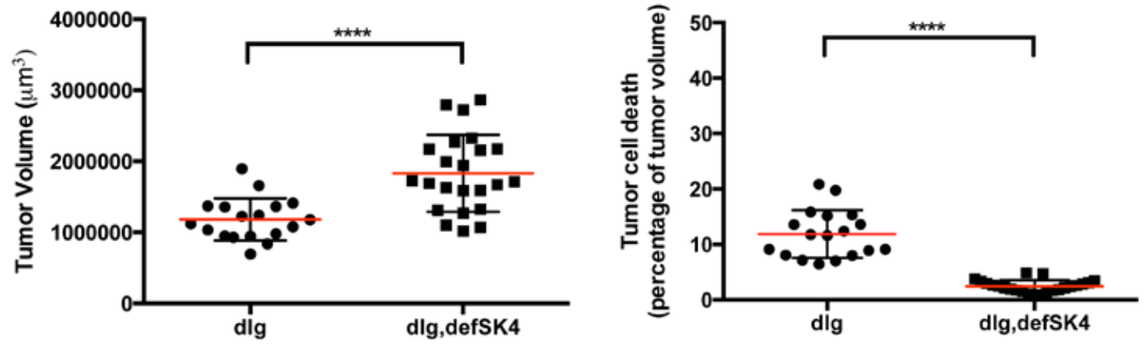

Figure 1
Tumour size quantification of wing disc tumours from disc large (dlg) mutant larvae either in a control background (left) or a Defensin mutant background (defSK4). Note that Defensin mutants display enlarged tumours with reduced apoptosis, indicating a tumour suppressor role of this gene.

tumour suppression via induction of intestinal stem cell quiescence. Interestingly, while the ligand Bursicon is expressed exclusively by enteroendocrine cells of the midgut, its receptor is expressed in many tissues, suggesting an endocrine role of Bursicon in mature adult flies.

We found that Bursicon expression and secretion is regulated specifically by dietary carbohydrates, while it is not affected by dietary protein (Fig. 2). Our results further indicated that enteroendocrine cells directly sense dietary carbohydrates via the sugar transporter GLUT1. This suggested a role in energy metabolism, so we next examined energy stores. Impairment in Bursicon signalling via mutations in *bursicon* or *dLGR2* resulted in hypoglycemia and a progressive loss of fat stores. In contrast, amino acid levels were normal. These reduced levels of sugar and fat occurred despite these flies being hyperphagic with increased nutrient absorption, and less physically active. Consistent with a role in energy metabolism and reduced fat stores, the impairment in Bursicon signalling resulted in hypersensitivity to starvation.

The receptor dLGR2 is expressed in the insulin producing cells of the fly brain, and silencing it in these cells phenocopied the reduced fat content of the mutants. This suggested that the effects of Bursicon could be mediated, in part, via insulin signalling, and consistent with this, we observed nuclear localisation of the transcription factor FoxO, indicative of reduced insulin/IGF activity. Moreover, the mutants displayed increased mitochondrial respiration, which might account for the reduced energy stores. Because the Bursicon receptor homologue LGR4 is expressed in the insulin producing pancreatic beta cells, and LGR4 mutants are hypoglycemic, the mechanism that we have identified in *Drosophila* might be evolutionary conserved and might have implications for therapies targeting certain cancers, obesity and type 2 diabetes.

Publications listed on page 93

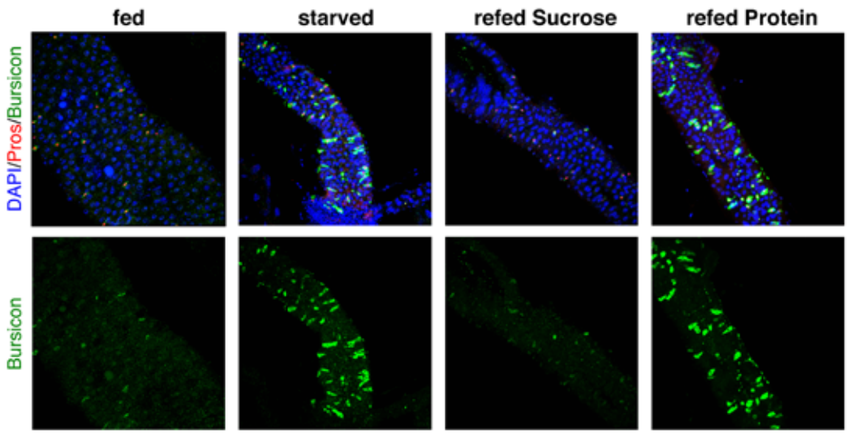

Figure 2
Bursicon is specifically regulated by dietary carbohydrates. Posterior midguts from animals with the indicated treatments were stained for Pros (enteroendocrine cells) and Bursicon. Note the increased retention of Bursicon in the case of carbohydrate deprivation.

TUMOUR MICROENVIRONMENT AND PROTEOMICS

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The tumour stroma is composed of many different cell types and extracellular matrix (ECM) components that work in concert to generate a microenvironment permissive to tumour initiation, progression and formation of metastasis, thus playing a key role in cancer pathology. Additionally, the tumour stroma can determine response to therapy. For these reasons, our group has a strong interest in understanding how the tumour stroma contributes to cancer progression and how to target this compartment for therapeutic benefit.

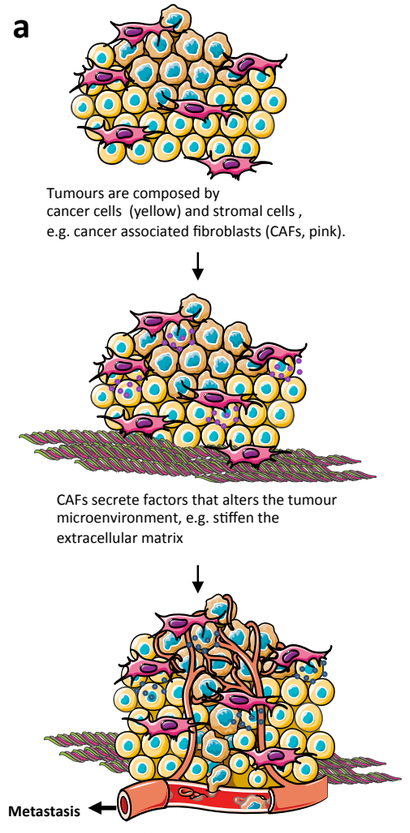
We study endothelial cells, which are key players in the growth of blood vessels in tumours or so called angiogenesis, a well-defined hallmark of cancer. Notably, the tumour vasculature is aberrant: providing a route for tumour cells to escape and form metastases, determining levels of hypoxia, and reducing
the delivery of drugs and the efficacy of radiotherapy. We also work on cancer-associated fibroblasts (CAFs), which secrete a plethora of factors that modify the tumour microenvironment and affect the behaviour of and communicate with surrounding cells, including endothelial, tumour and immune cells. Our group exploits its experience using high resolution mass spectrometry (MS) and accurate quantification methods, including stable isotope labelling with amino acids in cell culture (SILAC), in combination with *in vitro* and *in vivo* approaches to shed light on the complexity of the tumour microenvironment.

CAFs are abundant in the stroma of solid tumours and can alter the tumour microenvironment by secreting factors including ECM components, ECM modifiers and cell-ECM interaction regulators. For example, CAFs are responsible for the increased stiffness of tumours, a phenomenon observed in many cancer types. Notably, high stiffness enhances angiogenesis and tumour cell invasion, and thus has a significant impact on tumour development (Fig. 1a). It is therefore important to understand the molecular mechanisms regulating tumour stiffness and how stromal cells respond to these changes, in order to block cancer progression. To address this, we have used an unbiased MS-proteomic approach, which we have previously shown to be a powerful tool to investigate cellular secretomes (Zanivan et al, 2013) and the molecular mechanisms regulating endothelial cell functions (van den Biggelaar et al, 2013, Patella et al, 2015). Together with functional in vitro and in vivo assays, we have used this approach to characterise previously unknown mechanisms through which CAFs regulate the stiffness of the ECM, and also how endothelial cells respond to this.

Unravelling CAF-induced paracrine mechanisms of cell invasion

We have optimised protocols to perform an in-depth quantitative proteomic analysis of soluble secreted proteins (van den Biggelaar et al, 2013) and ECM components, and identified proteins specifically expressed or having altered levels in the secretome of CAFs. As a cellular model, we use cell lines of human mammary fibroblasts of different origins, normal (NF) and cancer-associated. We have identified more than one thousand proteins secreted by these cells; amongst them, ECM components and growth factors, such as collagens, fibronectin and transforming growth factor-beta, which are well known CAF markers and which were highly abundant in the iCAFs compared to iNFs. Intriguingly, we have shown a member of the chloride intracellular channel protein (CLIC) family, whose function so far has only been characterised intracellularly in tumour cells, is secreted by CAFs and deposited in the ECM. Detailed analysis of this protein revealed that CLIC is highly expressed in the stroma of aggressive breast and ovarian cancers, and is able to enhance angiogenesis and tumour cell invasion (in collaboration with Jim Norman's group) in vitro and in vivo. Moreover, we have found that these functions are mediated, at least in part, by the ability of CLIC to regulate ECM stiffness and cell-ECM interactions. We now have evidence that the secreted pool of CLIC acts as a redox enzyme, and this opens up the possibility of targeting its activity to reduce the pro-angiogenic and pro-invasive functions of CAFs in tumours.

Figure 1
a. Schematic representation of tumour progression that highlights the role of cancer-associated fibroblasts (CAFs) to induce tumour cell invasion and blood vessel growth by creating a stiff tumour microenvironment.
b. Tumour cell (TC) binding to endothelial cells (ECs) that have been cultured on fibronectin-coated polyacrylamide gels of low (400/1050 Pa) or tumour/high (22000 Pa) stiffness. The plot and representative immunofluorescence images show that TCs bind more to ECs exposed to high stiffness and that this can be blocked by depleting cells of the protein CCN (siCCN).

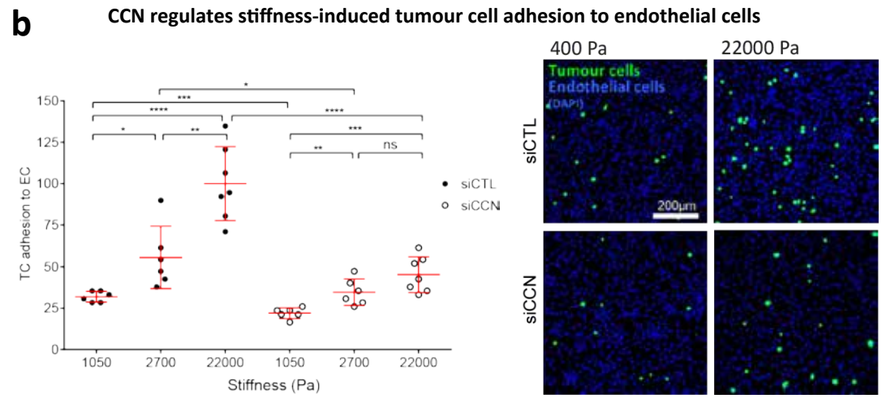


Tumour stiffness favours tumour cell invasion

Endothelial cells line the inner layer of the blood vessel wall and are in direct contact with the blood. Because of their particular location, endothelial cells play a key role in the intravasation of tumour cells into the bloodstream, a process that is required for the formation of metastases at distant sites from the primary tumour. It has previously been shown that tumour stiffness influences endothelial cell sprouting, the initial step in blood vessel growth from the surrounding vasculature into the tumour. However, it is not known if or how tumour stiffness could affect other endothelial functions.

Using MS proteomics, we have measured proteomic changes occurring in endothelial cells when adhering on fibronectin-coated polyacrylamide gels of physiological or tumour stiffness. Similar to studies with other cell types, our analysis showed that high stiffness induces proliferation and cell-ECM adhesion. Moreover, it revealed that several receptors involved in cell-cell interactions are upregulated by tumour stiffness. We have used a combination of cellular and molecular approaches to characterise one mechanism by which high stiffness induces increased levels of a member of the CCN protein family. This in turn enhances the expression and exposure to the plasma membrane of the transmembrane receptor of the cadherin family. Furthermore, using endothelial/tumour cells co-cultures *in vitro* and an *in vivo* model, we show that this mechanism is key to the binding of tumour cells to endothelial cells (Fig. 1b), and favours their intravasation into the blood stream to form metastases. Hence, we have discovered a new effect of tumour stiffness on the vasculature and a pathway that could be targeted to reduce or possibly block stiffness-induced intravasion of cancer cells.

Publications listed on page 94



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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Structural Biology
Peter Brown
Kenneth Cameron
Andrea Gohlke
Gillian Goodwin
Chris Gray
Marta Klejnot
Jen Konczal
Alexander Schuettelkopf
Ewa Warchol

Lab Support
Katrina McLean

The Drug Discovery team has established fragment-based hit identification and structure driven design capabilities in an integrated drug discovery platform. We interact closely with research groups within the Beatson Institute and beyond to identify novel and exciting targets for translation into the drug discovery paradigm. Many of our targets are challenging and complex, however if we can de-risk them by providing tool compounds and clinical development candidates this will ultimately deliver new medicines with clear benefit for patients.

Fascin is an actin bundling protein whose upregulation is known to correlate with advanced cancers and poor outcome, and is involved in cell movement and migration. In collaboration with Laura Machesky, we continue to make significant breakthroughs in this project increasing potency of our fascin small molecule inhibitors.

We have started a collaboration with Ahmed Ahmed (University of Oxford) and have obtained funding from the Medical Research Council Developmental Pathway Funding Scheme to develop selective SIK2 inhibitors for the treatment of ovarian cancer.

In ovarian cancer initial response rates of >70% are seen with platinum and taxane-based treatments (Muggia et al, 2000) but tumour recurrence is also observed in more than 70% of patients within a median of 12 months. These recurrences are typically resistant to chemotherapy and prove not to be amenable for surgical resection. Inhibition of salt-inducible kinase 2 (SIK2) may be an effective approach to overcoming the resistance seen to paclitaxel (Ahmed et al, 2010).

Fascin

Fascin is a pro-migratory protein whose expression is frequently upregulated when normal epithelial tissues become malignant. Its main cellular function is to crosslink filamentous actin into tightly packed bundles that drive the formation of cell surface protrusions involved in cancer cell migration and degradative invasion into the tumour extracellular matrix. Elevated fascin expression is strongly correlated with tumour invasiveness and poor clinical outcome in a variety of cancer types, therefore making fascin a compelling drug discovery target.

We have used a fragment-based screening approach incorporating surface plasmon resonance (SPR) to identify novel compounds that bind to fascin. X-ray co-crystallography of these hit compounds highlighted two distinct compound binding sites within the fascin protein (Fig. 1). We have concentrated our recent efforts on one site; a deeply enclosed pocket situated between domains 1 and 2 of the fascin protein. Fascin-compound complex crystal structures show that compound binding at this site forces significant conformational change between these two domains (Fig. 1B).

We have developed a fluorescence polarisation competition assay as an alternative to primary screening by SPR, thereby allowing the fast identification of compounds at binding site 2. A robust and reproducible actin bundling assay has allowed us to routinely determine the inhibitory effect of compounds on functional activity (Fig. 2). The combination of biochemical data with crystallography structures has helped to guide structure-based hit-to-lead chemistry, and furthered our understanding of the

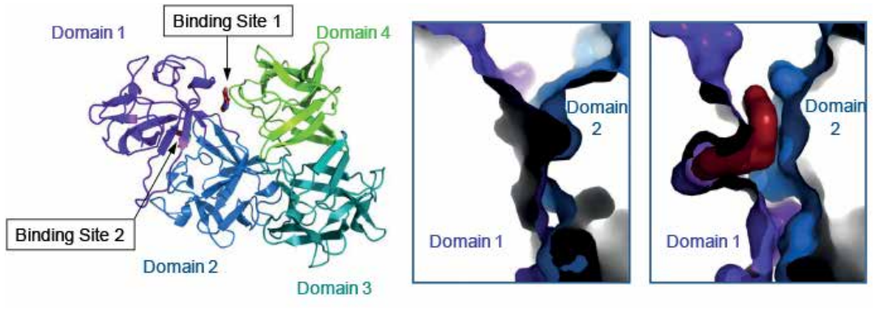

Figure 1
Structure of fascin showing the two ligand binding sites (left). A cut-through of *apo*-fascin (middle) versus ligand bound (right) shows how domain 1 moves following compound binding into a deep hydrophobic pocket within site 2.

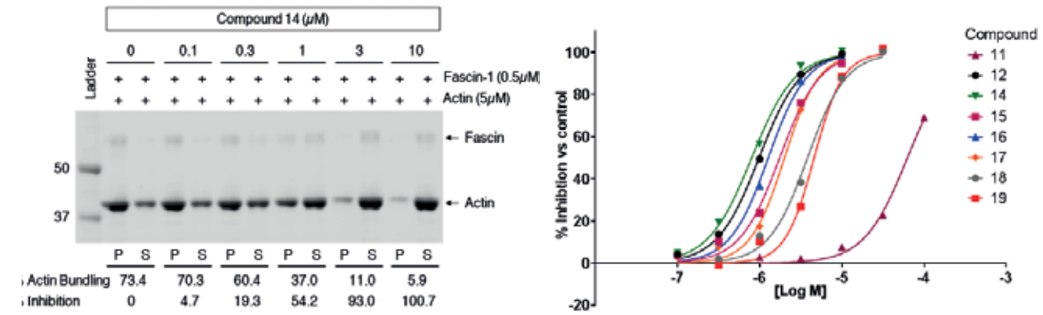

Figure 2
Inhibition of fascin-dependent actin bundling. In the absence of inhibitor, the majority of actin is bundled and in the pelleted fraction (P), with little in the supernatant (S). Addition of compound 14 inhibits actin bundling with the actin moving into the supernatant (left). Dose response curves showing the increase in potency of compounds relative to compound 11 (right).

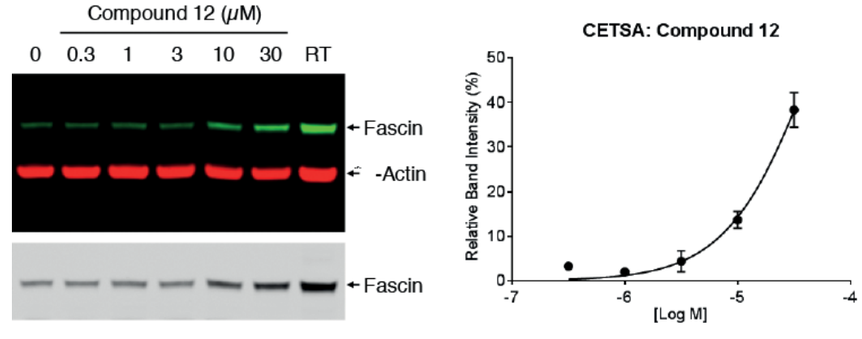

Figure 3
CETSA showing ligand engagement of fascin in cells. Compound binds and stabilises fascin 1 in the MDA-MB-231 D3H2LN breast tumour cell line.

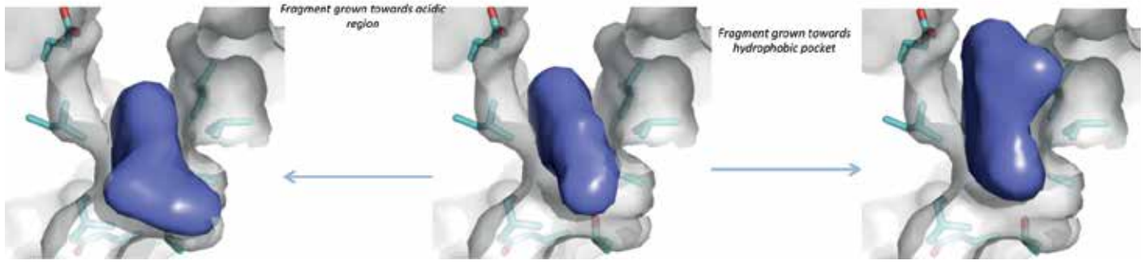

Figure 4
X-ray crystallography of fragment exemplars bound to SIK2 surrogate highlighting fragment 'growth'.

functional implications of different compound binding modalities. This has allowed us to develop a series of sub-micromolar compounds that inhibit fascin functional activity and also show compound engagement of fascin in a cellular environment using CETSA (cellular thermal shift assay) (Fig. 3).

Salt-inducible kinase 2

Ovarian cancer is diagnosed in more than 7000 women per year in the UK. Despite greater than 70% of patients responding to initial surgery followed by treatment with carboplatin and/or paclitaxel, tumour recurrence remains a problem, with a significant number of patients (70%) experiencing recurrence within 12 months.

Despite circulating in the blood, ovarian cancer cells metastasise specifically to the adipocyte-rich regions in the abdominal cavity (omentum and peritoneum) where the metastatic tumour can lead to bowel obstruction and subsequent malnutrition. This project is run in collaboration with Ahmed Ahmed (University of Oxford), who has shown recently that the serine/threonine protein kinase, SIK2 plays an important role in survival and proliferation of ovarian cancer cells and is upregulated in ovarian metastatic tumours. We are currently designing small molecule inhibitors of SIK2 to test the hypothesis that this approach will reduce proliferation and metastasis of ovarian cancer cells.

A recent fragment screen of our in-house fragment library identified a number of attractive chemical starting points for the SIK2 drug discovery project. Currently, the project is in the early stages of fragment evaluation and optimisation (hit-to-lead). To guide our medicinal chemistry approach, we are employing structure-based drug design. X-ray crystallography of our fragments bound to a SIK2 surrogate, derived from structurally related MARK2 (Fig. 4), is being employed to prioritise medicinal chemistry plans. Preliminary results are exciting, demonstrating that we can successfully 'grow' the initial hit fragment (SIK2 Ki 7μM in SIK2 biochemical assay) and improve the SIK2 potency (elaborated fragments show SIK2 Ki < 10nM in SIK2 biochemical assay). Work is continuing within the group to further improve the SIK2 potency of the elaborated fragments in preparation for cellular screening, whilst also evaluating their wider kinase selectivity and scope for optimisation.

Publications listed on page 84

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 - Functional Screening
Karen Blyth - Transgenic Models of Cancer
Douglas Strathdee - Transgenic Technology

BEATSON ADVANCED IMAGING RESOURCE (BAIR)

www.beatson.gla.ac.uk/bair

Head

Kurt Anderson
(see page 26)

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 (fluorescent proteins) has revolutionised research by enabling direct visualisation of any gene product. Concomitant with the development of new genetic tools, there have been tremendous advances in fluorescence imaging technology to visualise molecular dynamics in living cells, tissues and organisms.

These powerful techniques are increasingly sought after by researchers, who require assistance in both the evaluation and application of imaging technology in order to address fundamental questions in cancer biology.

Our mission is to support basic imaging and the development of advanced applications. Basic imaging support primarily consists of training new users in simple acquisition and analysis techniques, such as immunofluorescence microscopy, live cell time-lapse microscopy and the export of raw data into presentation software. Development of advanced applications requires close work with our users to understand their scientific questions and help them develop appropriate imaging strategies. The following advanced techniques have been identified through consultation with researchers as important: medium throughput long-term time-lapse imaging, high resolution live cell imaging, confocal microscopy, especially for the use of photo-activation, -bleaching, and -switching, total internal reflection fluorescence microscopy (TIRF), intravital microscopy, and fluorescence lifetime imaging for the determination of fluorescence resonance energy transfer (FLIM-FRET). Collectively, we provide our users with a powerful technology toolbox for cellular and molecular level investigations of disease and response to therapy *in vitro* and *in vivo*.

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

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

This year saw the arrival of several new imaging systems within the BAIR. First we installed a new long-term time-lapse (LTTL) system, based on a Nikon Eclipse microscope, as a replacement for an older system based on a Zeiss stand. The new system is identical to our three other Nikon-based LTTL systems, which simplifies training and maintenance, and includes the Nikon PerfectFocus autofocus mechanism, which is essential for maintaining focal stability over extend time-lapse acquisitions lasting up to one week. To complement the LTTL systems, we also installed two new IncuCyte systems from Essen Bioscience. The IncuCyte is a simplified microscope that sits within a conventional cell culture incubator, and therefore trades optical resolution and sensitivity for environmental stability. The system includes software modules optimised for quantitative analysis of wound healing, apoptosis and proliferation assays. Finally, we took delivery of a new Zeiss 880 LSM confocal microscope with an attached AiryScan super-resolution detector. The AiryScan achieves super-resolution by replacing the conventional PMT detector located behind the confocal pinhole with an array detector comprising 32 pixels. By over-sampling and re-summing the detected light using Sheppard sums a resolution improvement of ~1.4 times is achieved, which can be improved to ~1.7 times using deconvolution. The main appeal of the AiryScan for super-resolution is its extreme ease of use and sensitivity for live cell imaging. The system is compatible with conventional fluorophores and does not require any specialised sample preparation.

Highlights for the year include significant BAIR contributions to several high profile Beatson publications. The first involved the use of fluorescence lifetime imaging (FLIM) to quantify hydrogen peroxide levels using the GFP-based probe HyPer (Cameron et al, 2015). Imaging data showed that hydrogen peroxide levels are elevated in migrating cells, especially in cellular protrusions rich in filamentous actin. Furthermore, grey-level co-occurrence matrix (GLCM) analysis of immobilised actin gels was used to show that oxidation of cofilin by hydrogen peroxide inhibits actin filament severing. This technique has proven to be particularly useful for evaluating differences in the properties of filament-comprised gels. For example, we also applied GLCM to stratify human pancreatic ductal adenocarcinoma (PDAC) tumour samples based on the second harmonic signal (SHG) generated by collagen (Miller et al, 2015). PDAC is characterised by dense tumour stroma, and it was found that features such as tumour stage, lymph node positivity and vascular invasion could be predicted on the basis of the GLCM score.

Publications listed on page 82

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

www.beatson.gla.ac.uk/bioinformatics

Head

Gabriela Kalna

Informaticians

Ann Hedley

Matthew Neilson

We provide support for a range of research projects that require computational methods, advanced statistical analyses and mathematical modelling.

Despite the continuing demand for data analysis 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 or the clear presentation of results for use in theses and publications.

Our team focuses on exploratory data analysis, with the ultimate goal of providing insights that enhance our understanding of cancer biology. We offer routine processing of RNA and DNA sequencing data, including RNA differential expression analysis and splicing, and DNA copy number variations. In addition, we provide 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 and Statistics Toolboxes). We frequently 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. One of our routine tasks involves submitting lists of genes and metabolites for functional annotation, clustering, enrichment, ontology and pathway analysis using Ingenuity Pathway Analysis and GeneGo Meta Core. We also employ the Oncomine Research Premium Edition database and the Oncomine Gene Browser to satisfy the demands of researchers who wish to make use of publicly available datasets. Many tasks (such as motif incidence and isoform identification) require the use of online databases, and we regularly write customised data-mining scripts that download the relevant data and extract the appropriate information.

Over the last year, we have been involved in a number of projects that integrate data from RNAseq, siRNA screens and metabolomics. In a project led by Hing Leung and supported by a Prostate Cancer Foundation Challenge Award, we are examining gene expressions and genomic mutations of prostate cancer patients treated with docetaxel chemotherapy in an effort to identify prognostic biomarkers that predict chemotherapy sensitivity and *de novo* chemotherapy resistance. In addition, we will be analysing the metabolic profiles of prostate cancer cells and the contribution of metabolic alterations to chemotherapy resistance. In another project with Henry Däbritz and Eyal Gottlieb, we are developing RNA sequencing and untargeted metabolomics analyses of renal cancer clinical specimens.

Our computational model for pseudopod-centred cell migration and chemotaxis has been updated to include a signal-amplification term, which enables simulated cells to automatically adapt their chemotactic sensitivity in accordance with the concentration of any surrounding stimulus. In the presence of relatively high ambient concentrations, the signal-amplification term gives rise to receptor desensitisation, which allows the simulated cells to remain motile; in contrast, when the ambient concentration is relatively low, the signal-amplification term has the effect of boosting the gradient-sensing capabilities of the simulated cells. In a related development, this same computational model is being used (in conjunction with wet lab work) to test the hypothesis that phagocytosis and chemotaxis are driven by the same underlying process.

We aim to ensure that appropriate statistical methods are used and presented in publications. We provide an annual talk covering this topic, and offer advice on experimental design, statistical techniques and data presentation. We are also involved in teaching at the MSc course in Cancer Sciences, and provide personalised training in the use of specific methods, programming in R and Bioconductor.

Publications listed on page 86

METABOLOMICS

www.beatson.gla.ac.uk/metabolomics

Head

Gillian Mackay

Scientific Officer

Niels van den Broek

Targeted approaches to metabolomics and metabolite profiling can be used to demonstrate changes in metabolic pathways in cancer cells. We are part of Eyal Gottlieb's Cancer Metabolism Research Unit and work closely with the scientists in his and Karen Vousden's, Jurre Kamphorst's and Alexei Vasquez's groups.

We also support several other research groups within the Beatson, measuring metabolites in their samples. We have well established targeted LC-MS methods, where we measure approximately 100 metabolites per sample. For metabolite profiling, especially for clinical samples, we are investigating LC-MS methods and data analysis approaches using Nonlinear Dynamics' Progenesis QI software, to identify more metabolites that are changed in cancers.

With our targeted approach to metabolomics, we analyse a range of sample types including cells, medium, plasma, urine, cerebrospinal fluid, tumour and other tissues. We have identified over 300 metabolites in various samples on our LC-MS platform, by matching accurate mass and retention time with commercial standard compounds. In one analysis, we can determine a broad range of metabolites of different classes, including amino acids, organic acids, sugars, phosphates (glycolysis and pentose phosphate pathways), nucleotides and cofactors (such as CoA, NADH). We have also developed new LC-MS methods for specific applications, such as deoxynucleosides, using different LC columns and SIM mass spectrometry analysis, for improved sensitivity. We provide advice on sample preparation and training for data analysis using Thermo Scientific TraceFinder software.

Experiments using stable isotope tracers (often labelled 13C glucose in the medium) enable us to examine the intracellular kinetics and the proportional distribution of many metabolites produced from the tracer. We can calculate metabolite exchange rates between cells and the medium in which they are grown. Our methods for investigating cell metabolism using stable isotope tracers, including sample preparation, LC-MS analysis and quantification, have been published as a chapter in *Methods in Enzymology* (Mackay et al, 2015).

Metabolite profiling can be used to look for novel metabolic changes, by identifying compounds showing different abundances in cancer cells. We have developed two different LC methods for metabolite profiling to try to increase our coverage of the human metabolome. With Progenesis QI software, metabolites observed from the LC-MS/MS analysis are identified using various factors such as accurate mass, adducts, isotopes and fragmentation spectra, comparing with the Human Metabolome Database (HMDB) and other databases. The fragmentation spectra are compared with an *in silico* fragmentation database, to increase the confidence of the metabolite identification. This approach can be used with different types of samples and we are currently progressing with clinical samples.

We are also developing methods for GC-MS and GC-MS/MS analysis using our Agilent triple quad mass spectrometer. The method of sample preparation is more complex than for LC-MS, as samples need to be deriviatised to allow them to be volatile in the GC. We have methods in place for fatty acids, amino acids and acetate.

We have three Thermo Scientific LC-MS systems with their high resolution, accurate mass, Orbitrap technology. One is an Exactive mass spectrometer and the other two (Q-Exactive and Q-Exactive Plus) have increased sensitivity and the additional feature of fragmentation capability. These are complemented with our Agilent GC-MS/MS triple quad instrument.

Publications listed on page 89

NUCLEAR IMAGING

www.beatson.gla.ac.uk/gaurav_malviya

Head
Gaurav Malviya

Scientific Officer
Agata Mrowinska

In recent years, PET imaging techniques have gained wide acceptance for monitoring response to cancer therapy at the molecular level. The strength of PET lies in its high sensitivity and capacity to precisely quantify radiotracer distribution, while MRI allows acquisition of high-resolution anatomical images with superior soft tissue contrast. Both are established imaging modalities providing a wide variety of potential applications in themselves, and much more so when combined together.

Nuclear imaging involves the administration of tiny amounts of radioactive tracer followed by functional imaging using PET (positron emission tomography). A sequentially acquired MRI (magnetic resonance imaging) scan helps localise radioactive tracer uptake within the body. MRI provides superior quality images due to its high intrinsic soft tissue contrast, consequently giving us better anatomical information than CT (computed tomography), especially in abdominal tumour models, such as pancreatic, prostate, colon and intestinal cancers. MRI is also advantageous due to its lack of ionising radiation, and a wider range of acquisition sequences. Therefore, this year we acquired the UK's first preclinical dual modality PET/MRI imaging system. This new scanner has already been installed in our BSU and we are about to start several exciting cancer imaging projects. PET/MRI imaging and specific probes will allow us to make non-invasive, longitudinal assessments of biological processes, such as tumour metabolism, cell proliferation, angiogenesis, hypoxia and receptor dynamics.

In addition, we have increased our imaging armamentarium beyond ^{18}F-FDG and performed PET imaging using a wide variety of radiolabelled probes including ^{18}F-FLT (for cellular proliferation) and ^{18}F-NaF (for bone lesions). We also imaged lipid synthesis using ^{18}F-FAC (fluoroacetate) in a colon cancer model, in collaboration with David O'Hagan (University of St Andrews), Eyal Gottlieb, Gerry Gillen (NHS GG&C) and Kurt Anderson. Furthermore, cell proliferation arrest, following rapamycin treatment, was evaluated by ^{18}F-FLT PET scans in KC PTEN mice, in collaboration with Jennifer Morton and Owen Sansom.

Well-validated radiotracers may help provide proof-of-concept for target modulation in early stage research of novel therapeutics by helping test the underlying hypothesis, informing the rational selection of dose and schedule, aiding decision making, and possibly explaining or predicting therapy outcomes. Therefore, we have collaborated with Sally Pimlott (NHS GG&C), Anthony Chalmers and Andrew Sutherland (University of Glasgow), who have developed a novel translocator protein imaging PET tracer, ^{18}F-AB5186, which 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 novel tracer in a glioblastoma mouse model. Additionally, with the same group, we also evaluated other novel PET tracers - ^{123}I- FZ044 (for SPECT imaging) and ^{18}F-FZ236 (for PET imaging) - for poly ADP ribose polymerase (PARP) imaging. These tracers are particularly important because PARP inhibitors are being investigated in clinical trials as a means of sensitising tumours to chemotherapy.

In near the future, we are aiming to establish our new PET/MRI scanner and begin some exciting projects with novel radiotracers for imaging the tumour microenvironment in genetically engineered mouse models.

Publications listed on page 89

PROTEOMICS

www.beatson.gla.ac.uk/proteomics

Head
Sara Zanivan
(see page 44)

Scientific Officers
Sergio Lilla
David Sumpton

The Proteomics facility has strong expertise in using high-resolution mass spectrometry in combination with highly accurate quantification approaches and data analysis. We work in collaboration with research groups at the Beatson and externally to integrate state-of-the-art mass spectrometry (MS)-based proteomics approaches to their research in order to answer basic questions of cancer biology, thus contributing to the progress of cancer research.

We are actively working to use and develop MS-based proteomic platforms to help researchers better understand the mechanisms that regulate various aspects of cancer. To do this, the facility is well equipped with three LC-MS systems, LTQ Orbitrap Velos and LTQ Orbitrap Elite, which are coupled to Easy-nLC systems, and a 5600 triple TOF system, which is coupled to an Eksigent UPLC. We are also looking forward to using the newest Q-Exactive HF, which will be installed in 2016.

We have a number of dedicated software platforms: the MaxQuant software package for highly accurate quantitative analysis and a Mascot server for protein identification. We also use Skyline for the analysis of pRM data. Finally, we use Perseus and Scaffold for data compilation, analysis and dissemination.

During 2015, we worked with many groups at the Beatson to address a wide variety of questions including; single protein identification, identification of post-translational modifications on a single protein, protein-protein interactions, identification of kinase substrates, and quantitative global proteomics of two and three-dimensional cell culture systems as well as tumour tissue samples.

We are continuously striving to improve the methods we currently have in place in order to enrich the quality of the data that the facility can provide. We have recently introduced high pH reverse phase LC fractionation for in-depth proteomic analysis, which we will be optimising for low sample amount, and we are working to improve our expertise in the quantitative analysis of post-translational modifications.

Publications listed on page 87

FUNCTIONAL SCREENING

www.beatson.gla.ac.uk/emma_shanks

Head
Emma Shanks

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Kay Hewit[1]
Lynn McGarry

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Daniel James

Graduate Student
Matthew Davidson

[1] CRUK Glasgow Centre

The RNAi Screening facility couples high throughput RNA interference 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.

During 2015, we ran a total of eight screening campaigns in collaboration with both Beaton and CRUK Glasgow Centre groups. To date, the facility has generated over 2.2 million data points across 18,000 screening plates, which represents a substantial increase in output over the past year. Enhanced processing, analysis and reporting workflows have enabled us to deal with this increase in data. Furthermore we have the ability to link more seamlessly to HCS images during the analysis and reporting steps.

During the past year, we have also revised the way in which we engage in new projects, including revision of our proposal submission process and implementation of a proposal review panel.

Our screens continue to be a balance between those using high throughput screening approaches to address initial target identification and those using focused screening approaches to validate a shortlist of candidates identified from other approaches, i.e. bioinformatics or genomic sequencing. The latter is supported using bespoke, custom-built libraries and has seen us transition from the frequently used 'pooled' screening method, to using a 'deconvoluted' approach, i.e. screening composite siRNAs individually rather than together. Collectively, this approach has proved extremely informative in generating a rich and detailed dataset from which key decision points can readily be made.

We have also run a number of smaller screens using siRNAs and/or drugs identified from initial screening campaigns to support hit validation in multiple tumour and non-tumour cell lines. We continue to offer support to non-project researchers wishing to use our automation and imaging capabilities, and have a seen a marked increase in this capacity also.

In 2015, we have addressed the following questions in multiple cancer backgrounds:

- The role of endocytic trafficking in response to chemotherapy agents used to treat Her2+ breast cancer
- A focused approach to targeting non-small cell lung carcinoma
- A drug repurposing approach using 1) drugs affecting neurotransmitter pathways and 2) our wider drug repurposing collections to target TKI-resistant chronic myeloid leukaemia (CML)
- A chemotherapeutic repurposing approach to circumvent ponatinib resistance in CML
- Identification of a chemotherapeutic selectively efficacious under targeted amino acid depletion in colorectal cancer
- Investigating the mechanism of action underlying mutant TP53 addiction
- Synthetic dosage lethal partners in colorectal cancer
- Identification of radiosensitising partners as therapeutic candidates for glioblastoma (an externally funded collaboration between CRUK and Bayer)

Furthermore, through a longer-term collaboration with the Bryant group, we have developed a screening workflow to incorporate shRNA-based screens. The output of this screen necessitated development of automation in cell and three-dimensional spheroid culture processes, viral titre concentration and infection, all in 96-well format, as well as programmed confocal three-dimensional image stitching and analysis of the compound images.

The facility is currently exploring the integration of CRISPR/Cas9 technology into its workflow, and we have recently engaged in our first proof-of-concept model with which to evaluate this. While CRISPR/Cas9 screening will expand our existing resources, the emphasis will be on expanding our skillsets to efficiently process and analyse data arising from these screens.

Publications listed on page 92

TRANSGENIC MODELS OF CANCER

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Our lab uses *in vivo* models to study how signalling and metabolic pathways contribute to cancer development within a physiological setting. Particular projects in the lab have focused on the role of RUNX1, RUNX2 and MCL-1 in breast and prostate cancer.

Preclinical models

Epithelial cancers are three-dimensional entities that comprise cancer cells as well as other components of the tumour microenvironment including immune cells, stromal fibroblasts and blood vessels. In order to better understand cancer development, it is necessary to faithfully mimic such tumour complexity, which is not always possible in tissue culture. Therefore, our lab focuses on the use of xenograft, allograft and genetically engineered models. Using these models we can recreate the genetics known to cause cancer, or test the role of putative cancer-causing pathways. For example, this year, we collaborated with Eyal Gottlieb's group to explore how ACSS2 promotes tumourigenesis (Schug et al, 2015) and with Peter Adams' lab showing that oncogenic Ras promotes mitotic disruption and cell survival in senescent cells (Dikovskaya et al, 2015). We continue to collaborate with Eyal and Karen Vousden to study cancer cell metabolism, and for this purpose we have just taken delivery of a TSE PhenoMaster System of metabolic cages. It has been a busy year and so we were delighted to welcome Sandeep Dhayade to the group, who, along with Susan Mason and Dimitris Athineos, will work closely with the other groups at the Institute to exploit our *in vivo* models.

RUNX1 and RUNX2 in breast and prostate cancer

Nicholas Rooney and Alessandra Riggio in the lab are studying how a particular family of genes called the *RUNX* genes are involved in breast cancer. We have shown previously that high expression of these genes is associated with poorer prognosis in breast cancer patients (McDonald et al, 2014, Ferrari et al, 2014) and find that RUNX2 is involved in the regenerative potential of mammary cells, with a putative role in stem cell/progenitor cell function (Ferrari et al, 2015). The WNT signalling pathway is a key driver in breast and other epithelial cancers, and RUNX2 is specifically upregulated in WNT positive mammary tumours. It is relevant then, that RUNX2 is involved in mediating WNT pathway activation in mammary cells. There is a paradox in how RUNX1 may affect breast cancer - either by promoting or suppressing tumourigenesis - and we think that this may be dependent on the tumour subtype or the presence of other genetic lesions in the tumour. So, we are currently exploring this using breast cancer cell lines and genetic models, as well as the observation that RUNX2 may have a role in the tumour microenvironment.

Anne McKillop, a clinical research fellow, submitted her thesis this year in which she investigated the role of RUNX1 and RUNX2 in prostate cancer. Anne showed that higher levels of RUNX2 in patient samples associated with more aggressive tumours and poorer prognosis, and that deleting *Runx2* could slow tumour initiation in genetically altered models of prostate cancer. Conversely, loss of RUNX1 correlated with increased tumourigenesis and poorer patient survival.

MCL-1 as a novel target in breast cancer

In a project funded by a pilot grant from Breast Cancer Now, Kirsteen Campbell has been looking at the role of the *MCL-1* gene in breast cancer. *MCL-1* appears to be particularly prevalent in triple negative breast cancers for which no targeted treatment options are currently available. So it is very exciting that Kirsteen has shown that MCL-1 expression correlates with poorer prognosis in patients and that by targeting this gene it is possible to slow the growth of tumours using *in vivo* models.

Publications listed on page 82

TRANSGENIC TECHNOLOGY

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Graduate Student
Nicola Laprano

We make use of molecular genetic techniques to understand gene function and how this is altered in the development of cancer.

By using this type of technology we can generate precise genetic changes in cancer models that mimic those observed in the analysis of human cancers. The accumulation of a number of these genetic changes allow us to generate sophisticated models that are capable of more accurately modelling human cancers.

Generating cancer models with stem cells

Using embryonic stem (ES) cells allows the development of a number of useful tools for analysing the function of genes in the progression of cancer. The high rates of recombination allow us to modify endogenous genes in a highly predictable manner and make precise changes in genes that mirror those observed in human cancers. Once the desired genetic changes are introduced, the ES cells can then be differentiated into numerous different cell types from different tissues. Consequently, altered gene function can be investigated in a number of situations.

We are currently working in collaboration with a number of groups on projects using these technologies to introduce a variety of different types of genetically altered alleles including conditional knockouts, point mutations and inducibly expressed marker genes. Furthermore, we now routinely use genome editing technologies, which has improved the range and efficiency with which we can introduce alterations in targeted alleles.

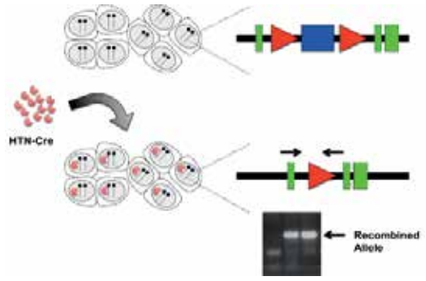

Figure 1
HTN-Cre deletion of a selectable marker in embryonic stem cells. Addition of His-TAT-NLS-tagged Cre protein to embryonic stem cells cultured in vitro leads to modification of a targeted allele by removal of a stop cassette. The Cre recombinase protein is taken up by the cells and directly translocates to the nucleus. There it mediates recombination at loxP sites (red triangles) in the targeted allele removing the Neo selectable marker cassette (blue box). PCR across the remaining loxP site confirms the removal of the cassette.

Enhancing recombination technology in models

In addition to making use of existing technologies, we are also updating and improving the methodologies that we use routinely, to allow experiments to work more efficiently. Site-specific recombinases such as Cre and Flp are routinely used for a variety of modifications to targeted alleles. This can be for the deletion of a critical exon to make a mutation in a floxed allele, or for the removal of a stop cassette or selectable marker. These recombinases are normally introduced into cells in the form of DNA and, although this process is frequently successful, it can be slow and laborious as it is often necessary to remove the recombinase DNA before the modified allele can be used in downstream applications.

In order to circumvent this, we have been making use of a recently developed method to allow the transduction of proteins directly into the nucleus of gene targeted cells. A full-length fusion protein was generated that contains an amino-terminal 11–amino acid protein transduction domain from the human immunodeficiency virus TAT protein. Proteins also have a 6xHis-tag to allow purification and a nuclear localisation signal. The Cre protein containing these three N-terminal modifications was termed HTN-Cre. Cells were exposed to HTN-Cre in tissue culture medium at 37oC for 30 minutes. Following exposure to HTN-Cre, DNA was isolated and analysed for recombination at the loxP sites (Fig. 1). Analysis showed successful recombination in greater than 90% of the cells. This demonstrated that we are able to induce recombination successfully using protein instead of DNA. Development of techniques such as this allow us to maximise efficiency and welfare, and enable us and our collaborators to get the most of out the models we develop as rapidly as possible.

Publications listed on page 93

BEATSON ASSOCIATES

UNIVERSITY OF GLASGOW

Peter D. Adams - Epigenetics of Cancer and Ageing
David Bryant - Molecular Control of Epithelial Polarity
Jurre Kamphorst - Cancer Metabolomics
Daniel J. Murphy - Oncogene-Induced Vulnerabilities
Stephen Tait - Mitochondria and Cell Death

EPIGENETICS OF CANCER AND AGEING

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Our 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 cancer incidence. While age is the biggest single risk factor for most cancers, the reason for this is current poorly understood.

Research in our lab primarily focuses on the interface between ageing, epigenetics and cancer. We are particularly interested in age-associated epigenetic changes that lead to increased cancer incidence with age. In addition, we are planning systems biology-based approaches to comprehensively understand age-associated epigenetic changes and to exploit this knowledge to develop interventions to promote healthy ageing and suppression of age-associated diseases, including cancer. We employ *in vitro* models, mouse models, human tissues and state-of-the-art analyses of large epigenomics datasets. We like to do collaborative, multidisciplinary research.

Why does cancer incidence increase with age?

The incidence of many cancers increases with age. Indeed, age is the biggest single risk factor for many cancers. However, surprisingly, the reason for this is poorly understood. Although current models for development of different cancers often quite accurately describe the step-wise acquisition of cancer-causing genetic and epigenetic changes from initiating lesion to advanced metastatic cancer (e.g. the so-called 'Vogelgram' for colon cancer), current models do not satisfactorily explain the decades-long time lag from birth to the earliest detectable neoplastic lesion. A substantial reduction in the incidence and deaths from cancer will likely ultimately come from a better understanding of cancer as a disease of ageing, thereby facilitating risk assessment, early detection and chemoprevention.

Focusing on epigenetics, we hypothesise that progressive age-associated changes to epigenetic programming and chromatin predispose to cell dysfunction and disease, and hence cancer. Conversely, we hypothesise that dedicated mechanisms operate to maintain dynamic chromatin in a cell type-specific steady state that is a pre-requisite for maintenance of cell phenotype, function, healthy ageing and suppression of disease. We have coined the term 'chromostasis' for these presumptive chromatin homeostatic mechanisms. Together, ongoing and future studies in the lab aim to: 1) map age-associated changes in chromatin structure, organisation and regulation; 2) assess the impact of these changes on cell phenotype, function and dysfunction, and predisposition to cancer; 3) understand the mechanisms employed by the cell to achieve chromostasis and so healthy ageing; and 4) ultimately exploit this knowledge in development of biomarkers to assess cancer risk and strategies for early detection and chemoprevention. Specifically, our studies in this area are based on a number of chromatin regulators implicated in cancer and ageing: namely, H4K20me3 and its histone methyltransferase SUV420H2; H4K16ac and its histone acetyltransferase MOF; histone chaperone HIRA and its substrate histone H3.3; DNA methylation and DNA methyltransferase DNMT3b. In all cases, we are employing a combination of cell culture studies, mouse models (including wild type and long lived Ames dwarf) and human tissues to define the role of these regulators in healthy ageing and suppression of cancer. We will also perform screens for novel regulators of chromostasis.

Epigenetic control of ageing and cancer

In collaboration with Ronen Marmorstein (Philadelphia), we have dissected the structure-function relationships between HIRA and its

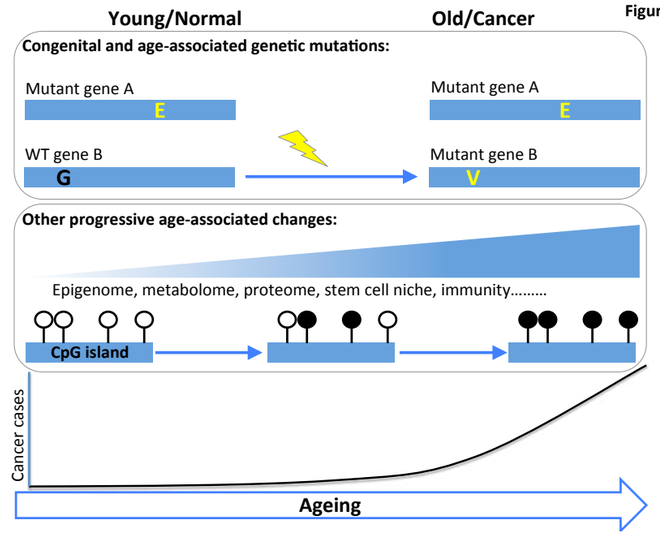

Figure 1

Figure 1
A model depicting the many factors proposed to contribute to age-associated increase in cancer incidence. These include congenital genetic mutations and mutations acquired in early growth and development; genetic mutations acquired during ageing; progressive age-associated changes to dynamic molecular, cellular and tissue systems, e.g. the epigenome, the metabolome, the proteome, stem cell niches, the immune system and others.

Figure 2
A section through human skin showing activated Wnt signalling in a dermal nevus. Red, beta-catenin (nuclear/cytoplasmic = activated Wnt signalling); Green, S100 (melanocytes); Blue, DAPI (DNA).

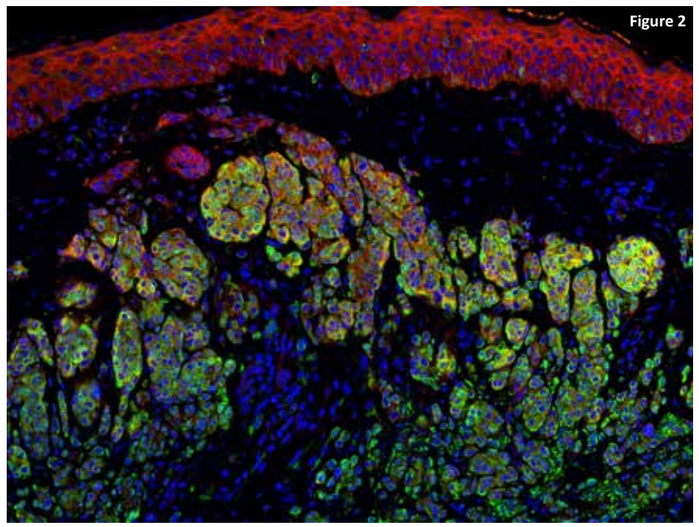

Figure 2

binding partners, UBN1, CABIN1 and ASF1a and substrate histone H3.3 (Zhang et al, 2005, Rai et al, 2011, Banumathy et al, 2009). This included a crystal structure of the HIRA/ASF1a interaction surface and more recently the UBN1/histone H3.3 interaction surface (Tang et al, 2006, Daniel Ricketts et al, 2015). We were the first to describe the distribution of the HIRA complex across the mammalian epigenome (Pchelintsev, 2013). In functional studies, we have demonstrated the role of this DNA replication independent histone chaperone complex in control of chromatin in non-proliferating senescent cells (Zhang et al, 2005, Rai et al, 2011, Banumathy et al, 2009, Ye et al, 2007, Rai et al, 2014). These studies have been facilitated by the mouse monoclonal and rabbit polyclonal antibodies that we have made to all subunits of the complex. More recently, we have generated the first conditional knockout mice of HIRA, UBN1 and CABIN1 and are using these to establish *in vivo* functions (Rai et al, 2014). Of particular note, we have revealed a function for HIRA in promoting healthy ageing and suppression of cancer (Rai et al, 2014).

Chromostasis

We coined the term 'chromostasis' to describe the presumptive homeostatic mechanisms that confer epigenetic stability and suppression of age-associated disease, including cancer, over the life course (Rai et al, 2014, Adams et al, 2015). Maintenance of cell phenotype and suppression of disease, including cancer, over the life course depends on a high level of epigenetic stability. However, since chromatin is inherently dynamic (Sedivy et al, 2008, Cruickshanks & Adams, 2011), this steady state stability likely reflects a challenge for the cell. Therefore, presumptive 'chromatin homeostasis' or 'chromostasis' mechanisms are predicted to actively maintain an epigenetic steady state over the life course, thereby suppressing age-associated disease (Rai et al, 2014, Adams et al, 2015). We have shown that histone chaperone HIRA is one such factor that contributes to epigenetic stability in non-proliferating cells (Zhang et al, 2005, Ye et al, 2007, Rai et al, 2014). Inactivation of HIRA predisposes mice to oncogene-induced neoplasia (Rai et al, 2014).

A 'tug of war'

We have demonstrated a 'tug of war' between tumour suppressive oncogene-induced senescence and oncogenic activated Wnt signalling in melanocytic neoplasia (Ye et al, 2007, Adams & Enders, 2008). The balance between these tumour suppressive and oncogenic activities determines the efficiency of senescence-mediated tumour suppression. For example, we showed that in oncogene-expressing melanocytes a low level of activated Wnt signalling promotes benign nevus formation (Pawlikowski et al, 2013). However, a high level of activated Wnt signalling, caused by germline sequence variants, promotes giant congenital nevi in the form of congenital melanocytic nevus (CMN) syndrome (Pawlikowski et al, 2015). In a mouse model that closely recapitulates the human genetics, we showed that activated Wnt signalling and an activated Ras oncogene (*NRasQ61K*) cooperate to drive CMN syndrome, and this is suppressed by acute post-natal treatment with MEK inhibitors (Pawlikowski et al, 2015). Based on these studies our collaborator Veronica Kinsler is preparing to test MEK inhibitors in babies afflicted by CMN syndrome.

Publications listed on page 94

MOLECULAR CONTROL OF EPITHELIAL POLARITY

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The loss of normal tissue architecture is a common feature of most epithelial cancers, the most predominant cancer type. The genomic revolution of the last 10 years has led to advances in identifying signalling pathways and genetic alterations driving cancer progression. However, how such changes affect cell polarity to turn highly organised tissues into disarrayed tumours remains poorly understood. Our research aims to understand the loss of cell polarity that is germane to cancer progression.

Our group focuses on epithelial polarity, particularly in lung and prostate tumours. We focus on two molecular pathways: the role of ARF GTPases (and their regulators and effectors, which we call the 'ARFome'), and the role of the cell surface protein, Podocalyxin. Both molecules are highly overexpressed in metastatic prostate cancer tumours. We utilise advanced microscopy techniques to characterise how these molecules control normal and aberrant prostate epithelial polarisation. Our ultimate aim is to investigate these molecules as potential future biomarkers of prostate cancer in patients, and possible targets for future therapeutic interventions.

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 methods have received much attention in recent years as methods for making spheroids from a variety of sources 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.

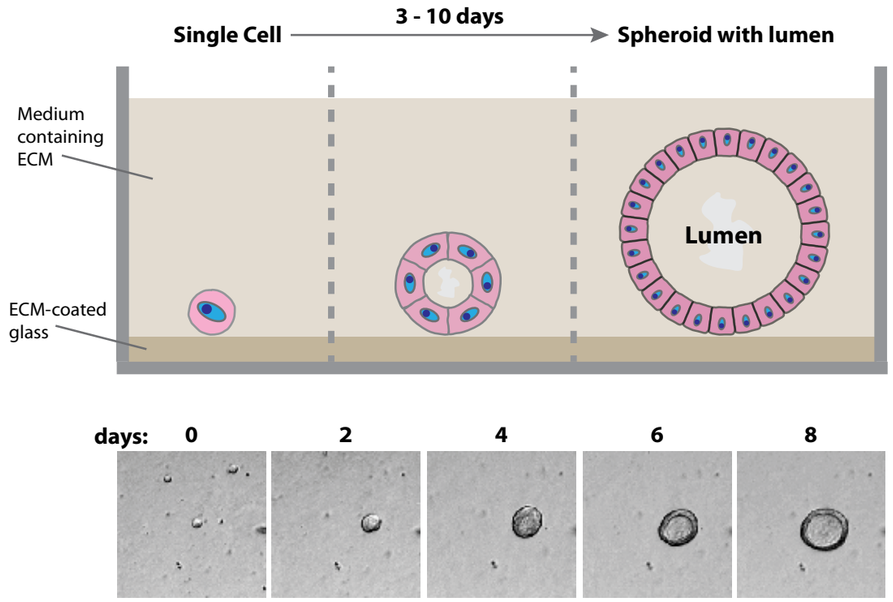
Our group is developing systems for examining gene manipulation in spheroids in a medium-to-high throughput fashion, to model genetic changes seen in patients with metastatic cancer. We are working closely with the Emma Shanks and the RNAi Screening facility to develop lentiviral shRNA arrays to manipulate gene expression in spheroids in a massively parallel format. 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

A normally 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 extracellular matrix. Such organisation is often lost as cancer progresses, though the exact mechanisms as to why remain elusive.

ARF GTPases make up a small, six-member family of GTPases that regulate aspects of vesicle trafficking and actin organisation. In collaboration with the groups of Shehab Ismail and Emma Shanks, we are systematically analysing the entire ARFome - all known ARF GTPases, activating (GEF) and inhibitory (GAP) proteins, as well as effectors – to determine their

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



function in prostate cancer cell invasion and spheroid formation. Notably, many such genes are altered in metastatic tumours of prostate cancer patients. Our current studies are aimed at investigating how ARF GTPases regulate normal cell polarisation in spheroids, and how they participate in the acquisition of invasive behaviours when oncogenes are expressed.

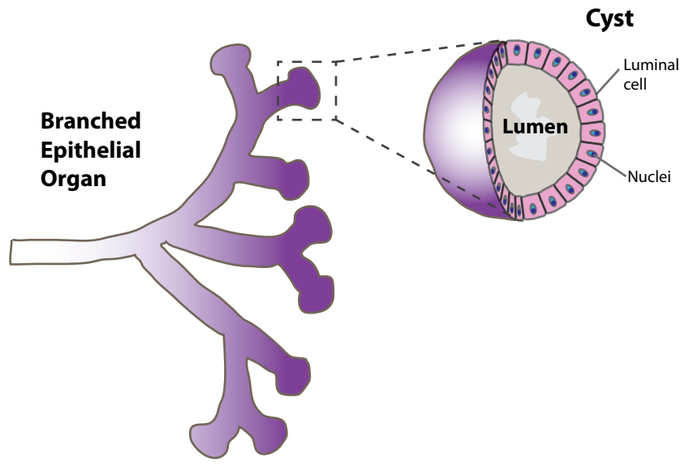
Function of Podocalyxin in cancer cell invasion

We previously demonstrated that Podocalyxin is a heavily glycosylated apical membrane protein with fundamental roles in cell polarity (Bryant et al, 2014). Podocalyxin expression is highly amplified in prostate, breast and pancreatic tumours, and is mutated in familial forms of prostate cancer. However, the mechanisms by which it contributes to cancer migration and invasion are largely unclear. Our work aims to examine, at the molecular level, how Podocalyxin controls polarisation of spheroids, and drives invasion and metastasis in prostate cancer.

In collaboration with Sara Zanivan's group, we are using proteomic approaches to understand the protein processing of Podocalyxin, its interacting network of proteins, and viral genetic techniques to manipulate its function. These studies are aimed at providing a detailed molecular dissection of Podocalyxin function and its contribution to cancer progression *in vitro* and *in vivo*.

Publications listed on page 95

Figure 2
Three-dimensional cultures of cells to form cysts (also called spheroids or organoids) allow us the model the basic structure of epithelial organs in the laboratory. This enables us to understand and manipulate factors that are altered in cancer patients and model their effect on tissue organisation.



CANCER METABOLOMICS

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[1] joint with Owen Sansom, support from Rosetrees Trust

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Cancer is a disease of uncontrolled cell proliferation. This means that cancer cells need to rearrange their metabolism to acquire the energy and cellular building blocks necessary for growth. In addition, the cells need to adapt to metabolic stress (limited nutrients and oxygen) imposed by the tumour microenvironment. Our hypothesis is that many of these metabolic adaptations are tumour-specific and therefore provide opportunities for new, selective therapeutic strategies. To characterise these metabolic alterations and to quantitatively understand them, we use state-of-the-art analytical methodology (i.e. metabolomics, lipidomics) and stable isotope tracing approaches, in combination with molecular biology.

Acetyl-CoA synthetase 2 recaptures acetate produced by cancer cells

Fatty acids are among the cellular building blocks that are required for the synthesis of cell membranes during cell division. The precursor of fatty acids is acetyl-CoA, which is mainly derived from glucose carbon. However, in hypoxic (i.e. low oxygen) conditions most glucose carbon is shunted towards lactate, causing a reduction in carbon available for production of acetyl-CoA and hence fatty acid synthesis. Exactly how cells cope with this is still incompletely understood. We previously reported a drastic increase in the apparent production of nucleo-cytosolic acetyl-CoA from acetate in hypoxic conditions (Kamphorst et al, 2014). This observation supported the findings made by others, including Zach Schug and Eyal Gottlieb, that the enzyme responsible for making acetyl-CoA from acetate, acetyl-CoA synthetase 2 (ACSS2), promotes tumour growth during metabolic stress (Schug et al, 2015). The favoured explanation for these results is that acetate acts as an alternative carbon source for nucleo-cytosolic acetyl-CoA when production from glucose is limiting.

To better understand the actual acetate carbon contribution to fatty acid synthesis, we next sought to quantify acetate uptake by cancer cells. Such measurements have thus far remained scarce because no robust and sensitive methods for analysis of acetate existed. We therefore developed a novel protocol based on derivatization of acetate with a propyl moiety and GC-MS analysis. This method is fast (<2 minutes preparation per sample, 3 minutes MS analysis time) and has a good limit of quantitation of 1 nmol, facilitating analysis of physiologically relevant acetate levels. Analysis of ^{13}C-acetate that was added to the medium of both normoxic and hypoxic cancer cells showed robust uptake in both conditions. Surprisingly, in both conditions cells simultaneously released unlabelled, ^{12}C-acetate into the medium. Thus, cells take up but also release acetate, indicating that significant exchange occurs. From experiments with cell lines expressing varying levels of ACSS2, we determined that its expression correlates with uptake of (^{13}C)-acetate but also inversely correlates with release of endogenously produced (^{12}C)-acetate. In combination with knockdown experiments, we demonstrated that ACSS2 not only functions to take up exogenous acetate but also to recapture endogenously produced acetate. Net uptake of acetate was observed in cell lines with very high ACSS2 expression, thereby confirming that acetate can be used as a carbon source. Curious to learn where the endogenous acetate was coming from, we discovered that a substantial fraction (40-50%) of released acetate came from histone deacetylation, and subsequently found that ACSS2 is crucial for maintaining histone acetylation during hypoxia, which has been shown to be important for survival and growth-promoting gene expression (Lee et al, 2014). In conclusion, acetate metabolism is highly dynamic (Fig. 1) and a relevant novel function of ACSS2 is recapturing acetate produced by the cancer cell. Follow-up work will include integrating data from labelling experiments with mathematical models of acetyl-CoA and acetate metabolism (together with Alexei Vazquez), as well as evaluating the effect of inhibiting ACSS2 in notoriously hypoxic tumours, such as pancreatic cancer.

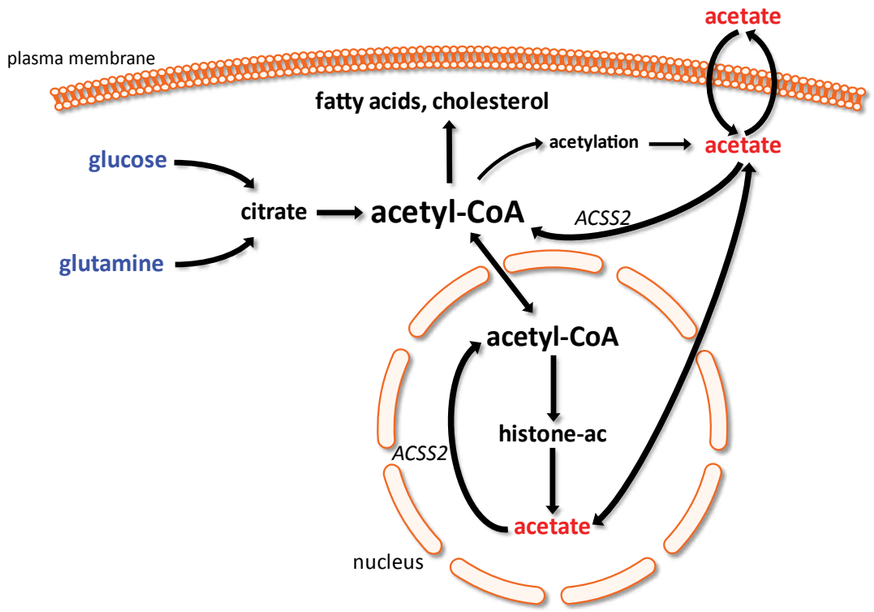

Figure 1
Acetate metabolism is highly dynamic. Schematic representation of acetate metabolism based on our new data. We found that cells not only take up exogenous acetate but they also release acetate following deacetylation reactions. A substantial fraction (40-50% depending on the cell line) of the released acetate is derived from histone deacetylation. A novel function of acetyl-CoA synthetase 2 (ACSS2) is to recapture the released acetate.

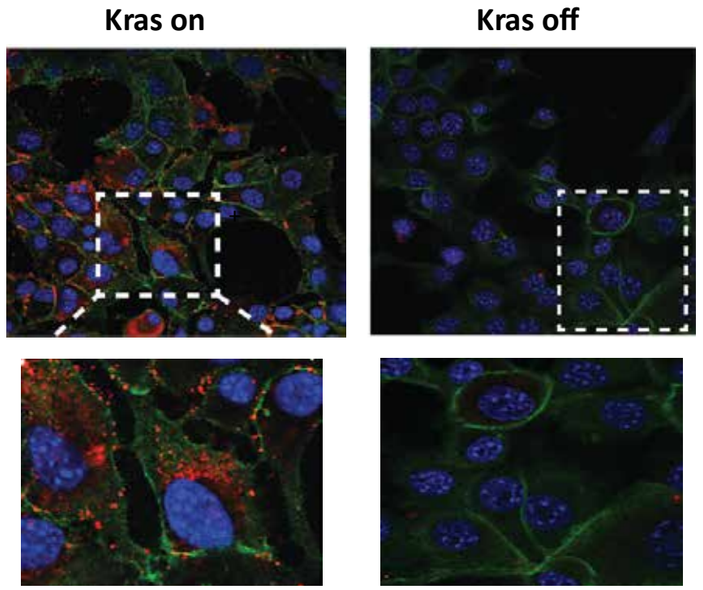

Figure 2
Macropinocytosis depends on oncogenic KRAS. Representative images of TMR-dextran internalisation through macropinocytosis, using doxycycline-inducible KRASG12D expressing cells (Lee et al, 2014). Macropinocytosis is evident in doxycycline treated (KRAS on) cells but absent in cells without doxycycline (KRAS off). Red is TMR-dextran (established marker for macropinocytosis), green is b1-integrin (to visualise cell periphery), blue is DAPI (to visualise nucleus).

Understanding the regulation and functional importance of macropinocytosis

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, 2013). Macropinocytosis is especially prominent in cell culture and *in vivo* models of pancreatic ductal adenocarcinoma (PDAC), a poorly vascularised, lethal KRAS-driven malignancy. We later showed that it also occurs in human PDAC tumours (Kamphorst et al, 2015). However, our knowledge of how macropinocytosis is regulated is limited, but will be crucial for studying its functional importance. Earlier reports have demonstrated that KRAS plays a role in the induction of macropinocytosis. To study the dependence of macropinocytosis on KRAS in a pancreatic setting, we used inducible KRAS PDAC cells (DePinho). Using these cells we could validate that in PDAC cells macropinocytosis is entirely KRAS-driven (Fig. 2). In collaboration with Owen Sansom, we are now investigating pathways downstream of KRAS and preliminary results suggest that the induction of macropinocytosis is through the GTPase RAC1. The next step will be to investigate if any of the RAC1 guanine nucleotide exchange factors (GEFs) selectively regulate macropinocytosis.

Publications listed on page 95

ONCOGENE-INDUCED VULNERABILITIES

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[1] joint with Owen Sansom

Oncogenic signalling profoundly alters how cells respond to their environment, typically putting tumour cells under tremendous pressure to reconcile conflicting cues. For example, tumour cells must re-organise their metabolic pathways to balance competing needs for biosynthetic precursors with energetic homeostasis, commonly while surviving in a milieu of limiting oxygen and nutrients.

Our overarching hypothesis is that such oncogene-induced biological perturbations can be exploited for cancer therapy, even in the absence of direct suppression of driver oncogenes. We use deregulated MYC as our paradigm oncogene coupled with a mixture of candidate and RNAi-based screening approaches to identify induced vulnerabilities in vivo and in vitro and are actively exploring several strategies for selective elimination of cells that overexpress MYC.

MYC in cancer

Overexpression of the transcription factor MYC occurs in a huge number of human cancers arising from almost every tissue type. MYC overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability or indeed increased signalling through upstream regulatory factors such as Ras, Notch or beta-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.

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. Curiously, the AMPK-related kinase ARK5/NUAK1 is also required for maintenance of ATP homeostasis in cells wherein MYC is overexpressed. NUAK1 plays a specific role in MYC-dependent activation of AMPK and also maintains mitochondrial respiratory capacity. Suppression of NUAK1 thus impairs the ability of MYC overexpressing cells to respond to declining ATP levels while simultaneously depriving cells of ATP-generating capacity, suggesting that suppression of NUAK1 may be an effective means to selectively kill cancer cells with high levels of MYC expression. We are presently testing this hypothesis in a mouse model of aggressive colorectal cancer, given that MYC deregulation is a near universal feature of such cancers.

In collaboration with the lab of Wei-Xing Zong (Rutgers University), we have also uncovered a non-canonical role of MYC that leads to production, rather than consumption, of glutamine. MYC-dependent transcription of the DNA demethylases, TET3 and TDG, drives demethylation of the glutamine synthetase (GLUL) promoter, thereby driving net glutamine production. *In vivo*, glutamine synthetase positive tumour cells are found interspersed amongst tumour cells that do not express GLUL, suggesting a potential symbiotic relationship whereby glutamine-producing cells support the viability of glutamine-consuming populations.

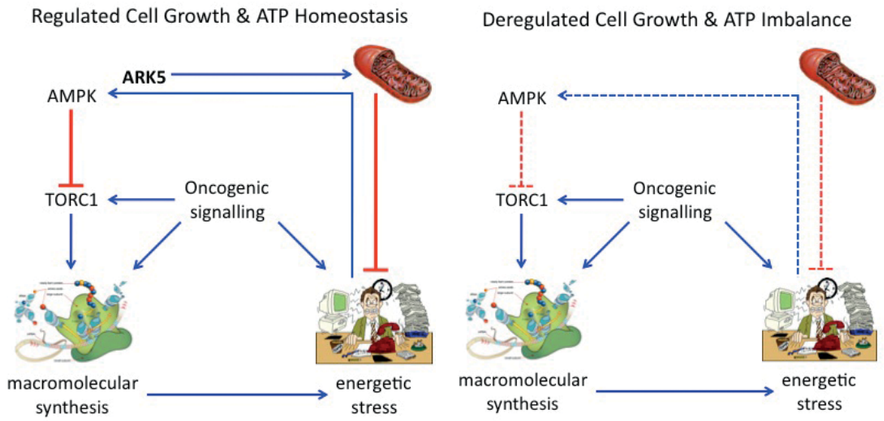


Figure 1
Induced dependencies need not reflect direct molecular interactions. Oncogene-induced cell growth, typically requiring signal transduction via the mechanistic target of rapamycin (mTOR) pathway, drives rampant ATP consumption, which must be compensated for through increased cellular intake of 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 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 the Gartnaval and Queen Elizabeth University Hospitals, 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.

Failed apoptosis has a role in tumour promotion

Apoptosis is triggered by a catastrophic permeabilisation of the mitochondrial outer membrane (MOMP), releasing cytochrome c and SMAC into the cytosol, followed by the consequent activation of caspases that effectively digest the cell from the inside. In collaboration with Stephen Tait's group, we showed that MOMP can also occur in a small percentage of mitochondria *in vivo* as well as *in vitro*. Such 'minority MOMP' drives low-level activation of caspases without reaching the threshold level of activity that is required to kill the cell. Caspase activation in turn activates the DNA endonuclease CAD, resulting in DNA damage and genomic instability, and the consequent acquisition of genetic mutations can cooperate with loss of tumour suppressors to accelerate cell transformation.

Major developments in 2015

While making excellent progress on our main projects (the role of NUAK1 in MYC-driven cancer and the mechanisms of early stage lung cancer progression), my team made important contributions to a number of collaborative studies that resulted in three primary publications, as well as a state-of-the-art review in FEBS Journal. My first graduate student, Nathiya Muthalagu successfully defended her PhD thesis in April and began her postdoc under joint mentorship with Owen Sansom, funded through a Worldwide Cancer Research grant. Sarah Neidler also successfully defended her PhD thesis in November. Meera Raja departed for a second postdoc at the University of Cardiff, while Bjorn Kruspig joined the group after defending his thesis at the Karolinska Institute. Masters degree student Martina Brucoli joined us from the University of Milan and Bachelors degree student Silvija Svambaryte completed an excellent summer internship with us.

Publications listed on page 96

MITOCHONDRIA AND CELL DEATH

www.beatson.gla.ac.uk/stephen_tait

Group Leader

Stephen Tait

Royal Society University Research Fellow

Beatson Associate

Research Scientists

Florian Bock

Gabriel Ichim[1]

Joel Riley

Graduate Student

Evangelos Giampazolias[2]

Visitor

Jonathan Lopez

[1] EMBO Fellowship

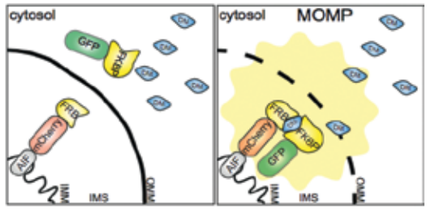
[2] CRUK Glasgow Centre

Cell death restrains tumourigenesis at multiple stages, ranging from transformation to metastasis. As such, cell death must be inhibited to allow cancer to develop. Importantly, cell death sensitivity also dictates how well anticancer therapies work. Our research focuses upon mitochondrial regulation of cell death. Mitochondria are key cellular organelles that power life but are also essential for the major form of regulated cell death called apoptosis. We aim to understand how mitochondria control cell death and define how this process is deregulated in cancer. The ultimate goal is use our findings to improve and develop new ways 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 cause 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. Importantly, this can be exploited therapeutically, allowing newly developed anticancer therapies that target these apoptotic blocks. For example, BH3-mimetic compounds exploit the Bcl-2 addicted state of certain cancer cells allowing tumour cell-specific killing.

Figure 1
A new method to detect permeabilised mitochondria. Left: mCherry, fused to a dimerisation domain (FRB), is targeted onto the mitochondrial inner membrane (IMM), while GFP, fused to a dimerisation domain (FKBP), is cytosolic. In healthy mitochondria, the intact mitochondrial outer membrane (OMM) prevents the two proteins from interacting. Right: In permeabilised mitochondria and in the presence of dimeriser (DM), GFP can now access the mitochondrial inner membrane and relocalise.



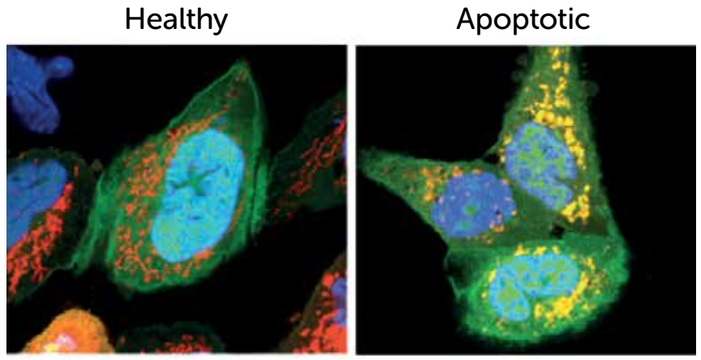
Sub-lethal stress can engage limited mitochondrial permeabilisation

Because of the key role MOMP has in apoptotic cell death, we developed a new approach that specifically detects permeabilised mitochondria. In this method, we co-express dimerisable mCherry targeted to the mitochondrial inner membrane together with cytosolic, dimerisable eGFP (Fig. 1). In the presence of chemical dimeriser, and following mitochondrial permeabilisation, GFP dimerises with mCherry thereby relocalising onto the mitochondrial inner membrane - this specifically reports permeabilised mitochondria (Fig. 2). Using this method we have found that sub-lethal apoptotic stresses can engage MOMP in a limited cohort of mitochondria - what we call minority MOMP. Importantly, cells can tolerate minority MOMP and survive.

A dark side of apoptotic signalling – DNA damage and genome instability

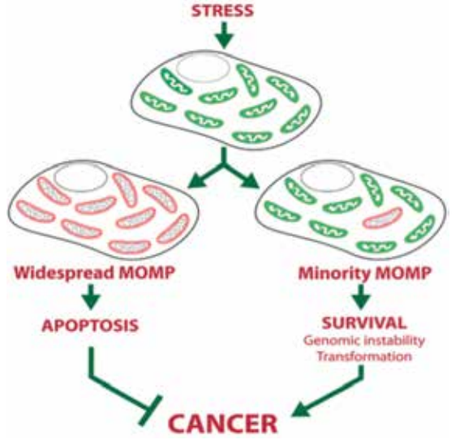
From our finding that stress can engage limited mitochondrial permeabilisation, we next wanted to investigate what its consequences were. Using different approaches we have found that minority MOMP can trigger caspase activation, again without killing the cell. During apoptosis, caspases cleave hundreds of different proteins leading to rapid cell death. One of the cellular hallmarks of apoptosis is massive DNA fragmentation that is mediated via caspase-driven activation of a protein called CAD (caspase-activated DNAse). We have found that minority MOMP driven-caspase activity can cause DNA damage and genome instability by activating CAD. Extending this, we find that repeated sub-lethal apoptotic stress, engaging minority MOMP and caspase activity, can actually promote cellular transformation. Collectively, our data argues that apoptosis has a dark side such that its initiation, but failure to execute can actually promote cancer. This tumour promoting effect of apoptosis may be important at various stages of tumourigenesis, ranging from initiation to tumour evolution. Moreover, it could also contribute to the acquisition of acquired resistance to targeted anticancer therapies, many of which act by engaging apoptosis. We predict that by understanding how tumour cells can engage minority MOMP/caspase activity and survive, we should be able to devise strategies to push them over the edge and kill them.

Figure 2
Lighting up mitochondria during cell death. Cells co-expressing mitochondrial targeted mCherry-FRB (red) and cytosolic GFP-FKBP (green) were left untreated (left) or treated with actinomycin D (to trigger apoptosis, right) in the presence of dimeriser. Specifically in apoptotic cells, GFP relocalises onto permeabilised mitochondria as visualised by co-localisation of mCherry and GFP.



Publications listed on page 96

Figure 3
Apoptosis and cancer: a double-edged sword. Left: Stresses engaging widespread MOMP kill the cell. By culling cells at risk of transformation, apoptosis forms an effective barrier to tumourigenesis. Right: Sub-lethal stresses engage minority MOMP allowing cell survival. Such initiation of apoptosis, but failure to execute promotes DNA damage and has oncogenic effects.



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 major new equipment for Flow Cytometry with the acquisition of a new Attune NxT cell analyser and Information Services have installed a new main storage server. In addition, the Histology Service and Molecular Technology have replaced routine processing equipment and Central Services have increased capacity for media preparation with an additional Mediaclave. Building Facilities have been active with a number of projects to refit laboratory areas and create additional laboratory space.

Building Facilities

Alistair Wilson, Alex Kernahan, Michael Daly

Building Facilities manage the outsourced provision of catering, cleaning and janitorial services. We provide maintenance support for the Beatson Institute buildings, manage alterations and refurbishments, and ensure that all statutory compliance issues with respect to buildings and systems are up-to-date. Use of the online helpdesk facility continues to be an effective means of logging reactive calls for maintenance and repair.

This year there have been projects to install new constant temperature rooms for the Structural Biology research groups, and upgrades to external drainage and security controls. There have also been several laboratory refits to facilitate large equipment installations such as the PET/MRI scanner, metabolic monitoring system and relocation of the X-ray generator. Space continues to be a major issue; a project to increase storage for the Drug Discovery unit has been completed, and an expansion project to provide additional laboratory space for Histology is scheduled to begin soon.

Central Services

Margaret Laing (Supervisor), Barbara Cadden, Elizabeth Cheetham, Dilhani Kahawela, Kirstie McPherson, Jonny Sawers, Linda Scott, Tracy Shields, Rose Steel, Robert Storey

Central Services perform a wide range of duties that are essential for the support of the research groups across the site. This includes cleaning and sterilisation of reusable laboratory glassware, sterilisation of consumables, and preparation of tissue culture solutions, bacterial culture media and Drosophila food. An additional Mediaclave was purchased this year to meet increased demand for Drosophila food. The team is also responsible for the cleaning and checking of equipment 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.

Flow Cytometry

Tom Gilbey, Tim Harvey

This year, major changes have occurred in the provision of the flow cytometry service at the Beatson. This has been separated from the Beatson Advanced Imaging Resource and combined with that in the neighbouring Wolfson Wohl building to create a new comprehensive Flow Cytometry service under the umbrella of Research Facilities.

The aim of the service is to provide advice, assistance and education to researchers on all aspects of flow cytometry. This can be divided into four distinct areas: pre-acquisition, when users can discuss their needs with facility staff before starting their experiments and get advice on which instruments, fluorochromes, controls and gating strategies to use; training and acquisition, so that before users begin working on their own they are trained to use the analysers correctly; post-acquisition, when facility staff can help with data analysis; and education, with regular seminars and workshops to outline the capabilities of the service.

The service has a number of cell analysers and sorters: the BD FACSCaliburs for simple flow experiments such as cell cycle and proliferation studies; the BD FACSVerse for routine flow experiments; and the Attune NxT (Applied BioSystems) for complex flow analysis; the BD FACSAria for sorting red fluorescent proteins; and the BD FACSAria Fusion for sorting samples requiring level 2 containment, such as virus transfected cells and human primary cells.

Histology Service

Colin Nixon, Barbara Cadden, 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 Beatson 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. Once received tissue samples are trimmed, processed and then orientated into paraffin wax blocks to facilitate tissue sectioning and staining. 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. All paraffin wax blocks sectioned are stained with haematoxylin and eosin in order to allow a general analysis of cell morphology and structure. After initial analysis more specialised histology stains can also be performed. If desired, mouse tissue microarrays can also be constructed using paraffin embedded tissue blocks. Where fixation is not required, 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.

A comprehensive immunohistochemistry service is also offered using our two large capacity autostainers. We are continually expanding the number of optimised antibodies that can be batch-stained using these. 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 so that individuals can perform staining to an acceptable and consistent standard.

Where no antibody is available for immunohistochemistry or a more specific technique is required, we 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, along with the necessary retrieval and amplification kits. The Institute also has a Leica LMD6500 laser microdissection system that allows subpopulations of cells to be isolated from histology slides under. Both DNA and RNA can be retrieved from tissue sections for downstream analysis.

We have installed a fully automated, large capacity Leica SCN400F slide scanner capable of capturing bright-field or fluorescent images. This allows high quality digital images to be scanned, stored and, if required, quantitative analyses performed. The image analysis software allows staining to be scored within specified areas. Consultation prior to designing the algorithms for this is necessary to make sure scoring is accurate and reproducible.

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 over 400 PCs on site comprising a mixture of Windows computers, Apple Macs and Linux machines. All have central authentication, central file storage and printer sharing. The servers have in excess of 500 TB of online storage with frequent backups, to provide support for microscopy, DNA sequencing and mass spectrometry data. Our central data store has been replaced with a multi-headed Isilon storage system, offering a much faster access to data and the ability to expand the network file space easily and in a manageable way.

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 El Capitan is being rolled out across the site, while holding Windows computers to Windows 7. All email 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.

Migration from physical servers to virtual ones using VMware® is complete. We offer 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 provide virtual desktops for OS X users requiring access to Windows-based packages. We are currently rolling out Microsoft vApps for specific proteomics and metabolomics applications.

Significant investment has been put into creating 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 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 network upgrade is currently underway, allowing computers to access central data file stores at 10Gb. Backbone network speeds are being moved up to 40Gb. This will allow us to allow users to access research data much faster.

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 lecture theatre.

Laboratory Management

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

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, relevant staff members have received certified training in manual handling, radiation safety and first aid. We also obtained a defibrillator for use within the Institute.

A major function of Laboratory Management is the overseeing of shared equipment servicing, replacement and the purchase 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 safety procedures, particularly those relating to COSHH. In addition, assistance is given to users to enable smooth processing of their orders and to ensure they comply with any requirements for import, and with any regulatory requests.

This year, one of our long-term stores staff, Joe McFadden retired and we welcomed a new member of staff, Michael Kilday who is proving a useful and valued member of the stores team. 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 researchers. This includes negotiating free samples from suppliers to enable the researchers 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, Jillian Murray, 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. Last year, we purchased an Illumina NextSeq500 platform, which has enabled us to sequence libraries at a lower cost with increased data output and a faster turnaround time. We also offer library production for NGS. Protocols currently used are ChIP-seq and RNA-seq. We recently purchased an Agilent Tapestation 2200 (to replace the Agilent Bioanalyser 2100) to validate the quality of RNA and libraries.

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 also 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 researchers. 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; an enzyme immunoassay against the four most common species of mycoplasma; and a colorimetric microplate assay to detect 16S ribosomal mycoplasma RNA.

Cell-derived matrices from Tiff 5 cells are prepared to order for researchers 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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Karen Blyth (page 62)
Transgenic Models of Cancer

Primary Research Papers

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Laura Machesky (page 34)

Migration, Invasion and Metastasis

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Gillian Mackay (page 57)

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Emma Shanks (page 60)

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Alexei Vazquez (page 20)

Mathematical Models of Metabolism

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Karen Vousden (page 22)
Tumour Suppression

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Jurre Kamphorst (page 70)
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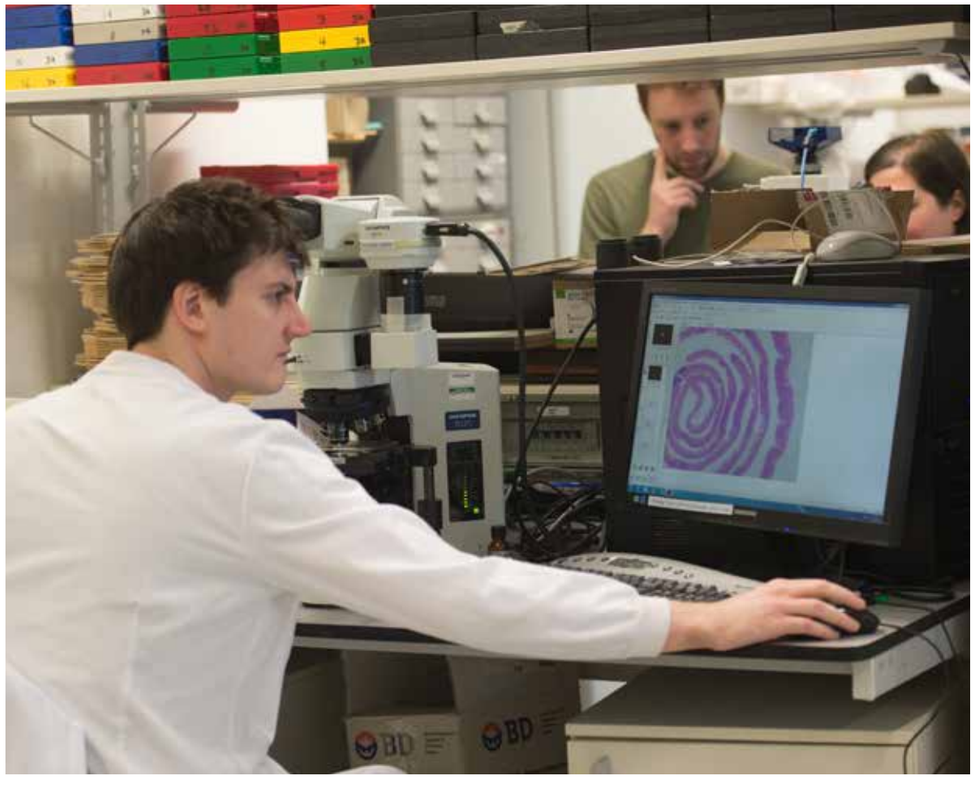

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Daniel J. Murphy (page 72)

Oncogene-Induced Vulnerabilities

Primary Research Papers

Bott AJ, Peng I-C, Fan Y, Faubert B, Zhao L, Li J, Neidler S, Sun Y, Jaber N, Krokowski D, Lu W, Pan J-A, Powers S, Rabinowitz J, Hatzoglou M, Murphy DJ, Jones R, Wu S, Girnun G, Zong W-X. Oncogenic Myc induces expression of glutamine synthetase through promoter demethylation. ***Cell Metabolism*** 2015; 22: 1068-77

Ichim G, Lopez J, Ahmed SU, Muthalagu N, Giampazolias E, Delgado ME, Haller M, Riley JS, Mason SM, Athineos D, Parsons MJ, van de Kooij B, Bouchier-Hayes L, Chalmers AJ, Rooswinkel RW, Oberst A, Blyth K, Rehm M, Murphy DJ, Tait SW. Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. ***Mol Cell*** 2015; 57: 860-72

Leikam C, Hufnagel AL, Otto C, Murphy DJ, Muhling B, Kneitz S, Nanda I, Schmid M, Wagner TU, Haferkamp S, Brocker EB, Schartl M, Meierjohann S. In vitro evidence for senescent multinucleated melanocytes as a source for tumor-initiating cells. ***Cell Death Dis*** 2015; 6: e1711

Monteverde T, Muthalagu N, Port J, Murphy DJ. Evidence of cancer-promoting roles for AMPK and related kinases. ***FEBS J*** 2015; 282: 4658-71

Other Publications

Murphy DJ, Muthalagu N. BIM's up first. ***Mol Cell Oncol*** 2015; 2: e975083

Stephen Tait (page 74)

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

Dikovskaya D, Cole JJ, Mason SM, Nixon C, Karim SA, McGarry L, Clark W, Hewitt RN, Sammons MA, Zhu J, Athineos D, Leach JD, Marchesi F, van Tuyn J, Tait SW, Brock C, Morton JP, Wu H, Berger SL, Blyth K, Adams PD. Mitotic stress is an integral part of the oncogene-induced senescence program that promotes multinucleation and cell cycle arrest. ***Cell Rep*** 2015; 12: 1483-96

Ichim G, Lopez J, Ahmed SU, Muthalagu N, Giampazolias E, Delgado ME, Haller M, Riley JS, Mason SM, Athineos D, Parsons MJ, van de Kooij B, Bouchier-Hayes L, Chalmers AJ, Rooswinkel RW, Oberst A, Blyth K, Rehm M, Murphy DJ, Tait SW. Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. ***Mol Cell*** 2015; 57: 860-72

Other Publications

Giampazolias E, Tait SW. Mitochondria and the hallmarks of cancer. ***FEBS J*** 2015. doi: 10.1111/febs.13603. Epub 2015 Nov 25

Lopez J, Tait SW. Mitochondrial apoptosis: killing cancer using the enemy within. ***Br J Cancer*** 2015; 112: 957-62

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 Emma Woodham from Laura Machesky's group. Her work has focused on the role of the protein Cdc42 in melanoblast migration and proliferation during mouse embryogenesis. See Woodham & Machesky, Current Opinion in Cell Biology 2014; 30: 25-32 for more details of the background to this project.

Theses

Brzezinska, Elspeth (2015) The role of β-catenin in prostate cancer tumourigenesis and treatment resistance [PhD thesis, University of Glasgow, Beatson Institute]

Clarke, Cassie (2015) An investigation into the role of the initiator methionine transfer RNA in cell migration and tumour growth [PhD thesis, University of Glasgow, Beatson Institute]

Gundry, Christine (2015) An investigation into the role of Rab-Coupling Protein and EphA2 during cancer cell migration [PhD thesis, University of Glasgow, Beatson Institute]

Heath, Nikki (2015) An investigation into the role of microvesicles in mutant p53 invasive gain-of-function [PhD thesis, University of Glasgow, Beatson Institute]

Julian, Linda (2015) Investigating the role of caspase cleavage of ROCK1 in tissue homeostasis and tumour development [PhD thesis, University of Glasgow, Beatson Institute]

Lee, Pearl (2015) The regulation of TIGAR [PhD thesis, University of Glasgow, Beatson Institute]

Marciano, Gabriele (2015) Structural characterisation of FACT histone chaperone complex [PhD thesis, University of Glasgow, Beatson Institute]

Muthalagu, Nathiya (2015) Understanding the mechanism of Myc induced vulnerabilities [PhD thesis, University of Glasgow, Beatson Institute]

Neidler, Sarah (2015) Identification of tumour progression genes in a mouse model for human non-small cell lung cancer [PhD thesis, University of Glasgow, Beatson Institute]

Nobis, Max (2015) In vivo analysis of pharmacodynamics and disease progression in mouse models of cancer using FLIM-FRET [PhD thesis, University of Glasgow, Beatson Institute]

Piscitello, Desiree (2015) Activated Akt pathway promotes genome instability through suppression of Mre11 [PhD thesis, University of Glasgow, Beatson Institute]

Salgueiro, Pedro (2015) Isolation and characterization of Lgr5 stem cells and cancer stem cells [PhD thesis, University of Glasgow, Beatson Institute]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Control of Cell Polarity and Movement in Cancer
5 - 8 July 2015
Bute Hall, University of Glasgow

Scientific Committee: Jim Norman, David Bryant, Robert Insall, Laura Machesky, Owen Sansom, Marcos Vidal

This year's conference highlighted 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. On the first evening, the Colin Thomson Memorial Keynote Lecture, sponsored by Worldwide Cancer Research (WCR), was given by Ian Macara (Vanderbilt) who described the role of the cell polarity protein PAR3 in breast cancer growth and invasion.

There was a range of excellent speakers throughout the meeting, including Alexis Gautreau, Andrew Reynolds, Yvone Tang, Guillaume Jacquemet Simona Polo, Paolo Armando Gagliardi, Kerstin Klinkert, Maria Tello-Lafoz, Martin Baumgartner and Paulo Ribeiro who gave selected short talks sponsored by WCR.

Following the poster session, Kerstin Klinkert (Paris), working on the role of Rab35 GTPase in the establishment of apical polarity, and Fanny Jaulin (Villejuif), who showed evidence of collective invasion in colorectal cancer, were jointly awarded the AMSBIO-Trevigen sponsored prize. The meeting was generously co-sponsored by Cancer Research UK and Worldwide Cancer Research.

The 2016 meeting will focus on the latest developments in the generation of sophisticated animal models of cancer, and emphasise how diverse aspects of the human disease can now be faithfully recapitulated in the laboratory (see www.beatson.gla.ac.uk/conf for more details and to register).

Postdoc Meeting

Scottish Biomedical Postdoctoral Researcher Conference
24 April 2015
CRUK Beatson Institute

Organisers: Beatson Institute and IGMM, University of Edinburgh Postdoctoral Societies

In April, postdocs from Glasgow and Edinburgh hosted a one-day meeting at the Institute sponsored by the Biochemical Society. This included research talks and poster presentations as well as keynote lectures by Mark Bradley (Chair of Chemical Biology, University of Edinburgh) and Susie Mitchell (Programme Director, Glasgow City of Science) and a careers/networking session. The postdocs plan to make this an annual event.

Beatson Retreat

An Institute retreat was held in our lecture theatre on 15th May, giving everyone a chance to hear about the latest, exciting work being done by researchers here. In a change from the usual format, there were elevator talks from some of the our students and postdocs, highlight talks by senior postdocs, a 'speed dating' event with groups coming together on the day to formulate and present ideas for potential grant applications, and a Question Time-style panel discussion. This all made for a very informative day and was nicely rounded off with a pub quiz and barbeque in the evening.

Beatson Models Symposium

The third in our series of mouse models symposia was held at the Institute on 17th November. This enabled all those who use the Biological Services Unit to present a talk or poster about their work. We were also delighted to welcome Lionel Larue (Paris) to give the plenary seminar at the meeting.

Open Evenings

As in previous years, to coincide with National Science and Engineering Week, we held an opening evening this year on 18th March.

Daniel Murphy (Group Leader), Rachana Patel (Associate Scientist), Linda Julian (PhD Student), Iain Macpherson (Clinical Lecturer) and Rob Fordham (Postdoctoral Scientist) described their work on breast and bowel cancers, how mouse models are used in cancer research, and how cancer affects the whole body and dying cancer cells their surroundings. Along with the lab tours and demos provided by our enthusiastic volunteers, this made for an engaging evening for the visitors.

We also hosted a similar open evening for Cancer Research UK supporters on 16th September, which was a great opportunity to highlight some of the work we are able to do with the funds they raise.

Poster for 2016 conference

SEMINARS AT THE BEATSON INSTITUTE

The following seminars were held at the CRUK Beatson Institute during 2015.

January

Giorgio Seano, Department of Radiation Oncology, Harvard Medical School, Boston, USA

Ciara Metcalfe, Department of Discovery Oncology, Genentech, San Francisco, USA

Claus Jorgensen, Cancer Research UK Manchester Institute, UK

Xavier Barril, Department of Physicochemistry, University of Barcelona, Spain

February

Marcin Iwanicki, Harvard University, Cambridge, USA

Simon Leedham, Wellcome Trust Centre for Human Genetics, University of Oxford, UK

March

Heidi Welch, Babraham Institute, Cambridge, UK

Mario Tschan, Institute of Pathology, University of Bern, Switzerland

Sandrine Etienne-Manneville, Pasteur Institute, Paris, France

Kim Jensen, Biotech Research and Innovation Center, University of Copenhagen, Denmark

Elizabeth Veal, Institute for Cell and Molecular Biosciences, University of Newcastle, UK

April

Richard Lamb, Molecular and Clinical Cancer Medicine, University of Liverpool, UK

May

Hans-Uwe Simon, Institute of Pharmacology, University of Bern, Switzerland

John Silke, Walter Eliza Hall Institute, Melbourne, Australia

June

Philippe Juin, University of Nantes, France

David Wedge, Wellcome Trust Sanger Institute, Cambridge, UK

Georges Lacaud, Cancer Research UK Manchester Institute, UK

July

Rosie Sears, Brenden-Colson Center, Oregon Health & Science University, Portland, USA

Arndt Friedrich Siekmann, Max-Planck Institute for Molecular Biomedicine, Münster, Germany

Armando Del Río Hernández, Department of Bioengineering, Imperial College London, UK

August

Simone Fulda, Institute for Experimental Cancer Research in Pediatrics, Goethe University, Frankfurt, Germany

Wael Rabeh, Assistant Professor of Practice of Chemistry, NYU Abu Dhabi, United Arab Emirates

David Carling, MRC Clinical Sciences Centre, Imperial College London, UK

Silvia von Karstedt, UCL Cancer Institute, University College London, UK

Emma Vincent, Department of Physiology, McGill University, Montreal, Canada

Christina Mitchell, Monash University, Australia

September

Stefan Raunser, Department of Structural Biochemistry, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany

Raul Rabadan, Department of Systems Biology, Columbia University, New York, USA

Gurinder Atwal, Cancer Center, Cold Spring Harbor Laboratory, New York, USA

Erik Schäffer, University of Tübingen, Germany

Berni Wehrle-Haller, Department of Cellular Physiology and Metabolism, University of Geneva, Switzerland

Anne Wheeler, Institute of Genetics and Molecular Medicine, Edinburgh, UK

Lucy O'Brien, Molecular and Cellular Physiology, Stanford University School of Medicine, USA

Andrew Gilmore, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

October

Francesco Cecconi, Department of Biology, University of Rome Tor Vergata, Rome, Italy / Danish Cancer Society Research Center, Copenhagen, Denmark

Brooke Emerling, Meyer Cancer Center, Cornell University, New York, USA

Gina de Nicola, Beth Israel Deaconess Medical Center, Boston, USA / Weill Cornell Medical College, New York, USA

Caroline Lewis, Massachusetts Institute of Technology, Cambridge, USA

Kostas Tokatlidis, University of Glasgow, UK
Christian Frezza, MRC Cancer Unit, University of Cambridge, UK

Holly Brown-Borg, University of North Dakota, Grand Forks, USA

Véronique Proux, Institut Jacques Monod, Paris, France

Matthias Mann, Department of Proteomics and Signal Transduction, Max-Planck Institute of Biochemistry, Martinsried, Germany

November

Martin Glennie, Cancer Sciences Unit, Southampton General Hospital, UK

Vera Gorbunova, University of Rochester, New York, USA

Leo Carlin, Inflammation, National Heart & Lung Institute, Imperial College London, UK

Olivia Rossanese, Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, UK

Carlos Sebastian, Harvard University, Cambridge, USA

December

Robert Wilkinson, Oncology Research, MedImmune, Cambridge, UK

Seth Coffelt, Netherlands Cancer Institute, Amsterdam, The Netherlands

Sir Paul Nurse, President, Royal Society, UK

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.

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

Finance

Peter Winckles ACA DChA,
Gary Niven CA, Richard Spankie CA, Nicki Kolliatsas, Jacqui Clare, Karen Connor, Lynn Wilson, Patricia Wylie

Human Resources

Angela Stuart CIPD, Elaine Marshall CIPD

Our Finance and Human Resources teams provide professional advice, support and services for everyone located at the Beatson Institute. The Director of Finance & Administration is also the Company Secretary.

Recently, our Finance team has been strengthened by the recruitment of two qualified and experienced staff members in the roles of Management Accountant and Systems Accountant. These two key posts supplement the experienced finance staff. Collectively they are responsible for the provision of all financial management information to Institute senior managers, budget holders and the Board of Directors (Trustees). They work with all managers, providing them with relevant information, to help manage and control their budgets and, thus, ensure that decisions concerning the allocation of the Institute's research resources provide the best use of stakeholders' funding. In 2015, the team implemented the first new finance system for over 15 years to ensure that the Institute's business processes and management information reporting were fit for purpose. The Institute has grown significantly over the last five to 10 years and in order to deliver CRUK's scientific research vision there was a need for fundamental change. The team put in a tremendous effort, which will improve financial reporting for the Institute as a whole.

Our professionally qualified Human Resources team provides support and advice across a wide spectrum of issues including recruitment, performance management, learning and development, pay and grading, absence management, employee relations and employee engagement. They also play a vital role in providing managers with the necessary budgetary and legal information with the aim of helping managers to more effectively manage their key resources - our people.

In addition, the Finance and Human Resources teams are also an important link in our association with the University of Glasgow through the coordination and administration of grants, payment of suppliers and staffing.

Administration

Sheila McNeill *(PA to Professor Vousden), Rebecca Gebbie, Eleanor Best, Barbara Laing, Sarah Price, Catriona Entwistle*

The Administration team, headed by the PA to the Director, provides an extensive range of secretarial and office services. These include assisting with staff recruitment, organising travel and accommodation, internal and external seminar arrangements, organisation of the Institute's annual conference, workshops and open evenings, 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 PhD, *Catherine Winchester PhD*

The Scientific Administration team consists of two former postdoctoral researchers who support scientists at the Beatson Institute by editing publications including the Scientific Report manuscripts and grants, taking minutes at a range of scientific and administrative meetings, maintaining an up-to-date website and publications database, 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 on all aspects of good practice in research. In 2015, this involved helping draft and implement publication and data management policies for the Institute.

Cancer Research Technology

Maria Lopalco PhD

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 Now, Royal Society

Martin Drysdale
Medical Research Council

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

Eyal Gottlieb
FEBS, Janssen Pharmaceutica NV, Metabomed

Danny Huang
European Community

Hing Leung
Medical Research Council

Laura Machesky
Danish Council, Medical Research Council, Pancreatic Cancer Research Fund

Jim Norman
Breast Cancer Now

Michael Olson
Medical Research Council, Worldwide Cancer Research

Kevin Ryan
Astellas Pharma Inc, EMBO, Worldwide Cancer Research

Owen Sansom
AstraZeneca, European Community, Gilead, Janssen Pharmaceutica NV, Novartis, Royal Commission for the Exhibition of 1851, 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

Sara Zanivan
Breast Cancer Now

Beatson Associates

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

Jurre Kamphorst
Rosetrees Trust

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.

Active Motif
Baljaffray Parish Church
Beckman Coulter (UK) Ltd
BioLegend
Jan Blackie, in memory of Mr Robert French
Legacy from the Estate of the Late Mr Joseph Boyle, in memory of his daughter Carol Anne Pethard
Margaret G Brown
James Campbell, in memory of his mum Anne Campbell
Clyde Travel
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Susan Donald & Andrew Kernahan, in memory of their mother Mrs Joan Kernahan
Agnes & Thomas Donaldson, in memory of friends who have died from cancer
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Christina McDougall, in memory of her husband John
GDS McIntyre
Fiona McNeill
S1 Enterprise Group, Mearns Castle High School
Legacy from the Estate of the Late Mrs Jean Merry
Legacy from the Estate of the Late Mr Roderick Morrison
Mat Morton
Mosshead Primary School
North View Housing Association, Castlemilk Family Day
Anne O'Hare, Hillpark Bowling Club
Mr & Mrs Olav Kerr's Charitable Trust
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PMV Pharmaceuticals
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Legacy from the Estate of the Late Robert Weir
West of Scotland Women's Bowling Association
William Grant & Sons
Legacy from the Estate of the Late Mr William Cluggie Wilson

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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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Electronic version of this report can be found at:
www.beatson.gla.ac.uk/annual_report

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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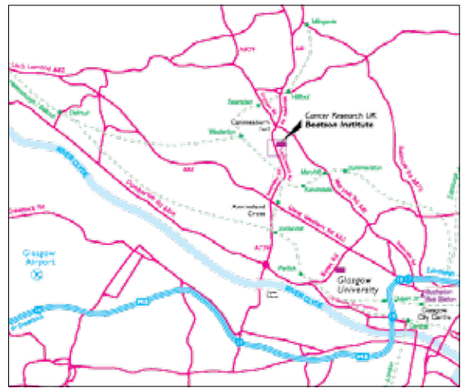
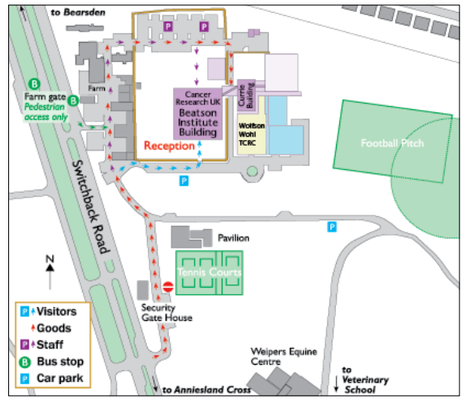
www.cruk.org





NOTES

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