SCIENTIFIC REPORT 2016

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COVER IMAGE

Yasmin Elmaghloob, second year PhD student in Shehab Ismail's group, who is studying the trafficking of Lck to the immune synapse

SCIENTIFIC REPORT 2016

BEATSON INSTITUTE

University of Glasgow

CANCER RESEARCH UK | BEATSON INSTITUTE

Cancer Research UK Beatson Institute building

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DIRECTOR'S INTRODUCTION

Interim Director of the Cancer Research UK Beatson Institute

Professor Owen Sansom FRSE

It has been a year of considerable change at the Beatson.

In July, Karen Vousden was appointed Cancer Research UK's new chief scientist, a role in which she will be responsible for overseeing the whole of CRUK's scientific research portfolio. Sadly, this means that Karen stepped down from her role as Institute Director, a post she had held since 2003. She will also be moving her research group to the Francis Crick Institute early next year. Meanwhile, until a new appointment has been made, I will be leading the Institute as interim director.

During the past 13 years, Karen has overseen a considerable expansion of the Beatson, including the move to a new, state-of-the-art building in 2008. This has enabled a number of excellent research groups focused on key aspects of cancer biology to be established here along with some key advanced technology teams to underpin their work. Overall, this has allowed the Institute to become an internationally renowned research centre of the highest quality. Karen has also continued to run a world-leading research programme studying the tumour suppressor gene p53, and been instrumental in the establishment of the CRUK Glasgow Centre, encompassing different aspects of cancer research across the city. During her time here, Karen has received a number of well-deserved awards and accolades in recognition of her work. She will be much missed by everyone here but we wish her every success in her new role and look forward to continuing interactions with her.

We welcomed several new junior group leaders this year. Leo Carlin joined us from Imperial College London as the head of our imaging facility. Leo is an expert in intravital imaging with an interest in tumour immunology. Cancer Centre-funded Beatson Associate Seth Coffelt, who studies the role of immune cells in metastasis, came to us from the NKI, while Saverio Tardito, who was a postdoc with Eyal Gottlieb, established his own laboratory focused on cancer metabolism, particularly in glioma and hepatocellular carcinoma. Finally, Tom Bird joined us as a Wellcome Trust intermediate clinical research fellow with an interest in the mechanisms that control liver regeneration.

There were a number of successes for our researchers in 2016. Two of our senior group leaders were recognised for considerable contributions to their fields - Laura Machesky was elected as a Fellow of the Academy of Medical Sciences, while Kevin Ryan was elected as a Fellow of the Royal Society of Edinburgh. The outcome of Kevin's quinquennial review, held in October, was also extremely positive. Hing Leung had his research programme successfully reviewed, and was awarded a Marie Sklodowska Curie Actions Innovative Training Network to support early career researchers in prostate cancer.

We continue to develop and support our early career researchers and this year, held very helpful mid-term reviews for two of our junior group leaders - Jurre Kamphorst and Shehab Ismail. We are particularly grateful to the external reviewers who contributed to these important reviews. A number of our postdoctoral and clinical fellows were awarded fellowships this year, including Juliana Blagih (Canadian Institutes of Health Research Fellowship), Bjorn Kruspig (Marie Sklodowska Curie Fellowship), Joshua Leach (MRC Clinical Research Fellowship), Susan MacDonald (Medical Research Scotland Daphne Jackson Fellowship), Mohammed Mansour (Royal Society Newton International Fellowship) and Johannes Meiser (DFG Individual Fellowship).

Professor Karen Vousden CBE, FRS, FRSE, FMedSci, Cancer Research UK's new chief scientist.

The CRUK Glasgow Centre was successful reviewed towards the end of the year and will be renewed at the same level as previously, allowing us to continue to provide key infrastructure support to areas of cancer research that are particularly strong in Glasgow. Our Drug Discovery Unit also received support from the CRT Pioneer Fund to work with researchers in Frederick in the USA on the optimisation of RAS inhibitors. Finally, we made a number of major funding applications with colleagues from here and elsewhere, including large CRUK Grand Challenge and Precision Panc bids, and look forward to hearing the outcome of these early next year.

We hosted a number of excellent meetings and workshops in 2016, including our own international conference, which focused on modelling cancer *in vivo*, and SEARCHBreast and FLIM-FRET-FSC workshops. These events allow us to showcase some fantastic science but also to highlight the many positives both the Institute and the city of Glasgow have to offer.

Finally, huge thanks must to go our many supporters who both remember us in the form of legacies and work so hard to raise funds for us, including especially Clyde Travel, Mosshead Primary School and the West of Scotland Women's Bowling Association who do this so tirelessly and to such effect each year. Without this and Cancer Research UK's considerable support our work would not be possible.

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

Cammareri P, Rose AM, Vincent DF, Wang J, Nagano A, Libertini S, Ridgway RA, Athineos D, Coates PJ, McHugh A, Pourreyron C, Dayal JH, Larsson J, Weidlich S, Spender LC, Sapkota GP, Purdie KJ, Proby CM, Harwood CA, Leigh IM, Clevers H, Barker N, Karlsson S, Pritchard C, Marais R, Chelala C, South AP, Sansom OJ, Inman GJ.
Inactivation of TGF-beta receptors in stem cells drives cutaneous squamous cell carcinoma. *Nat Commun* 2016; 7: 12493

This work, done in collaboration with Gareth Inman's group at the University Dundee, identifies frequent TGF-beta receptor mutations in human skin cancers and uses mouse models to demonstrate how switching off TGF-beta signalling can be a critical early event leading to the development of skin cancer. The results of the study support the idea that TGF-beta acts as a tumour suppressor, which could have significant implications for how skin cancers are treated in the future.

Clarke CJ, Berg TJ, Birch J, Ennis D, Mitchell L, Cloix C, Campbell A, Sumpton D, Nixon C, Campbell K, Bridgeman VL, Vermeulen PB, Foo S, Kostaras E, Jones JL, Haywood L, Pulleine E, Yin HB, Strathdee D, Sansom O Blyth K, McNeish I, Zanivan S, Reynolds AR, Norman JC.
The initiator methionine tRNA drives secretion of type II collagen from stromal fibroblasts to promote tumor growth and angiogenesis. *Curr Biol* 2016; 26: 755-65

Expression of the initiator methionine tRNA [tRNA(i)(Met)], which is involved in protein synthesis, is deregulated in cancer. In this paper, the authors generate a mouse expressing additional copies of tRNA(i)(Met) gene to investigate the contribution of elevated stromal tRNA(i)(Met) to tumour progression. They show that growth and vascularisation of subcutaneous tumour allografts is enhanced in these mice. Then, in a series of *in vivo* and proteomic experiments, they show that tRNAi(Met) does this by enhancing the ability of stromal fibroblasts to synthesise and secrete a type II collagen-rich extracellular matrix that supports endothelial cell migration and angiogenesis. In addition, the study finds that collagen II expression predicts poor prognosis in high-grade serous ovarian carcinoma.

Fansa EK, Kosling SK, Zent E, Wittinghofer A, Ismail S.
PDE6 delta-mediated sorting of INPP5E into the cilium is determined by cargo-carrier affinity. *Nat Commun* 2016; 7: 11366

In this paper, the authors investigate the mechanism underlying the sorting of farnesylated cargo between cilia and other cell compartments, something that is not well understood. *In vitro* experiments show that the G-proteins Arl2 and Arl3 regulate the release of farnesylated proteins shuttled by the phosphodiesterase 6 delta subunit (PDE6D), where Arl3 selectively releases ciliary proteins. Furthermore, the specific targeting of ciliary proteins to their destination depends on their strong affinity for PDE6D and the presence of active Arl3 in cilia. Structural and mutational analyses of PDE6D/cargo complexes also reveal the molecular basis of the sorting signal, which depends on the residues at the -1 and -3 positions relative to farnesylated cysteine.

Maddocks ODK, Labuschagne CF, Adams PD, Vousden KH.
Serine metabolism supports the methionine cycle and DNA/RNA methylation through de novo ATP synthesis in cancer cells. *Mol Cell* 2016; 61: 210-21

In this work, the authors consider the contribution of serine metabolism to the methionine cycle and thus to the methylation of DNA and RNA, a fundamental component of epigenetic regulation. Using stable isotope tracing, mass spectrometry and nutrient modulation of cancer cells, they show that serine not only provides one-carbon units to generate methionine for the pathway but also, surprisingly, supports it through the de novo synthesis of ATP, an important source of ATP in cancer cells. In addition, serine starvation causes a dramatic decrease in total ATP levels in rapidly proliferating cells, which can lead to changes in methyl group transfer. Overall, this paper highlights the crosstalk between one-carbon metabolism and epigenetic regulation, and its impact on cancer.

Steele CW, Karim SA, Leach JD, Bailey P, Upstill-Goddard R, Rishi L, Foth M, Bryson S, McDaid K, Wilson Z, Eberlein C, Candido JB, Clarke M, Nixon C, Connelly J, Jamieson N, Carter CR, Balkwill F, Chang DK, Evans TR, Strathdee D, Biankin AV, Nibbs RJ, Barry ST, Sansom OJ, Morton JP.
CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. *Cancer Cell* 2016; 29: 832-45

The study of pancreatic cancer is a major focus of several labs at the Beatson, and this paper investigates the role of CXCR2 - a molecule that primarily regulates cells of the immune system - in its development and spread. The results identify a key role for CXCR2 in establishing and maintaining the metastatic niche, and suggest that inhibiting CXCR2 could be used to treat this devastating disease. This information will now form the basis of future clinical trials.

Tweedy L, Knecht DA, Mackay GM, Insall RH.
Self-generated chemoattractant gradients: Attractant depletion extends the range and robustness of chemotaxis. *PLoS Biol* 2016; 14: e1002404

Chemotaxis is a fundamental process in biology and in cancer progression. Using both computational simulations and wet lab experiments, the authors of this paper analyse self-generated chemotaxis, in which cells respond to gradients they have made themselves by breaking down available attractants. They describe these self-generated gradients in some detail, along with the indicators that allow them to be identified. Importantly, they also show that gradients of this type are found in real biological contexts and guide cells over much longer distances than are possible by other means.

Veltman DM, Williams TD, Bloomfield G, Chen BC, Betzig E, Insall RH, Kay RR.
A plasma membrane template for macropinocytic cups. *eLife* 2016; 5: e20085

Macropinocytosis is a fundamental mechanism that allows cells to take up extracellular liquid into large vesicles. This work, done jointly in collaboration with Robert Kay's group in Cambridge, uses lattice light sheet microscopy to investigate how macropinocytic cups form in the amoeba *Dictyostelium*. The authors show that this depends on the formation of a ring of protrusive actin beneath the plasma membrane along with coincident patches of signalling molecules, including PIP3 and Ras, which is often mutated in cancer. They also show that expressing mutant, active Ras in *Dictyostelium* cells leads to the formation of larger macropinocytic cups.

Institute of Cancer Sciences

Lopez J, Bessou M, Riley JS, Giampazolias E, Todt F, Rochegue T, Oberst A, Green DR, Edlich F, Ichim G, Tait SWG.
Mito-priming as a method to engineer Bcl-2 addiction. *Nat Commun* 2016; 7: 10538

This paper describes the development a powerful new technique, called mito-priming, that renders cells addicted to anti-apoptotic Bcl-2 proteins and highly sensitive to their inhibition. This allows the authors to survey the efficacy of a range of anti-cancer BH3 mimetic compounds in triggering mitochondrial apoptosis, and to identify potent and specific MCL-1 inhibitors. They also use mito-priming to demonstrate that tBID and PUMA can preferentially kill in a BAK-dependent manner.

Nelson DM, Jaber-Hijazi F, Cole JJ, Robertson NA, Pawlikowski JS, Norris KT, Criscione SW, Pchelintsev NA, Piscitello D, Stong N, Rai TS, McBryan T, Otte GL, Nixon C, Clark W, Riethman H, Wu H, Schotta G, Garcia BA, Neretti N, Baird DM, Berger SL, Adams PD.
Mapping H4K20me3 onto the chromatin landscape of senescent cells indicates a function in control of cell senescence and tumor suppression through preservation of genetic and epigenetic stability. *Genome Biol* 2016; 17: 158

In this study, the authors investigate the function of the histone modification H4K20me3 and its methyltransferase SUV420H2 in senescence and tumour suppression. They use immunofluorescence and ChIP-seq to determine the distribution of H4K20me3 in proliferating and senescent cells. They show that elevating SUV420H2 and H4K20me3 does not accelerate senescence but it does reinforce oncogene-induced senescence-associated proliferation arrest and slow tumourigenesis *in vivo*. Overall, the study does not support a major role for H4K20me3 in the initiation of senescence. Although, the authors do speculate that it might have a role in contributing to long-term senescence-mediated tumour suppression.

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

www.beatson.gla.ac.uk/eyal_gottlieb

TUMOUR METABOLISM

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[2]Beatson West of Scotland Cancer Centre Endowment Fund

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

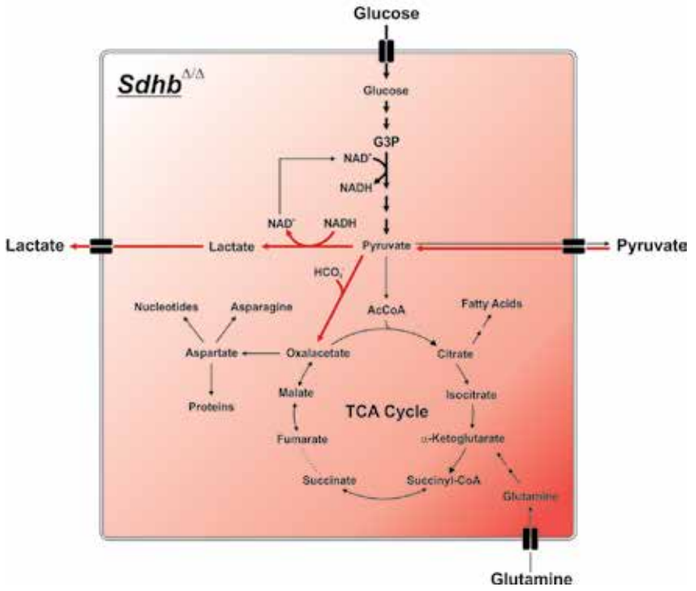

Figure 1
Schematic representation of metabolic rewiring observed in Sdhb-null cells. Lack of SDH activity commits cells to consume extracellular pyruvate, which sustains Warburg-like bioenergetic features. Moreover, pyruvate carboxylation diverts glucose-derived carbons into aspartate biosynthesis, thus 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.

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 95

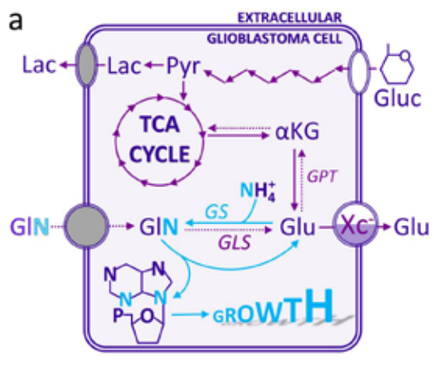

Figure 2
Glutamine metabolism in glioblastoma. (a) Glutamine-restricted glioblastoma cells utilise glucose-derived carbons to produce alpha-ketoglutarate (solid arrows). This keto acid is transaminated to glutamate, mostly by the glutamate pyruvate transaminases (GPT). Glutamate is then either secreted 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.

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UBIQUITIN SIGNALLING

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

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes - E1, E2 and E3 (Fig. 1). Our group is interested in understanding the regulation and mechanistic functions of RING E3s with particular focus on RING E3s that have been linked to cancer.

Regulation of Cbl proteins by tyrosine phosphorylation and mutation

Activation of receptor tyrosine kinase (RTK) signalling cascades is important for cellular processes such as cell proliferation, differentiation, migration and survival. Prolonged or aberrant activation of RTKs is commonly associated with cancer. Cbl proteins (Cbls) - c-Cbl, Cbl-b and Cbl-c - are RING E3s that negatively regulate RTKs, tyrosine kinases and a host of other proteins by promoting their ubiquitination and subsequent degradation by the proteasome or via endocytosis. Independent of their E3 activity, Cbls also function as adaptor proteins.

All Cbls share a highly conserved N-terminal SH2-containing tyrosine kinase-binding domain (TKBD), a linker helix region (LHR) and a RING domain (Fig. 2a) followed by a variable proline-rich region (PRR). The TKBD mediates substrate specificity by binding to proteins containing phosphotyrosine motifs commonly found in RTKs or tyrosine kinases, while the PRR recruits proteins containing an SH3 domain. The LHR and RING domain play central roles in recruiting E2~Ub and in mediating target ubiquitination, where phosphorylation of a conserved LHR Tyr (Tyr371 in c-Cbl) activates Cbl's ligase activity. Recent studies showed that c-Cbl mutations are found in human patients with myeloproliferative diseases (MPD) and these mutations abrogate E3 ligase activity and induce cell transformation (reviewed in Kale *et al.*, Cancer Res 2010; 70: 4789). Notably, Tyr371 mutation is the major hotspot.

Over the last few years, we have determined several new crystal structures of Cbl to understand how LHR Tyr phosphorylation regulates Cbl. We showed that in the unphosphorylated state, 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 Cbl's activity. LHR Tyr phosphorylation activates Cbl's ligase activity by inducing dramatic LHR conformational changes that (1) enhance overall E2~Ub binding affinity by eliminating auto-inhibition and forming a new phosphoTyr371-induced platform for E2~Ub binding; and (2) place the RING domain and E2 in proximity of the substrate-binding site. Notably, the LHR phosphor-Tyr participates directly in Ub binding to stabilise E2~Ub in the closed conformation that is critical for optimising E2~Ub thioester bond for nucleophilic attack by the substrate lysine. This interaction alone contributes to 200-fold enhancement in Cbl's catalytic efficiency explaining how Cbl is regulated by LHR Tyr phosphorylation (Dou *et al.*, Nat Struct Mol Biol 2012; 19: 184; Dou *et al.*, Nat Struct Mol Biol 2013; 20: 982).

Based on these results, we postulated that Tyr371 mutation could decouple Cbl's regulation. First, the integrity of LHR conformation in the native state is dependent on LHR Tyr-TKBD interaction. Tyr371 mutation could loosen LHR-TKBD interaction leading to the release of LHR in the native state thereby disrupting auto-inhibition. Second, all Tyr371 mutations cannot initiate the pTyr371-interaction network to generate the new E2~Ub binding platform and hence all Tyr371 mutants lack the ability to activate E2~Ub for catalysis. Using small-angle X-ray scattering and E2~Ub binding analyses, we showed that MPD Tyr371 mutants (Y371S, Y371D, Y371H, Y371C and Y371A) adopted an elongated conformation and exhibited enhanced E2~Ub binding affinity as compared to wild type c-Cbl, suggesting that MPD Tyr371 mutations disrupt LHR-TKBD interaction in the native state. Moreover, we showed that MPD Tyr371 mutants induced transformation. Surprisingly, a Y371F mutant, not found in MPD patients, displayed a similar conformation and E2~Ub binding affinity as wild type c-Cbl and induced transformation at a negligible rate as compared to MPD Tyr371 mutants. Our work suggests that in addition to the loss of ligase activity, disruption of LHR-TKBD interaction in the native state is essential to transform c-Cbl into an oncogene (Buetow *et al.*, 2016).

Publications listed on page 96

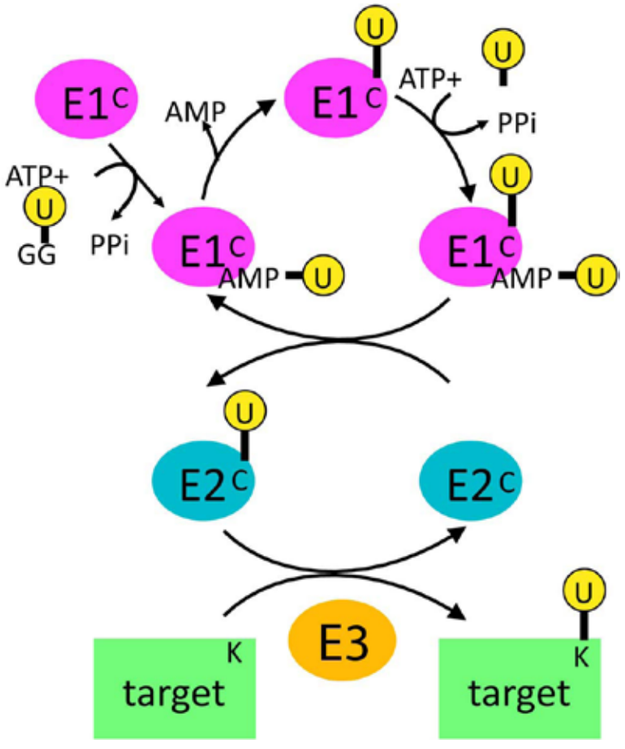

Figure 1
Enzymatic cascade for Ub modifications. E1 initiates the cascade by adenylating Ub's C-terminus in the presence of Mg^{2+} and ATP, followed by the formation of a covalent thioester intermediate with Ub. E1 then recruits an E2 and transfers the thioesterified Ub to the E2's catalytic cysteine forming an E2~Ub thioester intermediate (~ indicates thioester bond). E3 plays a pivotal role in determining substrate fate. In general, E3 consists of an E2-binding module (HECT, RING or U-box domain) and a protein-protein interaction domain that can recruit substrate directly or indirectly. With this configuration, E3 recruits E2~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.

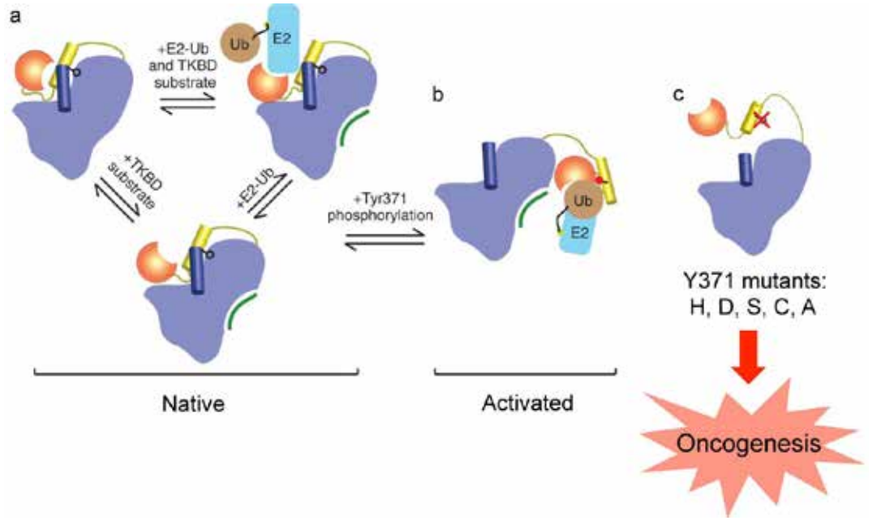

Figure 2
Regulation of Cbl by LHR Tyr phosphorylation and mutation. (a) In the unphosphorylated state, c-Cbl's LHR (yellow) is bound to the TKBD (blue) thereby restricting the RING domain (orange) to a region distal from the TKBD substrate (green) binding site. In this state, the E2-binding surface of the RING domain is occluded thereby reducing E2 binding affinity. (b) LHR Tyr phosphorylation (red ball stick) releases LHR to form a pTyr-LHR-RING platform to optimise E2~Ub into a closed active conformation for catalysis. Additionally, flexibility in the LHR linker enables juxtaposition of E2~Ub and TKBD substrate for Ub transfer. (c) MPD Tyr371 mutants disrupt LHR-TKBD interaction in the native state to induce transformation.

PROSTATE CANCER BIOLOGY

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[4]MRC Clinical Research Training Fellowship

Prostate cancer remains a major global health issue, resulting in significant morbidities and premature deaths among prostate cancer sufferers. Research in our group has a uniquely comprehensive, cross-disciplinary strategy encompassing preclinical laboratory model systems, analysis of clinically obtained samples and timely clinical trials based on our research findings (Fig. 1).

Our research objective is to identity, characterise and validate key aberrant cellular signalling events to inform and facilitate the development of novel therapies. We are actively recruiting patients into the SPECTRE trial to study the efficacy of statins in castration-resistant prostate cancer. Our group is also responsible for the coordination of the TRANSPOT consortium, a Horizon 2020-funded Innovative Training Network for 10 research groups across Europe.

Treatment resistance in prostate cancer through altered lipid metabolism – 'Fat within the tumour'

Research in our laboratory builds on the important and clinically relevant finding that combined inactivation or deficiency of the tumour suppressor genes *PTEN* and *Sprouty2 (SPRY2)* is sufficient to drive aggressive prostate cancer. Additionally, we discovered that this subtype of prostate cancer is also resistant to hormonal therapy (or androgen deprivation therapy, ADT), resulting in castration-resistant prostate cancer (CRPC), which remains incurable.

Studying the molecular basis of treatment resistance, deregulated lipid metabolism is implicated in CRPC, not just in our models but also in published genomic and transcriptomic clinical datasets. Detailed mechanistic analysis points to enhanced production and utilisation of cholesterol in CRPC. Based on positive preclinical efficacy evaluation of statin in *PTEN* and *SPRY2* mediated CRPC, we are now conducting a proof-of-concept translational study (SPECTRE, Combined suppression of cholesterol bioavailability and androgen deprivation therapy to treat castration resistant prostate cancer) on the use of statins as a potential treatment for patients showing early sign of CRPC. The SPECTRE study is now open to recruitment, for up to 24 months. A range of liquid biopsy markers as well as prostatic biopsies are planned. This study will inform us with the feasibility of statins in treating CRPC.

Treatment resistance in prostate cancer through obesity – 'Fat around the prostate'

Besides the specific molecular class of prostate cancer highlighted by *PTEN* and *SPRY2*, clinical obesity is associated with poorer outcome in patients with prostate cancer. To date, the relationship between local peri-prostatic fat and tumour response to ADT has not been tested. We have studied patients with advanced prostate cancer at the time of diagnosis and investigated the amount of fat surrounding the prostate gland among patients treated with ADT for their cancer. Extracting information from magnetic resonance imaging routinely performed clinically, we were able to 'calculate' the volume

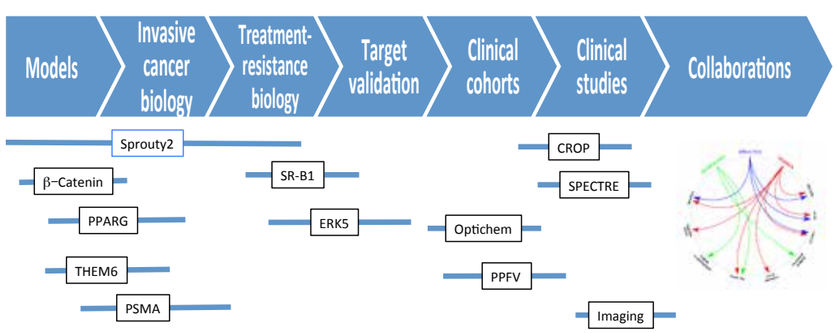


Figure 1
Illustration of the comprehensive 'bench to bedside' nature of our programme of translational research in prostate cancer (boxed text represents individual interconnected translational projects).

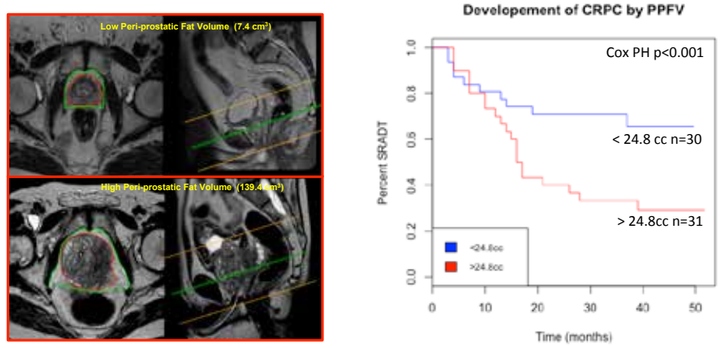


Figure 2
Analysis of time to castration resistant prostate cancer (CRPC) and its relationship to PPFV (Salji *et al.*, 2017). Examples of high and low PPFV measured on staging MRI (A). Kaplan Meier analysis of time to CRPC for two groups of patients divided by the median PPFV (B) (n= 31 red >24.8 cm^3, n = 30 blue <24.8 cm^3). Patients with higher PPFV showed greater and faster development of CRPC. Examples of low (<15cc), medium (15-45cc) and high (>45cc) PPFV biopsy materials immunohistochemistry for chemokine receptor 3 showing increased staining in patients with high versus low PPFV (p=0.05 Wilcoxon Rank Sum Test) (C).

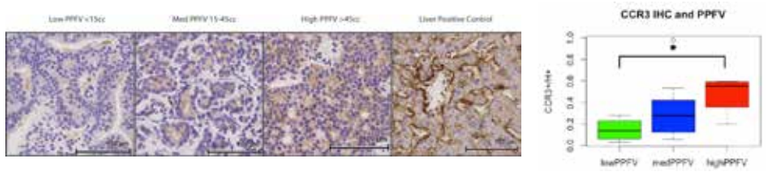
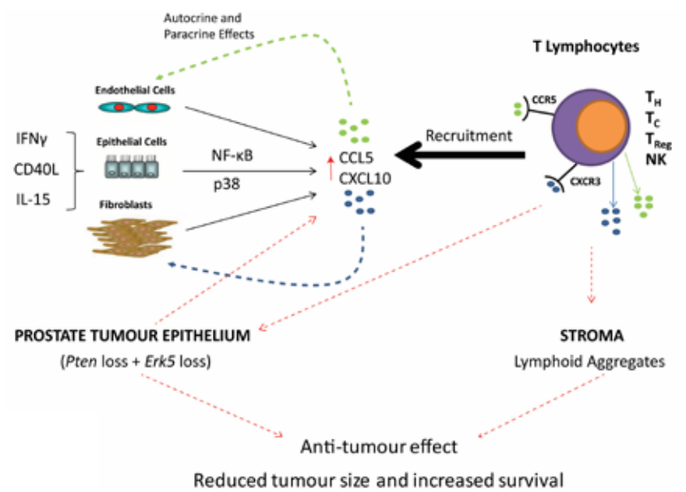


Figure 3
Schematic representation summarising the compartmentalisation and potential role of cytokines in mediating the effects of targeting Erk5 in prostate carcinogenesis (Loveridge *et al.*, 2017). Loss of *Erk5* in combination with loss of *Pten* leads to increased levels of expression of the cytokines, CCL5 and CXCL10, within the malignant epithelium. CCL5 and CXCL10 can exert autocrine and paracrine effects on the cells from which they were produced but their major function is to recruit T lymphocytes to their site of production. Recruitment of T cells (predominantly $CD4^+$) to the tumour epithelium (and distinct sites within the stroma) is enhanced in the context of *Erk5* loss in combination with *Pten* loss. The enhanced T cell infiltration is associated with an anti-tumour effect in terms of reduced tumour size and increased survival.

of peri-prostatic fat (PPF), i.e. the amount of fat that the prostate is 'embedded' in *in situ*, and analysed the PPF volume against the tumour response to ADT. We found that the volume of PPF was significantly higher in patients who subsequently developed CRPC (p<0.0001, Wilcoxon rank sum test, when compared to patients with no sign of CRPC) (Fig. 2). Multivariate analysis using Cox Proportional Hazards models to incorporate known predictors of CRPC also identified PPF volume as an independent marker for CRPC as well as the most significant predictor of time to CRPC.

It is increasingly appreciated that the proximity of fat near (around/surrounding) the developing tumour can fuel tumourigenesis. An interesting example of this is illustrated in the involvement of how the chemokine/chemokine receptor (CCL7/CCR3) axis interacts with high-grade prostate cancer. Consistent with this, we also found upregulated CCR3 (chemokine receptor 3) expression in tumours associated with elevated PPF volume (p=0.04, Wilcoxon Rank Sum test).



Collectively, for the first time, we have linked clinical imaging to tumour-host interaction in the context of treatment resistance, supporting a role for PPF in CRPC.

Validation study for ERK5 as a therapeutic target in prostate cancer

Extracellular signal-regulated protein kinase 5 (ERK5/MAPK7) is a key regulator of important biological processes during development and homeostasis. Research from our lab and others has proposed ERK5 as a target for therapy in a number of cancer types, including prostate cancer.

We have been collaborating with colleagues in the Northern Institute for Cancer Research (Herbie Newell, Stephen Wedge) and Cancer Research Technology to develop potent, specific inhibitors against ERK5 in prostate cancer. Using a genetically modified mouse model, we validated that in a *Pten* driven prostate cancer model, prostate tumorigenesis was impaired by the loss of *Erk5* (Fig. 3), resulting in extended survival in mice with combined lost of *Erk5* and *Pten*, thus further supporting the notion of ERK5 as a potential target for therapy.

The Optichem (Optimizing the use of taxane chemotherapy in prostate cancer) study

Finally, as a result of the Treatment Sciences Challenge Award that we secured from the Prostate Cancer Foundation (USA), we have launched a project aimed at dissecting the molecular basis of chemoresistant prostate cancer. We have two parallel approaches, with transcriptomic analysis of clinical materials from the STAMPEDE trial, and the application of genome-wide screening using preclinical *in vitro* and *in vivo* models. This project will provide us with unrivalled understanding of chemoresistance in prostate cancer.

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TUMOUR CELL DEATH

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[1]Worldwide Cancer Research
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[3]left during 2016

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.

Glycosylation control in chemotherapy-induced death

There are multiple mechanisms within cells that determine viability. One of the most studied is a process termed apoptosis, which is an evolutionarily conserved mechanism of cell suicide. It is now well established that apoptosis has a very important function in protecting us against tumour development by causing the eradication of damaged cells that if not removed may go on to form a tumour. In addition, apoptosis also has an incredibly important role in cancer therapy, with many standard forms of chemotherapy causing death of tumour cells via apoptotic pathways.

A major regulator of apoptosis during tumour development, and in response to cancer therapy, is the tumour suppressor p53. Following various forms of cellular stress, such as DNA damage, p53 becomes activated. It then mediates tumour suppressive effects either via protein interactions in the cytoplasm or through transcriptional activation of a series of target genes that include factors that directly engage the apoptotic machinery. To search for additional factors that mediate p53's tumour suppressive effects in a way that may be connected to induction of apoptosis, we performed microarray screens to identify genes induced by p53. Amongst the genes identified, we were intrigued by one gene that encodes the lysosomal glycosidase alpha-L-fucosidase 1 (FUCA1). This enzyme cleaves fucose linked moieties from N-linked glycans (sugar structures linked to proteins via asparagine residues). Since a link between p53 and glycosylation control had not previously been established, we chose to examine FUCA1 in greater detail.

Closer analysis of the relationship between p53 and FUCA1 revealed that *FUCA1* is induced by wild type, but not by tumour-derived mutants of p53. Furthermore, we found that this effect was mediated by direct p53 binding to a DNA element within *FUCA1*'s first intron, which we found when cloned in isolation was p53 responsive. Importantly, we also discovered that a number of important chemotherapeutic drugs also induce *FUCA1* expression and that this is accompanied by an increase in fucosidase activity within cells. Both these effects we found to be largely p53-dependent.

Inspired by these findings, we were naturally excited to understand what role FUCA1 might play downstream of p53 and in response to chemotherapy. In the first instance, we cloned *FUCA1* so that we could overexpress the gene in cells to examine its effects on cell viability. We found, however, that in short-term apoptotic or long-term clonogenic assays, expression of *FUCA1* has no impact on cell viability, at least in the cells examined.

Undeterred by the fact that overexpression of *FUCA1* does not kill cells, we considered that the enzyme might still have a role in p53-mediated cell death responses in conjunction with the effects of other target genes. To test this possibility, we reduced the levels of endogenous FUCA1 by RNA interference (RNAi). This showed indeed that downregulation of FUCA1 decreased apoptosis induced by p53 as measured by assessing the number of cells with sub-G1 DNA content and the levels of cleaved caspase 3 and poly-ADP ribose polymerase.

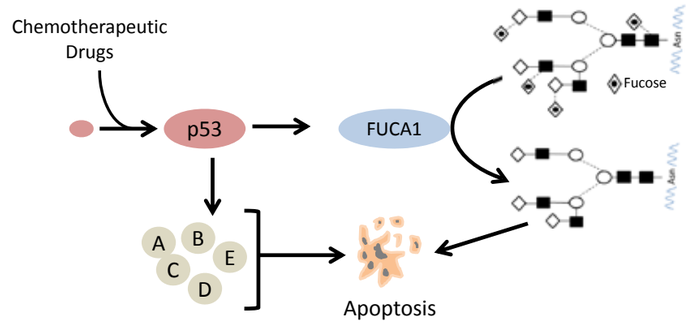

Figure 1
Glycosylation control contributes to p53 and chemotherapy-induced apoptotic death. Chemotherapeutic drugs increase the levels of p53, which transactivates *FUCA1*. FUCA1 cleaves fucose moieties from N-linked glycans and together with additional p53 target genes (A, B, C, D, E, etc.) this leads to apoptosis.

Using the same RNAi approach, we also examined whether FUCA1 has a role in chemotherapy-induced cell death. Cisplatin and etoposide are two major chemotherapeutic drugs that we had shown induced fucosidase activity. We therefore examined the impact of FUCA1 knockdown on the response to these and found that induction of cell death was significantly reduced (Fig. 1).

These findings highlight that modulation of glycosylation is an additional arm in p53's phenotypic effects. What is unclear at this stage, however, is how FUCA1 might mediate these effects. As glycans are emerging as modulators of signalling effectors, akin to the roles of acetylation and ubiquitylation, it is tempting to speculate that FUCA1 contributes to cell death by changing glycan linkages as a mechanism to change protein function. More studies in this area are clearly required, as the prospect of enhancing chemotherapeutic responses by modulating glycosylation makes this quest undoubtedly worthwhile.

The role of autophagy during tumour development

In addition to apoptosis, another process that can regulate cell viability is autophagy. This process and more specifically the form called macroautophagy, is a mechanism of cellular traffic that delivers cytoplasmic material to lysosomes for degradation (Fig. 2). As such, autophagy is a major mechanism for the removal of damaged proteins and organelles thereby preserving cellular fidelity. In addition, autophagy can be modulated in response to various forms of stress and can mitigate the effects of this stress to promote cell viability.

It is now well established that autophagy has an important role in both tumour suppression and tumour development. In the protection against cancer, autophagy's effects on the preservation of cellular integrity are clearly tumour suppressive. However, in established tumours, it is also considered that in certain contexts autophagy can be oncogenic by promoting cancer cell survival.

In our previous studies, we discovered that p53 status could determine the role of autophagy in tumour development. This naturally prompted us to question whether the status of other tumour suppressors can have a similar effect. To test this we generated mice that were hemizygous for the PTEN tumour suppressor and either wild type or null for the essential autophagy gene *Atg7* in their pancreas. This revealed that PTEN status, like p53 status, could affect the role of autophagy in pancreatic cancer development. In PTEN wild type animals, loss of Atg7 blocks tumour development. In contrast, animals hemizygous for PTEN combined with simultaneous ablation of autophagy accelerates the disease. These findings underscore the fact that the role of autophagy in cancer is context-specific and dependent on additional genetic factors associated with the development of the disease.

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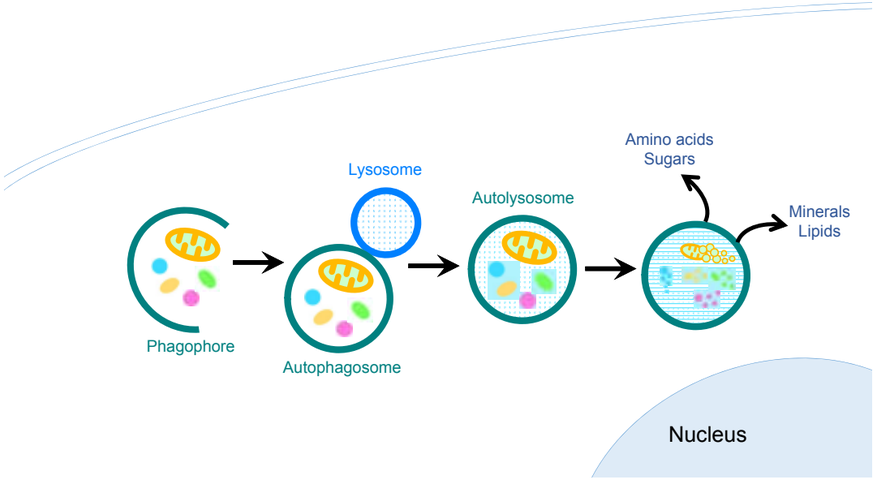
Figure 2
The process of macroautophagy. Membranous structures form and encapsulate cytoplasmic material in ball-like organelles termed autophagosomes. Autophagosomes then fuse with lysosomes to form autolysosomes within which macromolecules are broken down into constituent parts. These are either further catabolised or recycled into biosynthetic pathways.



ONCOMETABOLISM

Group Leader
Saverio Tardito

Research Scientist
Victor Villar Cortes

At the foundation of cellular and tissue growth stands the transfer of chemical energy from nutrients into macromolecules. Tumours are no exception to this principle and unavoidably seek metabolic states that support anabolism and growth. Our vision is that the tissue of origin influences the biochemical pathways utilised by tumours to grow in two ways. On the one hand, by imposing environmental constraints, the tissue of origin exposes metabolic vulnerabilities of the tumour. On the other hand, enzymes normally restricted to a defined population of differentiated cells, and required for tissue physiological functions, can be hijacked by cancer cells to enhance their metabolic fitness.

Glutamine and glutamate metabolism in brain and liver tumours

Glutamine and glutamate are instrumental to physiological processes, such as neurotransmission in the brain (Fig. 1) and ammonia homeostasis in the liver (Fig, 2), but are at the same time obligate substrates for anabolism of tumours originating in these organs, such as glioma and hepatocellular carcinoma. In particular, we plan to investigate the role of glutamine synthetase (GS) in the initiation and progression of glioma and hepatocellular carcinoma tumour models. GS catalyses the ligation of glutamate and ammonia, and is the only known enzyme able to synthesise glutamine in mammalian cells. We have previously shown that in glioblastoma, the most aggressive type of glioma, GS-derived glutamine provides the nitrogen required for nucleotide biosynthesis under glutamine restriction. In particular, GS is central to a metabolic crosstalk between normal astrocytes and glioblastoma cells with low expression of this enzyme. We also showed that in the majority of glioblastoma patients GS expression is retained. Therefore, we aim to identify the metabolic and pro-tumourigenic role of this astrocytic enzyme, by interfering with its activity in primary-derived orthotopic xenografts of glioblastoma as well as in advanced cell culture systems (Fig. 3).

While in normal liver the expression of GS is strictly confined to pericentral hepatocytes (Fig.2), in liver tumours this metabolic zonation is disrupted. In particular, a subset of hepatocellular carcinoma with an overactive WNT signalling pathway show a widespread and sustained GS expression among cancer cells. By means of an HPLC-mass spectrometry based metabolomic approach, we will study the rewiring of carbon and nitrogen metabolism imposed by GS expression in murine and cellular models of hepatocellular carcinoma. This approach will pave the way for the identification of metabolic vulnerabilities exacerbated by the aberrant activity of GS in liver cancer.

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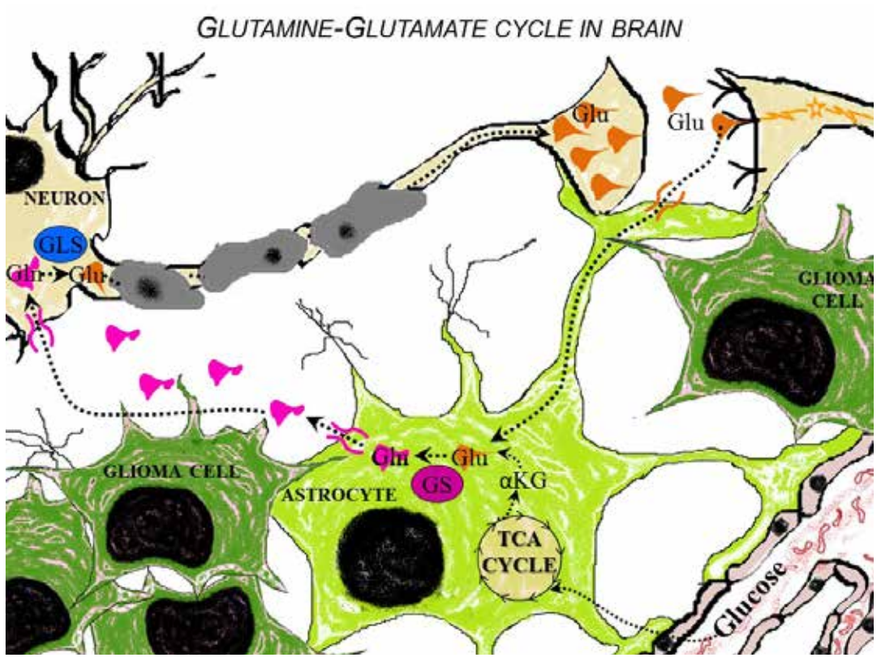

Figure 1
Glutamine-glutamate cycle in the brain. Glutamate (Glu) is the most abundant neurotransmitter in the central nervous system. It is released by glutamatergic neurons to activate postsynaptic receptors. In the synaptic cleft, astrocytic transporters with high affinity for excitatory amino acids mop up glutamate in order to prevent excitotoxicity. Astrocytes efficiently convert glutamate into glutamine (Gln) *via* glutamine synthetase (GS). Glutamine, which is not an active neurotransmitter, is released by astrocytes and re-uptaken by neurons, through cell type-specific transport systems. Finally, glutamine is deaminated by glutaminase (GLS) to close the cycle and replenish the pre-synaptic pool of glutamate. What happens to this finely tuned metabolic cycle when astrocytes transform into glioma cells is still poorly understood.

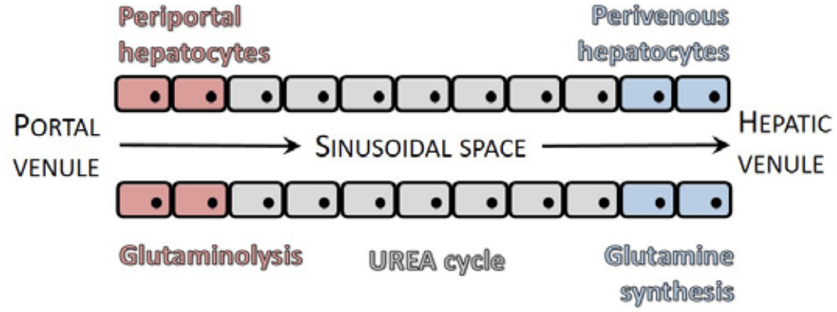

Figure 2
Liver zonation of glutamine metabolism. The liver is an ammonia-detoxifying organ and maintains homeostatic levels of circulating ammonia and glutamine. The functional unit of the liver constitutes an elegant example of metabolic zonation. In fact, the periportal region expresses a liver-type glutaminase that converts the excess of circulating glutamine into glutamate and ammonia. The latter flows downstream through hepatocytes competent in the urea cycle. These cells, despite having a low affinity for ammonia, constitute the bulk of the liver and convert large amounts of ammonia into urea. The ammonia escaping this metabolic zone is captured by a few rows of hepatocytes surrounding the central vein, which express high levels of glutamine synthetase. This enzyme has high affinity for ammonia and fixes it into the non-toxic glutamine, which is finally returned to the circulation.

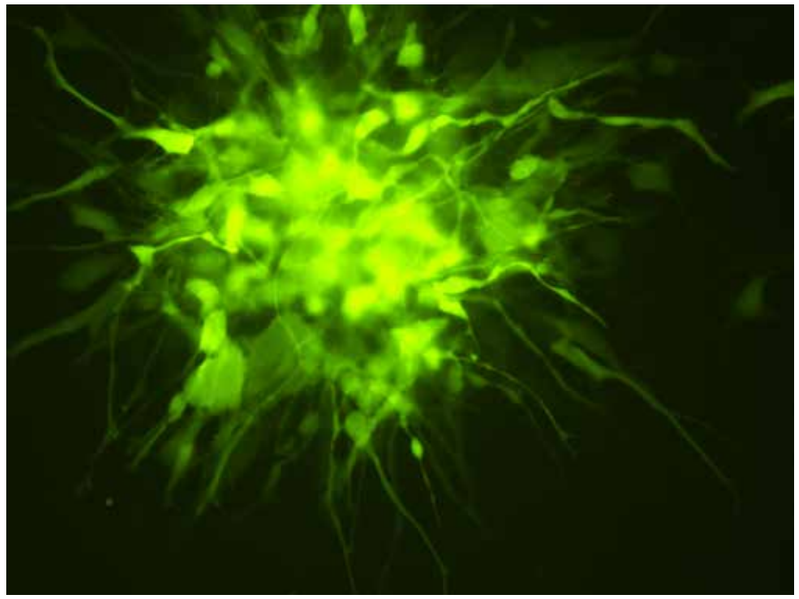

Figure 3
A spheroid human glioblastoma cells expressing GFP, obtained from a primary derived xenograft and cultured in a newly formulated and chemically defined medium with physiological levels of nutrients.

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MATHEMATICAL MODELS OF METABOLISM

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

Serine one-carbon catabolism with formate overflow

Serine catabolism to glycine and a one-carbon unit has been linked to the anabolic requirements of proliferating mammalian cells. However, previous genome-scale modelling from our group predicted a catabolic role for energy generation with one-carbon release as formate. We have now experimentally proven that in cultured cells the majority of serine derived one-carbon units is released from cells as formate, and that formate release is dependent on mitochondrial complex I and reverse 10-formyl-tetrahydrofolate synthetase activity. We have also demonstrated that in mice, 50% of plasma formate is derived from serine and that serine starvation or complex I inhibition reduces formate synthesis *in vivo*. These observations have significant implications to our understanding of one-carbon metabolism, energy metabolism and the use of complex I inhibitors to treat cancer.

Our work shows that cells run serine one-carbon catabolism in excess of the biosynthetic demand of one-carbon units. Most of the excess one-carbon units are released as formate in the conditions tested. For every formate molecule released from cells one ADP molecule is phosphorylated to form ATP via reverse mitochondrial 10-formyl-THF synthetase. The coupling of the mitochondrial NAD$^+$ dependent 5,10-methylene-THF dehydrogenase to mitochondrial oxidative phosphorylation can contribute with an additional 2.5 molecules of ATP per formate molecule released. In fact, the only cell autonomous phenotype we have observed in MTHFD1L knockdown cells is an increase in glycolysis, a canonical pathway for energy generation. While this evidence is not conclusive, it supports the idea that serine one-carbon catabolism with formate overflow can contribute to energy generation. Further work is required to investigate if this contribution is enhanced in certain cancers relative to normal tissues.

The phenomenon of serine one-carbon catabolism with formate overflow resembles the well known phenotype of glucose catabolism with lactate overflow (the Warburg effect). Both phenotypes are characterised by the apparent 'waste' of carbon atoms and energy production. They are different with respect to localisation. The Warburg effect contributes to cytosolic and the serine one-carbon catabolism to mitochondrial energy generation. They also differ in the magnitude of the pathway rate, glucose catabolism being significantly higher than serine catabolism. However, this rate difference is not unexpected considering that mitochondria represent a low percentage of the biomass content of normal proliferating cells and cancer cells. Finally, similar to the re-synthesis of glucose from lactate in the liver via the Cori cycle, serine could be re-synthesised in the liver, contributing to the organism's balance of energy and one-carbon units.

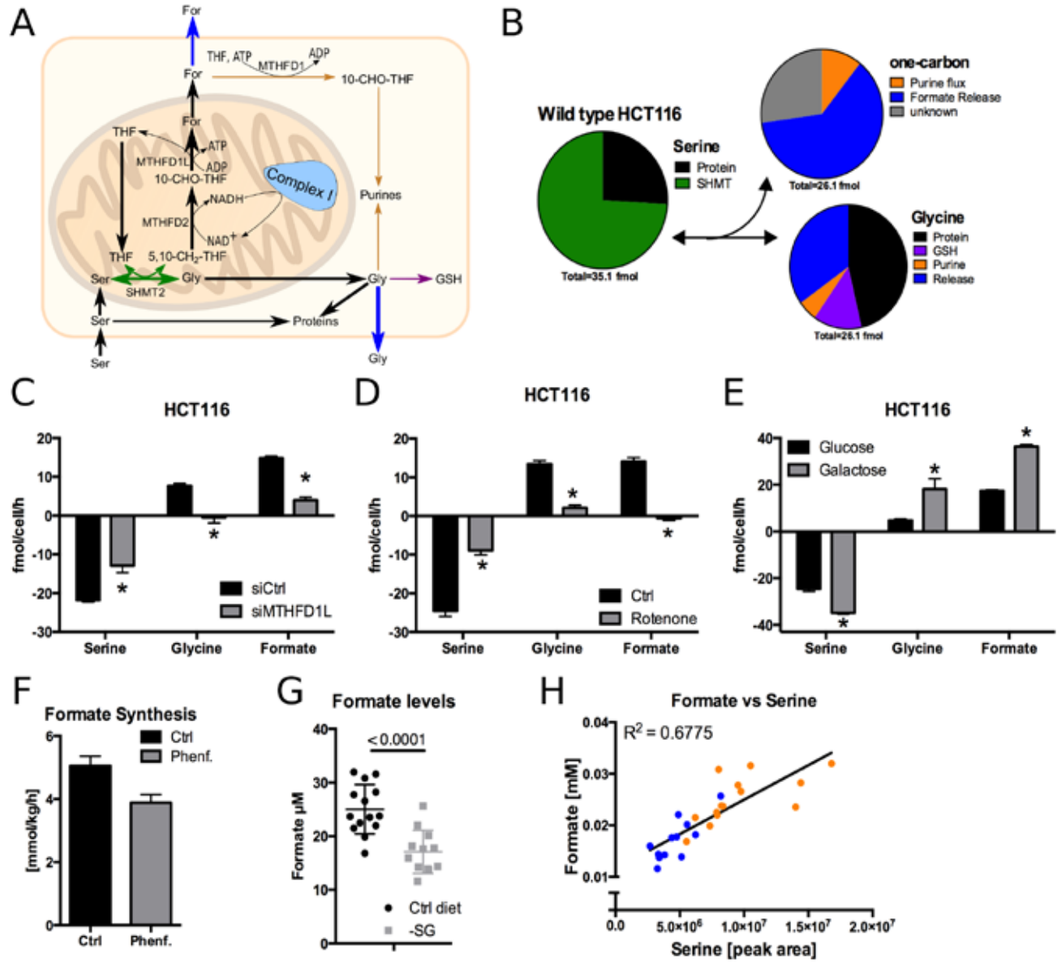

Figure 1
Metabolic flux analysis of serine one-carbon catabolism to formate. A) Model illustrating mitochondrial one-carbon metabolism and its interactions with other pathways. B) Pie chart representing relative fluxes of serine, glycine and one-carbon moieties in HCT116 colorectal cancer cells. C-E) Serine one-carbon catabolism is increased by a switch from glucose to galactose, decreased by rotenone treatment and by MTHFD1L knockdown. F) Estimated serine-derived formate synthesis rate in phenformin-treated or untreated mice. G) Formate concentration in the serum of control, and serine and glycine starved mice. H) Linear relationship between serum serine and formate levels in the serum of control, and serine and glycine starved mice. * $P < 0.01$ by multiple t-test.

The demonstration of serine catabolism with formate overflow has several implications to our understanding of mammalian metabolism in normal physiology and disease states. Of most importance is the use of metformin, a mitochondrial complex I inhibitor, for the treatment of cancer. A study in mice has shown that metformin treatment synergises with serine deprivation in the growth inhibition of mouse xenograft tumours. Our data suggest a reduction in plasma formate synthesis from serine as a consequence of treatment with complex I inhibitors. Taken together, this evidence indicates that both treatment strategies inhibit the serine one-carbon catabolism to formate, potentially contributing to the observed synergy.

Our future research will focus on uncovering the function of serine one-carbon metabolism with formate overflow and its relevance for cancer development and treatment.

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TUMOUR SUPPRESSION

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There is an increasing interest in how changes in metabolism can help support tumour growth, and whether cancer cells acquire metabolic dependencies that could be targeted for therapeutic intervention. Oncogenic transformation is accompanied by an increased dependency of cancer cells on certain nutrients, including several non-essential amino acids (NEAAs). Our previous studies have shown that many cancer cells are dependent on an exogenous supply of the NEAA serine for optimal growth, and that serine starvation can impede their proliferation and survival. Consistently, *in vivo* experiments showed that dietary restriction of serine could slow tumour growth and enhance survival.

Serine one-carbon catabolism with formate overflow

To understand more fully this requirement for serine in cancers, we have investigated more closely how serine in utilised in these cells. Serine has numerous uses in the cell – it is one of the building blocks for protein synthesis, it can help the cell synthesise other amino acids and it supports the production of phospholipids. Serine is also the major source of one-carbon units, which are essential for nucleotide synthesis and help support the methylation reactions that drive the modification of proteins, DNA and RNA. The role of epigenetic modifications such as methylation in cancer development has gained prominence over the past years, with encouraging therapeutic effects of small molecule inhibitors of this process. Methylation reactions depend on the generation of S-adenosyl- methionine (SAM), which donates a methyl group to an acceptor molecule, generating homocysteine. The contribution of a one-carbon unit from serine can remethylate this homocysteine to regenerate methionine and complete the cycle (Fig. 1).

Our work revealed an unexpected additional role for serine in this pathway, demonstrating that the synthesis of ATP (which also depends on serine-derived one-carbon units) is important to allow the production of SAM. We found that serine supported the methionine cycle in the presence and absence of methionine through *de novo* ATP synthesis. Serine starvation increased the methionine/S-adenosyl methionine ratio, decreasing the transfer of methyl groups to DNA and RNA. While serine starvation dramatically decreased ATP levels, this was accompanied by lower AMP and did not activate AMPK. This work highlights the difference between ATP *turnover*, and new ATP *synthesis*, and a vital function of nucleotide synthesis beyond making nucleic acids. While regeneration of ATP from ADP/AMP has been recognised as a key regulatory step in many biochemical reactions, less well appreciated is the potential role of *de novo* ATP synthesis.

In the past year, we have also made progress in understanding how oxidative stress contributes to cancer development. The generation of reactive oxygen species (ROS) in cells can lead to damage of protein and nucleic acids, and can help promote oncogenic changes. Based on these observations, it has been suggested that anti-oxidants could help to limit cancer development. However, excessive levels of ROS drive cell death - and malignant transformation of cells is often accompanied by increased ROS generation - that is compensated in the cancer cell by increased antioxidant defence. There has therefore been great interest in the concept that increasing ROS (by limiting antioxidant capacity) could result in tumour-specific cell death. The complexity of this system is further enhanced by the observation that in addition to damaging macromolecules, ROS can also play an integral role in mitogenic signalling, so supporting abnormal proliferation seen in cancers. Our previous work identified TIGAR as a component of the cell's antioxidant defence. Loss of TIGAR in the intestine resulted in elevated ROS levels and a failure to effectively repair damage following irradiation. A similar defect in proliferation was also seen in intestinal tumour models driven by deletion of the tumour suppressor gene APC, which results in Wnt-driven intestinal hyperproliferation. In this model, loss of TIGAR increased ROS and reduced proliferation, thereby limiting the development of adenomas and extending overall survival. Similarly, the proliferation of *in vitro* intestinal organoids was inhibited by TIGAR deletion, a response that was enhanced or suppressed by treatment with different antioxidants. By contrast, previous studies showed that inhibition of proliferation in response to deletion of RAC1 results from a decrease in NOX-mediated ROS production. Like RAC1, TIGAR is upregulated by the Wnt signalling pathway, consistent with the observation that both proteins help to support proliferation, despite having opposing effects on ROS. To unravel these two paradoxical roles of ROS, we used 3D organoid cultures and *in vivo* genetic models to show that ROS produced through different pathways can differentially affect the cell response. Indeed, despite having opposite effects on ROS levels, TIGAR and RAC1 loss cooperated to further reduce proliferation. Our results show ROS from different sources can have differential effects on cell growth, and suggest that ROS signalling is more nuanced than simply a variable response to overall ROS levels.

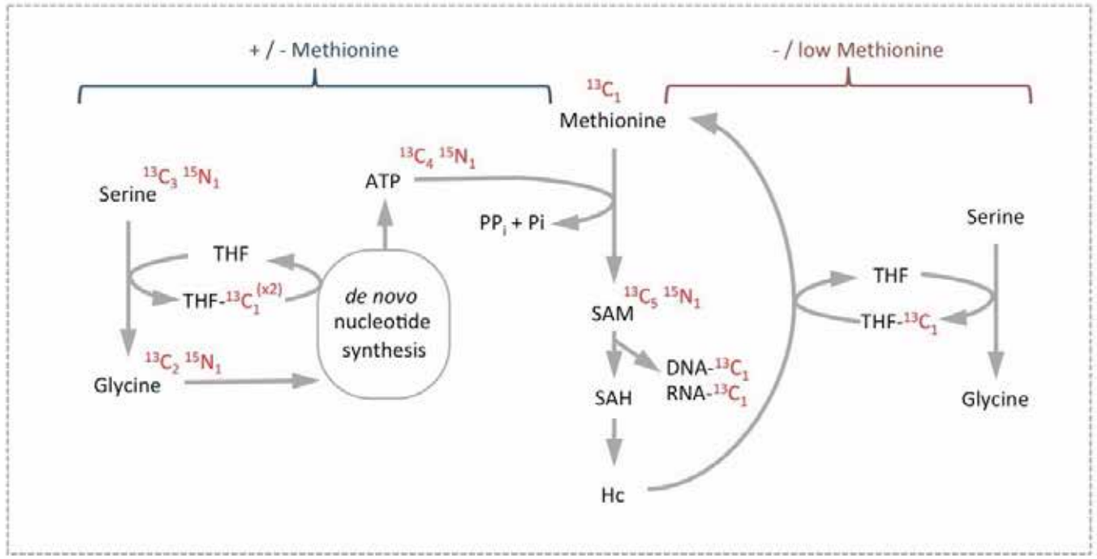

Figure 1
The contribution of serine metabolism to the SAM cycle. RHS: Under conditions of low methionine, one-carbon units derived from serine are used to remethylate homocysteine (Hc) to regenerate methionine. LHS: Serine also contributes to the synthesis of ATP, which is required for the generation of SAM from methionine. This contribution of serine is important under both methionine fed and starved conditions.

Our work suggests that the response to ROS modulation is complex, but supports the concept that enhancing damaging ROS (for example, by inhibition of TIGAR) could be beneficial for cancer therapy. Our previous work showed that the adaptation to serine starvation is dependent on ROS control, and that increasing ROS in combination with serine starvation can more effectively limit tumour cell growth *in vitro*. We have now translated these observations into more clinically relevant autochthonous tumours in genetically engineered mouse models. These models showed improved survival following dietary serine restriction alone, and survival was further improved by antagonising the antioxidant response following TIGAR deletion. We are now exploring the potential of combining ROS increasing chemotherapies with dietary serine starvation to control cancer development and progression.

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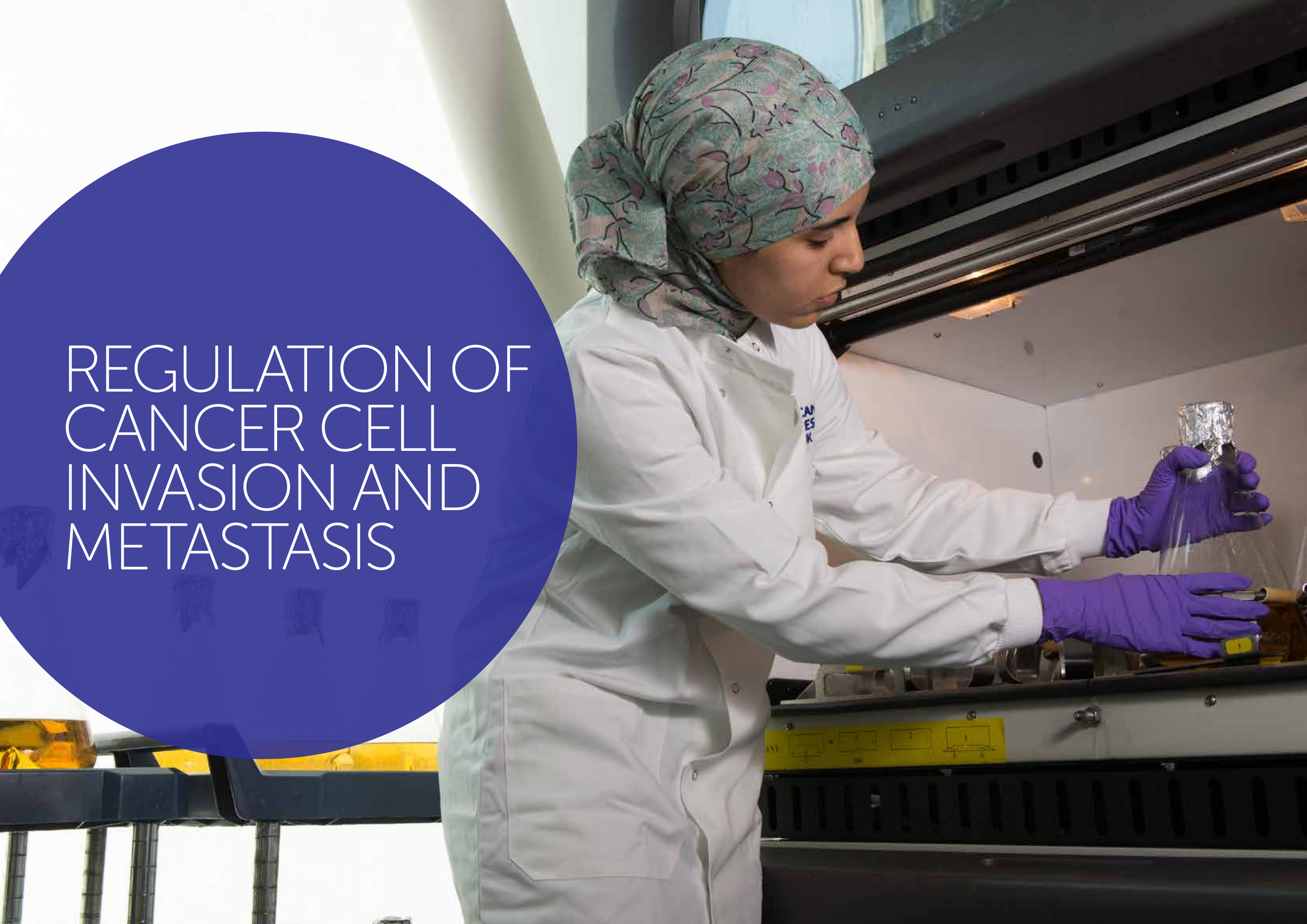
REGULATION OF CANCER CELL INVASION AND METASTASIS



LIVER DISEASE AND REGENERATION

Head
Tom Bird

Wellcome Trust Intermediate Research Fellow

Liver cancer is the second most common cause of cancer-related death worldwide and liver cirrhosis is the third most common cause of premature death in the UK. The focus of my group is to understand what makes the liver regenerate in health, and how abnormalities in specific signalling pathways can lead to liver cancer. We study the role of the main functional cell, the hepatocyte, and what makes some hepatocytes regenerate in health, and disease and what can prevent them from doing so.

Hepatocytes are the key target for regenerative therapy for patients with liver disease and are the source of liver cancers (hepatocellular carcinoma). These cells show immense regenerative capacity but despite our current understanding of the mechanisms that control liver regeneration, no therapeutic breakthroughs have been achieved to date. It is the aim of my group to understand what makes some hepatocytes regenerate whilst others do not, and to unpick some of the molecular pathways that underpin the transformation of regenerating hepatocytes into malignant hepatocytes.

Characterising the regenerative hepatocyte

Regeneration in other organs (e.g. gut) is now highly characterised, aided specifically by an understanding of the structural hierarchy of regenerating cells related to microanatomy. In the field of liver research, significant debate still exists regarding which cells regenerate, where they reside and how they are controlled. Our data identifies that repeated hepatocyte division occurs principally in specific areas of the liver (Fig. 1). We are investigating how regeneration occurs both when damage occurs elsewhere in the liver but also what happens when the areas of the liver that normally regenerate in health are damaged by disease.

Mechanisms controlling hepatocyte proliferation

The Wnt/β-catenin signalling pathway is crucial for establishing and maintaining the zones of the liver in which we believe that the regenerative cells reside. Activation of the Wnt pathway is sufficient to cause hepatocytes to divide and the liver to grow. However, when this occurs anti-proliferative pathways are also activated preventing ongoing liver growth. We are investigating the nature of these pathways, and how they might be controlled therapeutically.

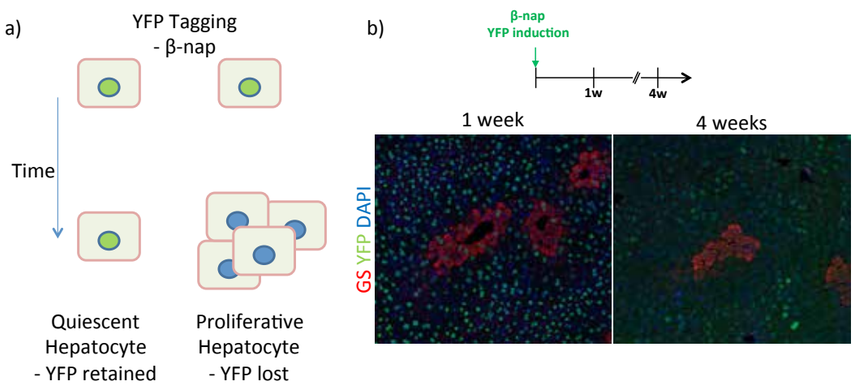


Figure 1
A transgenic model (AhH2-YFP) used to track proliferation in adult hepatocytes. a) Schematic of transgenic model – two separate hepatocytes with different proliferative fates are tracked over time for YFP loss. b) Images taken from early and late time points following induction showing zonal depletion of the YFP signal – indicative of proliferation in these pericentral (GS, glutamine synthetase) areas.

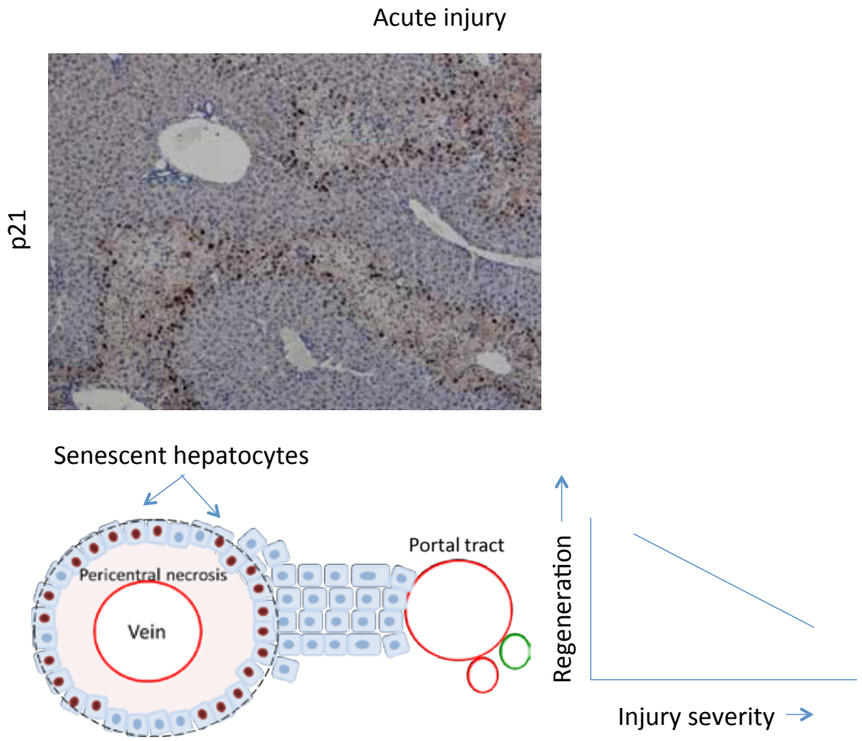


Figure 2
Damage-induced senescence in the liver. Following acute paracetamol toxicity a rim of senescence develops around the area damaged by the toxic insult (pericentral). Instead of local regeneration, hepatocellular proliferation is activated elsewhere. However, when injury becomes increasingly severe liver regeneration reduces progressively.

The process of preventing proliferation may result in a state of permanent cell cycle arrest known as senescence. This state leaves many of the functional abilities of the hepatocyte preserved but render it incapable of participating in regeneration. In severe liver injury we have shown that senescence may occur in response to injury (Fig. 2). We are investigating the pathways by which this process is activated and are currently performing preclinical trials in models of acute severe liver injury to prevent senescence formation and improve regeneration.

Transformation of regenerative hepatocytes into malignancy

Whilst the Wnt/β-catenin pathway plays a role in regeneration it is also the most frequent site of mutations in liver cancer. The actions of active β-catenin in hepatocytes are different to those in other organs. We are investigating how the blockade of proliferation imposed by β-catenin on hepatocytes may be broken during cancer formation.

Publications listed on page 92

LEUKOCYTE DYNAMICS

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President's PhD Scholarship

Paradoxically, the immune system can both benefit and antagonise the growth of cancer. Therefore, understanding how the cells of the immune system interact with the cancer microenvironment is of crucial importance. Our lab uses cutting-edge light microscopy to image these interactions directly.

In their updated seminal review 'Hallmarks of Cancer: The Next Generation', Hanahan and Weinberg underline the importance of 'avoiding immune destruction' and 'tumour-promoting inflammation' to tumour biology. The immune cell compartment of cancer is composed of the resident immune cells of the tissue and leukocytes that infiltrate from the circulation. The development of the cancer immune environment is inherently dynamic and the processes that regulate immune cell recruitment and function are not well understood. In recent years, the field has discovered that immune cells play roles in initiation of primary tumours, maintenance and growth of the tumour and in aiding cancer metastasis. Additionally, recent success in directing and strengthening the immune system's anti-cancer functions (e.g. tumour infiltrating lymphocyte (TIL) therapy and immune checkpoint inhibition) highlight the potential for new therapies that can come from a better understanding of how leukocytes are (dys) regulated.

Specialised vasculature and leukocyte dynamics

Our group has a particular interest in the lung both as a site of primary tumour development and cancer metastasis. Since it was first studied by microscopy almost 120 years ago, leukocyte extravasation has been refined in molecular detail in the post-capillary vessels, the major sites of immune cell infiltration in most (but importantly not all) anatomic sites. The extensive capillary network of the lung is unusual in several ways. Alveolar capillaries are of exceptionally small diameter (~5μm) and are in such close proximity to external mucosa that they share a basement membrane with the epithelium. In contrast to other organs, pulmonary capillaries are thought to be a major site of leukocyte extravasation with markedly different mechanisms to the general paradigm of leukocyte recruitment. Moreover, localisation and regulation of leukocytes within the pulmonary capillaries is not fully described or well understood.

Neutrophils pose the first-line of defence against many pathogens and play a key role in initiating the host immune response. In addition to potent effector mechanisms, including phagocytosis, degranulation and the recently described process of NETosis, neutrophils can contribute to the inflammatory milieu in a number of ways. They can produce and consume chemokines and cytokines, and modify the extracellular matrix to produce and modulate matrikines. Additionally, the accumulation of apoptotic neutrophils and their subsequent clearance is thought to directly contribute to anti-inflammatory programmes at the end of acute inflammatory responses. Taken together, these features mean neutrophils have the potential to contribute to both tumour antagonism and tumour-promoting inflammation, and recent work in the field of onco-immunology has demonstrated an important role for neutrophils in the process of lung metastasis.

Neutrophils have been described to 'marginate' in the pulmonary capillaries in a steady state to provide a rapidly mobilised pool that could supplement emergency granulopoiesis in the bone marrow in cases of systemic inflammation. Indeed, some studies - where isolated and radiolabelled neutrophils have been tracked through the circulation - have shown they have an extended transit time though the pulmonary vasculature but it is not clear whether this is simply a product of the exceptionally small capillaries that must be transited through in the lung or potentially an artefact of *ex vivo* purification and radiolabelling.

In contrast to other vascular beds that have been directly imaged by intravital microscopy (IVM), the lung microvasculature has not been extensively observed in this way due to the obvious technical difficulty of imaging a rapidly moving organ. It has been shown that neutrophils can be regulated by their interactions with both immune and non-immune cells. Thus, a thorough

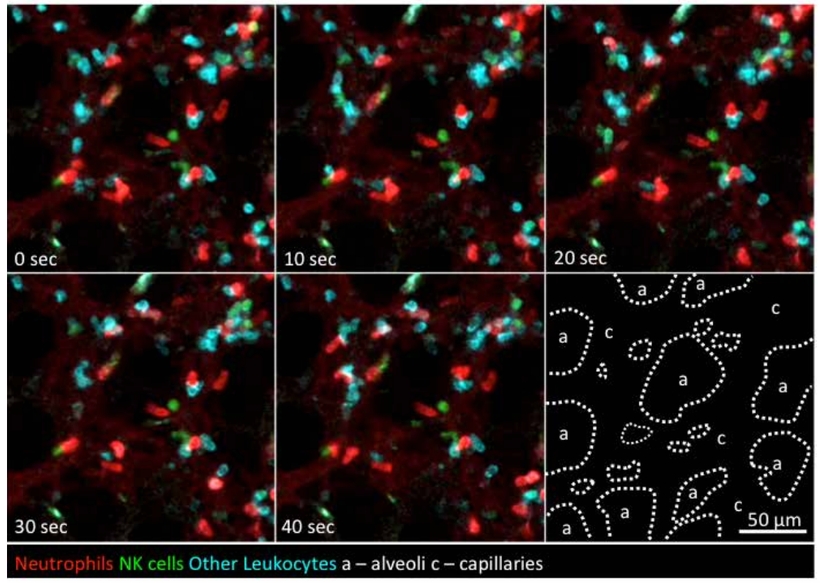

Figure 1
Lung intravital microscopy to monitor leukocyte dynamics.

examination of the localisation and regulation of pulmonary neutrophils and other leukocytes *in vivo* is a clear unmet need to understand the fundamental mechanisms underlying lung onco-immunology.

As mentioned above, many of the advances in our understanding of how leukocytes are recruited from the blood into tissue *in vivo* have been underpinned by IVM experiments in the post-capillary venules of the cremaster muscle or mesentery that are at least 20-40μm in diameter. However, these mechanisms do not necessarily apply to smaller diameter capillaries. For example, it has been known for some time that liver sinosoids that are 7-15μm in diameter are able to support selectin-independent leukocyte adhesion. Advances in optical microscopy and associated techniques have made it possible to image leukocytes directly in the context of intact organs in living organisms by IVM. In this way, several recent studies have revealed previously unknown intravascular leukocyte functions for NKT cells, monocytes, regulatory T cells and neutrophils. Our lab uses lung IVM (Fig. 1.) in combination with other experimental approaches (flow cytometry, transcriptomics, biochemical analysis) to better understand how the regulation of leukocyte dynamics contributes to the tumour environment in the context of both 'avoiding immune-destruction' and 'tumour-promoting inflammation'.

Having said this, the tumour microvasculature itself is as different and specialised as that of the lung. As mentioned above, leukocyte function is thought to be as important in the primary tumours as at secondary sites. Therefore, an additional important goal for our group is to also address the above regulatory mechanisms in the neo- and co-opted vasculature of the primary tumour.

As we only joined the Institute in September this year, much of our effort so far has been in identifying the most relevant cancer models to use, establishing our assays here and embarking on promising collaborations with other scientists at the Beatson and outside. This year, in our previous lab at Imperial College London, we hypothesised that pulmonary neutrophils were regulated by interactions with other leukocytes present in the alveolar capillaries both in a steady state and during inflammation. Our preliminary data are consistent with this and we look forward to uncovering more about this mechanism and its functional consequences in the coming year.

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TRANSLATIONAL CANCER THERAPEUTICS

Group Leader
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Associate Scientist
Jennifer Morton

Scientific Officer
Saadia Karim

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 anti-cancer 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 local control of inoperable disease.

Optimising molecular targeted therapies

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.

However, clinical trials of mTORC1 inhibitors in pancreatic cancer have not demonstrated significant anti-tumour efficacy in unselected patients, raising questions about this therapeutic approach. We employed a genetic approach to delete the obligate mTORC2 subunit *Rictor* and identified the critical times during which tumourigenesis requires mTORC2 signalling and showed that *Rictor* deletion resulted in profoundly delayed tumourigenesis. Whereas previous studies showed that most unselected pancreatic tumours are insensitive to rapamycin, treatment with a dual mTORC1/2 inhibitor strongly suppressed tumourigenesis. In late-stage tumour-bearing models, combined mTORC1/2 and PI3K inhibition significantly increased survival. Thus, targeting mTOR may be a potential therapeutic strategy in advanced pancreatic cancer.

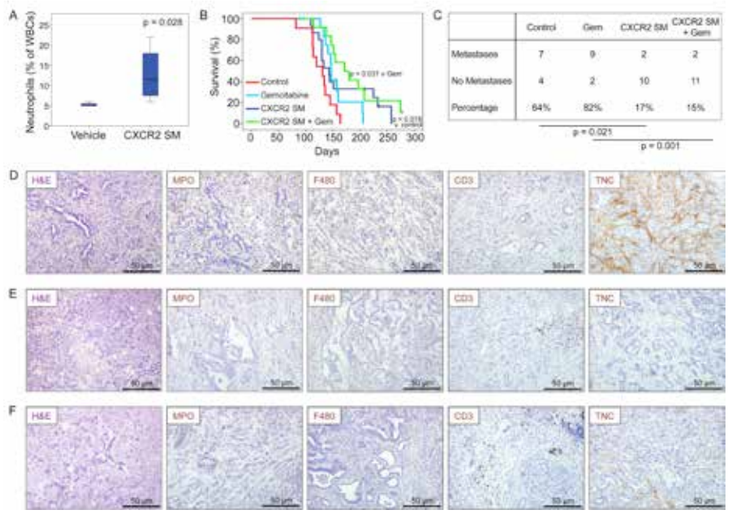

Figure 1
Therapeutic targeting of CXCR2 inhibits metastasis and prolongs survival. A] Boxplot showing circulating neutrophils in models (n = 4) treated with CXCR2 inhibitor. B] Kaplan-Meier survival analysis of KPC models treated from age of 10 weeks with vehicle (n = 11), gemcitabine (n = 14), CXCR2 inhibitor (n = 15) or CXCR2 inhibitor + gemcitabine (n = 12) C] Table comparing incidence of metastases in KPC models treated as indicated. D-F] H&E staining and IHC for MPO, F4/80, CD3 and Tenascin C in tumours in response to vehicle (D), CXCR2 inhibitor (E) and CXCR2 inhibitor + gemcitabine (F).

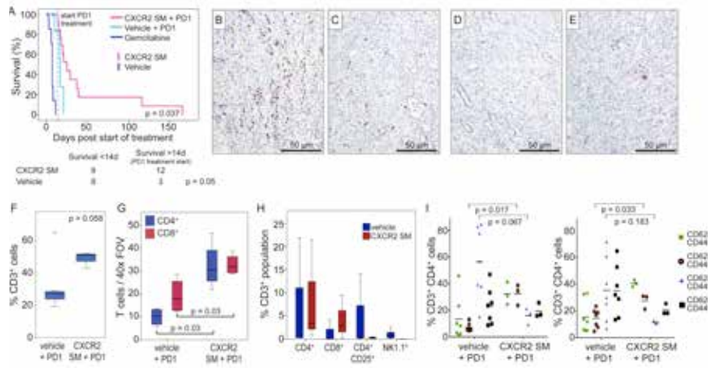

Figure 2
CXCR2 blockade promotes T cell infiltration into tumours and sensitivity to immunotherapy. A] Kaplan-Meier survival analysis of tumour-bearing KPC models treated with either gemcitabine, CXCR2 inhibitor alone for 2 weeks, and then in combination with anti-PD1, vehicle alone for 2 weeks, and then combined with anti-PD1, CXCR2 inhibitor alone or vehicle alone. B-C] IHC for Ki67 in tumours from KPC models treated with vehicle + PD1 (B), or CXCR2 inhibitor + PD1 (C). D-E] IHC for cleaved caspase 3 in tumours from KPC models treated with vehicle + PD1 (D) or CXCR2 inhibitor + PD1 (E). F) FACS analysis of intra-tumoural CD3+ cells in models treated as indicated. (G) Boxplot showing quantification of IHC for CD4+ and CD8+ T cells in tumours from KPC models treated as indicated. (H) FACS analysis of intra-tumoural CD4+, CD8+, CD4+CD25+ and NK1.1+ cells (% of CD3+ cells) in models treated as indicated. (I) FACS profile of CD4+ and CD8+ T cells isolated from tumours in models treated with either vehicle + anti-PD1 or CXCR2 inhibitor+ anti-PD1. staining and IHC for MPO, F4/80, CD3 and Tenascin C in tumours in response to vehicle (D), CXCR2 inhibitor (E) and CXCR2 inhibitor + gemcitabine (F).

Optimising immunotherapy

We are also targeting myeloid cells to enhance 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, and monocyte and granulocyte myeloid-derived suppressor cells (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.

CXCR2 is a G-protein-coupled receptor for the human CXC chemokines CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8. The primary immune function of CXCR2 is the regulation of neutrophil migration, as it controls the egress of these cells from the bone marrow and their recruitment to sites of inflammation. CXCR2 also regulates the migration of MDSCs. CXCR2 has been suggested to have both tumour-promoting and tumour-suppressive properties. We have shown that CXCR2 signalling is upregulated in human pancreatic cancer, predominantly in neutrophils/ MDSCs but rarely in tumour cells. Genetic ablation or inhibition of CXCR2 abrogated metastasis, although only inhibition slowed tumourigenesis. Depletion of neutrophils/MDSCs also suppresses metastasis suggesting a key role for CXCR2 in establishing and maintaining the metastatic niche. Importantly, loss or inhibition of CXCR2 improved T-cell entry and combined inhibition of CXCR2 and PD1 in murine models with established disease significantly extended survival. We have also shown that CXCR2 signalling in the myeloid compartment can promote pancreatic tumourigenesis and is required for pancreatic cancer metastasis. Our data also suggest that therapeutic targets that may cause senescence escape will not have deleterious effects in late stage disease in PDAC because tumours have already escaped this checkpoint.

Based on our data, we propose two potential therapeutic opportunities for PDAC: firstly, the addition of CXCR2 inhibitors into the adjuvant setting in patents who have undergone surgical resection of potentially curative disease and who have no visible evidence of metastases; secondly, the combination of CXCR2 inhibition and anti-PD(L)-1 antibodies could be explored in patients with advanced (metastatic) disease.

Clinical trial designs

We will exploit our preclinical 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. Critical to these approaches will be identifying potential genotype-specific biomarker signatures in murine models and confirming the clinical relevance of these in human tissue microarrays, and developing robust assays for patient selection to select or enrich the clinical trial population.

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 ECMCs in Belfast, Leicester and Guy's & St Thomas'. In the initial phase 1 part of the study, we are determining 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.

Publications listed on page 93

CELL MIGRATION AND CHEMOTAXIS

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Olivia Susanto
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Scientific Officer
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Graduate Students
Clelia Amato
Yvette Koh

Visitor
Vojtech Dostal

[1]CRUK Multidisciplinary Award

Cell migration in multicellular organisms must be suppressed, so that the architecture of tissues and organs remains consistent. In most early-stage cancer, it remains supressed. However, when tumours become metastatic, suppression of cell migration may be lost - cancer cells invade nearby tissues, and spread into the blood and lymph systems to form secondary tumours. Our group brings together multiple tools, from different disciplines, to improve understanding of how cell migration is controlled. We use *in vivo* models, cancer cells, model organisms and computational simulations, and apply a wide range of techniques, from genetics through biochemistry and microscopy to quantitative analysis of microscope movies and computational modelling.

We are 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, in particular actin.

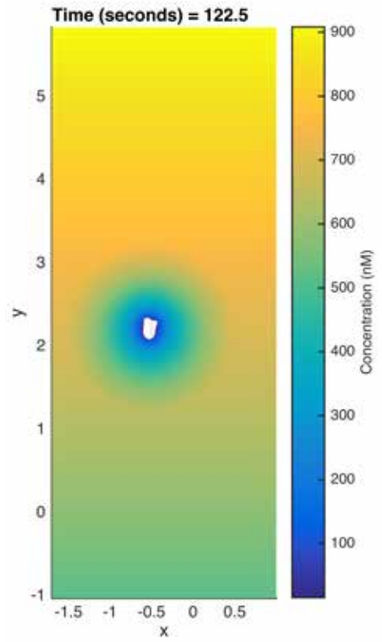
Mechanisms underlying chemotaxis: Pseudopods and self-generated gradients
Chemotaxis is emerging as a major driver of tumour metastasis. In the past, we have found that chemotaxis in *Dictyostelium* cells works by a different mechanism than that which is usually described. Pseudopods are constantly generated in random directions, then the ones that point in the best directions are selected and maintained. We are currently testing whether the same is true for cancer cells, using several melanoma lines. We have 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. Our initial results suggest that the melanomas move like *Dictyostelium*, with many randomly generated pseudopods whose behaviour is biased to change the cell's direction.

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 now studying the details of self-generated gradients, using mathematical models to identify the range of possible behaviours, and experiments with a wide range of different cell types including melanoma, pancreatic ductal adenocarcinoma, immune cells and *Dictyostelium*.

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

Figure 1
Computational modelling of the local gradient caused dynamically when a cell breaks down the chemoattractant in its vicinity.



newly associated with motility and invasion every year so we urgently need a mechanism to determine which are the most important.

Regulators of actin and the Arp2/3 complex

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. 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 activator 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. We are using the Beatson's expertise in mass spectrometry to identify proteins that crosslink to SCAR in living cells at different migration rates. 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.

Publications listed on page 96

STRUCTURAL BIOLOGY OF CILIA

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Tamas Yelland

Scientific Officer
Michael McIlwraith

Graduate Student
Yasmin Elmaghloob

Our group investigates the cellular mechanisms that maintain the distinct composition of cilia and immunological synapses. In particular, we are interested in trafficking of lipid-modified signalling proteins by a group of proteins called GDI-like solubilising factors. In our lab, we address our research questions using a combination of structural, biochemical and cellular biology approaches.

The phosphodiesterase 6D (PDE6D) protein is involved in the shuttling of ciliary and non-ciliary proteins. However, the sorting mechanisms that determine the ultimate destinations of these cargoes were unknown. Together with Alfred Wittinghofer's group (Max Planck Institute), we put forward a sorting model for prenylated INPP5E delivery to cilia. The model depends on the affinity of cargo for PDE6D, the presence of an active Arl3 found exclusively in cilia and the specific release of ciliary cargo by active Arl3.

GDP dissociation inhibitor (GDI)-like solubilising factors (GSFs) are a family of proteins, including PDE6D, UNC119a and UNC119b, which solubilise lipid-modified proteins and share structural homology with the Rho GDP dissociation inhibitors, a class of protein known to bind prenylated Rho proteins. PDE6D binds to and is involved in trafficking of prenylated proteins whereas UNC119a and UNC119b are specific for myristoylated proteins.

Arl2 and Arl3 are small G-proteins that belong to the Arf (ADP ribosylation factor)-like small G-protein subfamily, have a 52% sequence identity and share several interactors. Amongst Arl2 and Arl3 interactors are PDE6D, UNC119a and UNC119b, where the interactions are guanosine triphosphate (GTP)-dependent and do not involve lipid moieties. In my past work, I showed that Arl2 and Arl3 function as allosteric release factors for lipidated proteins bound to PDE6D and UNC119a/b in a GTP dependent manner. Furthermore, it has been reported that the ciliary protein Arl13b can act as a specific guanine nucleotide exchange factor (GEF) for Arl3.

PDE6D is involved in shuttling ciliary proteins, including INPP5E, GRK1 and PDE6 catalytic subunits, and non-ciliary proteins, such as Ras proteins. This raised the question of how cargoes are targeted to different destinations yet shuttled by the same protein. Using INPP5E and Rheb as examples for ciliary and non-ciliary proteins, respectively, we show that lipidated cargoes are solubilised by binding to GSFs in the cytosol. If a cargo binds to GSFs with a low binding affinity, the complex will be disrupted by active Arl2GTP in the cell body. In the case of ciliary proteins, which bind to GSFs with strong binding affinities, the soluble complex can diffuse into the cilia where it is released by Arl3, which is in turn activated by the ciliary protein Arl13b. The released cargo is then retained in cilia by associating with the ciliary membrane.

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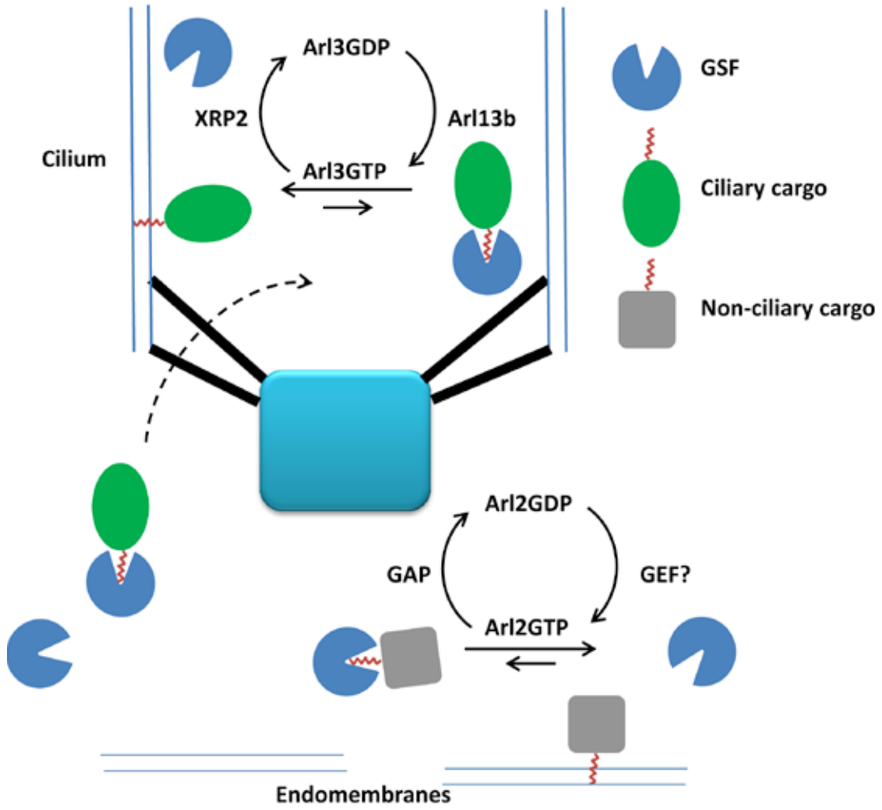

Figure 1
Sorting and shuttling of prenylated/myristoylated ciliary cargo into the cilium. The GSF (blue; e.g. Unc119b) binds to the lipid-modified tail of the ciliary cargo (green). The complex diffuses into the cilium where Arl3, maintained in a GTP bound state by Arl13b, binds the GSF, forcing a conformational shift that releases the ciliary cargo to the ciliary membrane. Binding of Arl3 to its GAP, XRP2, results in its inactivation. Non-ciliary cargo (grey) is solubilised by the GSF in the same manner before being released to endomembranes by Arl2GTP. The GEF required to activate Arl2, in its GTP bound state, is not known.

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MIGRATION, INVASION AND METASTASIS

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[1]Pancreatic Cancer Research Fund
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We urgently need to improve our understanding of the mechanisms of metastatic cancer dissemination in order to develop new therapeutic strategies to improve patient outcomes. Our research addresses 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.

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, the actin cytoskeleton 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 associated stromal cells 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 of proteins (including WASP/N-WASP, Scar/WAVE, WASH, WHAMM and JMY) transmit signals to the Arp2/3 complex to trigger actin assembly. We aim to understand the mechanisms of regulation and the involvement of these proteins in invasion and metastasis of cancer as well as their normal cellular function. WASP family proteins regulate actin assembly in multiple cellular processes,

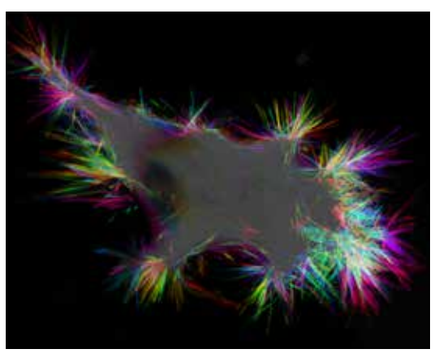

Figure 1
Fascin localises in highly dynamic filopodia in a MiaPaCa human pancreatic cancer cell.
Photo credit: Nikki Paul

such as endocytic trafficking, protrusion of lamellipodia and filopodia, cell division and assembly of invasive structures such as podosomes and invadopodia. Postdoc Ben Tyrrell studied the role of the WASH complex in assembling actin on endocytic vesicles to mediate trafficking of receptors and integrins. Deletion of the strumpellin subunit in melanocytes had no effect during development, despite defects in trafficking at the cellular level. Native gels revealed that, surprisingly, strumpellin null cells contained a partial WASH complex that appeared to recruit actin to endocytic vesicles, calling into question the previous view that each subunit of the WASH complex is essential for its function (Tyrrell *et al.*, 2016).

PhD students Loic Fort and Jose Batista (Robert Insall's group) have discovered a new highly conserved regulator of the Scar/WAVE complex that is responsible for controlling communication between the Rac1 GTPase and the Scar/WAVE complex. This protein allows cells spatial regulation of Rac1 signalling and subsequent actin assembly and protrusion. Future experiments will determine how this new regulator modulates cell migration, polarity and Rac1 signalling.

Role of actin regulatory proteins in colorectal and pancreatic cancer

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*. MRC-funded clinical research fellow Hayley Morris found that loss of N-WASP accelerated tumour progression of APC-driven colorectal cancer in a mouse model. Her findings suggest that N-WASP could have a tumour suppressive role in colorectal cancer, even though it is a promoter of invasion and metastasis in other models. Postdoc Amelie Juin is studying the role of N-WASP in pancreatic

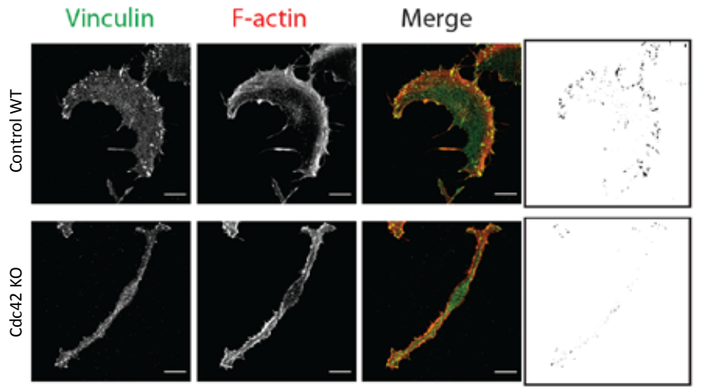

Figure 2
Vinculin and F-actin (actin filament) staining in normal and Cdc42 knockout melanocytes. Loss of Cdc42 causes a disruption of focal adhesion organisation in melanocytes.
Photo Credit: Nikki Paul

ductal adenocarcinoma (PDAC). Amelie has discovered a role for N-WASP in tumour invasion and metastatic spread to secondary sites. Using cells cultured from the tumours, she uncovered how N-WASP drives matrix remodelling by tumour cells and potentiates chemotactic signalling loops that mediate cell egress from the primary tumour. In collaboration with Ines Anton (Barcelona), we have uncovered a role for the N-WASP interacting protein (WIP) in cancer cell invasion and regulation of the formation of matrix degrading invadopodia protrusions (Garcia *et al.*, 2016).

Fascin is an actin bundling protein that is not expressed in normal epithelial cells, but is often upregulated in the most invasive and aggressive cancers (Fig. 1). PhD student Loic Fort and Pancreatic Cancer Research Fund postdoc Nikki Paul are studying how fascin upregulation affects the initiation of pancreatic cancer from precursor lesions (pancreatic intraepithelial neoplasia). Nikki leads our ongoing efforts to develop and test fascin-1 inhibitor compounds together with Drug Discovery. The team has developed some exciting new inhibitor compounds that can potently inhibit actin bundling *in vitro* and which are being tested in cellular assays.

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. We continue to investigate the roles of RhoA and Cdc42 in melanoblasts with PhD student Emma Woodham, postdoc Nikki Paul and scientific officer Heather Spence, together with Cord Brakebusch (BRIC, University of Copenhagen). Cdc42 is crucial for melanoblast migration and cells lacking Cdc42 show defects in polarity, migration and integrin-based adhesion (Fig. 2). Postdoc Karthic Swaminathan has discovered a role for the Scar/WAVE complex in melanoblast migration and melanoma tumour development and progression, suggesting that the Scar/WAVE complex may be an important melanoma target downstream of Rac1. New postdoc Jamie Whitelaw will study the role of the Scar/WAVE complex in cancer cell invasion and explore possible links between this complex and protein translational control. New PhD student Anh Le will study how Rac1 P29S, a driver mutation found in around 5% of sun-exposed melanomas, affects cell migration pathways.

Role of extracellular matrix in migration and invasion of tumours

Pancreatic ductal adenocarcinomas contain a dense fibrous stroma rich in collagen, fibronectin and other components. This is thought to serve both as a barrier to chemotherapeutic treatment and an inducer of more aggressive behaviour of the tumour cells. PhD student Vassilis Papalazorou has been engineering surfaces of various stiffness to test how this affects pancreatic cancer cell migration (Fig. 3). Vassilis is co-supervised with Manuel Salmeron-Sanchez (Engineering, University of Glasgow). Our goal is to better understand the crucial properties of desmoplastic stroma so that PDAC treatments could be improved in the future.

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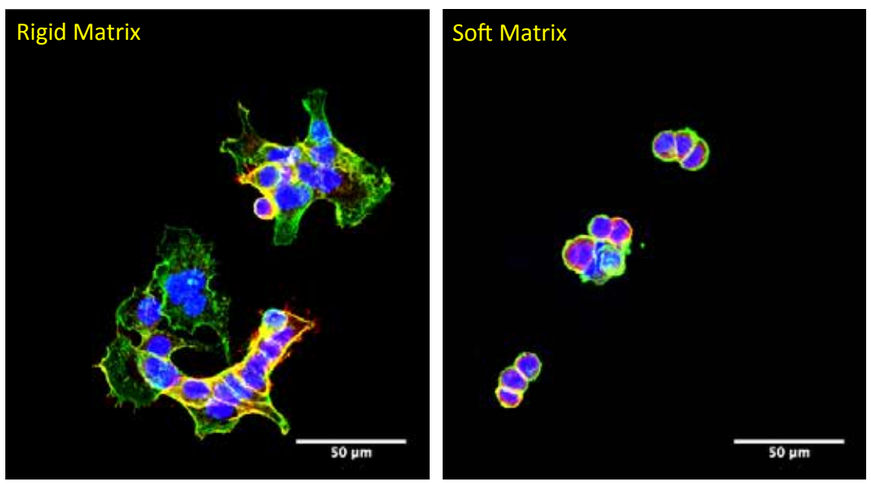
Figure 3
ASPC1 human pancreatic cancer cells on rigid matrix (40kPa) or soft matrix (1kPa) showing phospho-FAK (red), Actin filaments (green) and the nucleus (DAPI)
Photo Credit: Vassilis Papalazorou



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 integrin trafficking in the fibroblasts of other organs leading to extracellular matrix remodelling and the priming of metastatic niches. 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.

Expression of mutant p53 in tumours promotes production of exosomes that alter integrin trafficking in fibroblasts and drive extracellular matrix remodelling in distant organs

Primary tumours can influence the microenvironment in distant organs so as to promote the seeding of metastasis. We have been investigating the influence of tumours driven by particular oncogenes on the microenvironmental and extracellular matrix (ECM) organisation of various tissues using second harmonic generation microscopy. Interestingly, we have found that the presence of a mutant p53-expressing tumour in the pancreas leads to particular and quantifiable changes in organisation of the extracellular matrix in the lungs. Moreover, these alterations to lung ECM are consistent with those that we find to support invasive growth of tumour cells. Further investigation indicated that mutant p53-expressing tumours release exosomes in a manner that is dependent on the small GTPase, Rab35. These exosomes influence integrin trafficking in fibroblasts in a way that enables these cells to deposit collagen with the characteristics of the ECM found in the lungs of mutant p53 tumour-bearing animals. Quantitative proteomics indicate that mutant p53 influences levels of the sialomucin, podocalyxin on exosomes in a manner that enables them to promote integrin trafficking in target cells. Moreover, the target cells must express the Rab11 effector, Rab-coupling protein (RCP) and the phosphatidic acid generating enzyme, diacylglycerol kinase-α (DGKα) in order to alter their integrin trafficking in response to exosomes from mutant p53-expressing tumour cells. These findings indicate that a small population of mutant p53-expressing tumour cells are potentially able to influence integrin trafficking in the cells of other organs so as to prime metastatic niches.

Glutaminolysis drives receptor recycling to promote cancer invasion

The role of glutaminolysis in providing metabolites to support tumour growth is well established, but the involvement of glutamine metabolism in invasive processes driving metastasis is yet to be elucidated. We have found that normal mammary epithelial cells consume glutamine, but do not secrete glutamate. Indeed, low levels of extracellular glutamate are necessary to maintain epithelial homeostasis, and provision of glutamate drives disruption of epithelial morphology and promotes key characteristics of the invasive phenotype such as lumen-filling and basement membrane disruption. By contrast, primary cultures of invasive breast cancer cells convert glutamine to glutamate, which is released from the cell through the system Xc- antiporter to activate a metabotropic glutamate receptor. This contributes to the intrinsic aggressiveness of these cells by upregulating Rab27-dependent recycling of a transmembrane matrix metalloprotease to promote invasion and metastasis. Thus, acquisition of the ability to release glutamate is a key watershed in disease aggressiveness. Our recent findings 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.

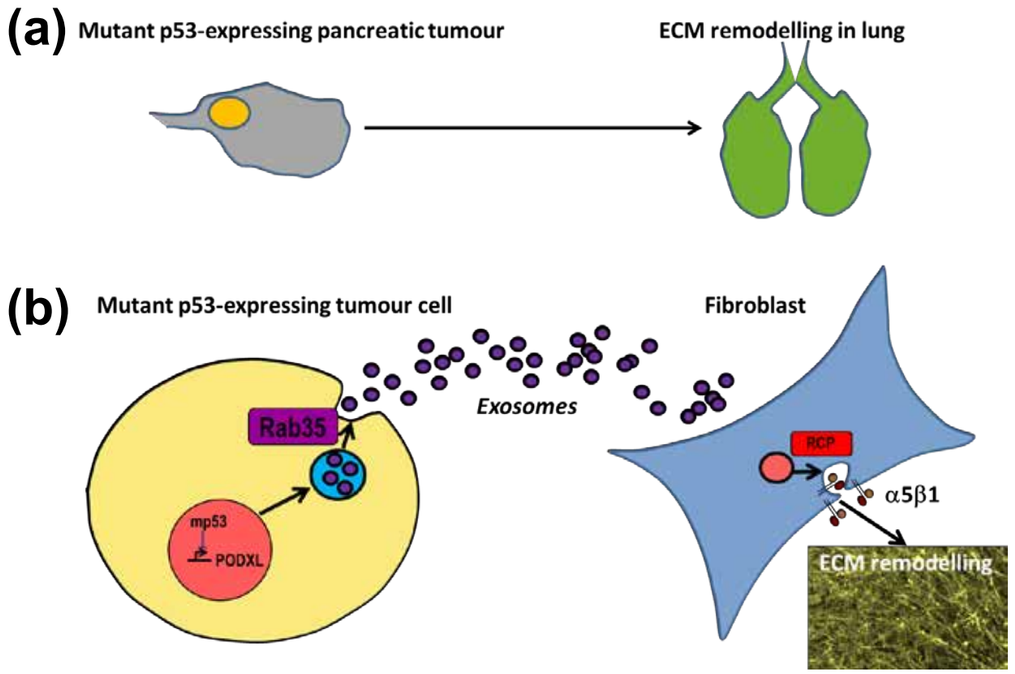

Figure 1
Exosomes from mutant p53-expressing tumours influences integrin trafficking in fibroblasts to promote ECM remodelling in other organs. (a) The extracellular matrix (ECM) in the lungs of mice is influenced by the presence of a mutant p53-expressing tumour in the pancreas. (b) The tumour cell depicted on the left expresses mutant p53, whereas the fibroblast on the right expresses wild type p53. In the tumour cell, mutant p53 activates a Rab35-dependent process tht=at leads to exosome release. Exosomes may then be transferred to the fibroblast. The fibroblast responds to these exosomes by activating RCP-dependent α5β1 recycling to promote ECM remodelling.

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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 influences, 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 important regulators of actin cytoskeleton dynamics that contribute to processes, which 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.

Actomyosin-induced gene expression responses

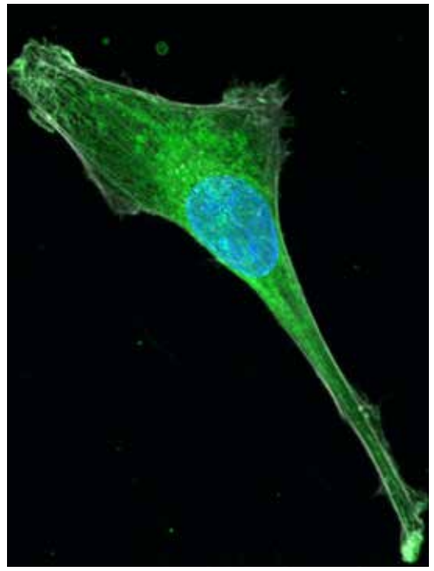
The RhoA and RhoC GTPases act via the ROCK1 and ROCK2 kinases to promote actomyosin contraction, resulting in directly induced changes in cytoskeleton structures and altered gene transcription via several possible indirect routes. Elevated activation of the Rho/ROCK pathway has been reported in several diseases and pathological conditions, including disorders of the central nervous system, cardiovascular dysfunctions and cancer. To determine how increased ROCK signalling affected gene expression in pancreatic ductal adenocarcinoma (PDAC) cells, we transduced mouse PDAC cell lines with retroviral constructs encoding fusion proteins that enable conditional activation of ROCK1 or ROCK2, and subsequently performed RNA sequencing (RNA-Seq) using the Illumina NextSeq 500 platform. Activation of ROCK1 or ROCK2 signalling induced significant changes in gene expression that could be used to determine how actomyosin contractility influences gene transcription in pancreatic cancer. Gene ontology analysis revealed a coordinated programme of ROCK-induced genes that facilitate extracellular matrix remodelling, with greatest fold-changes for matrix metalloproteases (MMP), Mmp10 and Mmp13. MMP inhibition not only decreased collagen degradation and invasion but also reduced proliferation in 3D contexts. These findings reveal an ancillary role for increased ROCK signalling in pancreatic cancer progression to promote extracellular matrix remodelling that facilitates proliferation and invasive tumour growth.

MRCK inhibitor development and biomarker identification

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

In collaboration with Drug Discovery, funding was obtained from Worldwide Cancer Research to characterise inhibitors of the MRCK proteins, and to discover and characterise biomarkers that would be informative of MRCK activation status. By expressing and purifying active or kinase-dead MRCKα followed by mass spectrometry, phosphorylation events were identified in wild type MRCKα that did not occur in the kinase-dead protein. By raising rabbit polyclonal antibodies against one of these sites, we were able to validate the post-translational modification as an autophosphorylation that reported on MRCKα activation. Validation of the antibody with MRCKα knockout cells grown as subcutaneous tumours, allowed us to confidently use this antibody reagent for immunohistochemical analysis of tissue microarrays to examine the relationship between MRCK activity and cancer. In addition, we were able to demonstrate that MRCKα autophosphorylation in mouse skin was a valid pharmacodynamic biomarker to examine the *in vivo* efficacy of MRCK small molecule inhibitors developed by Drug Discovery. *In vivo* pharmacological proof-of-concept experiments are currently underway to characterise the anti-tumour efficacy and pharmacodynamic biomarker modulation by the most promising MRCK inhibitory lead compound.

Figure 1
MDA MB 231 human breast cancer cells were labelled with BCN-E-BCN (green), a novel cell-permeable biscyclooctyne that can be used for the detection of sulfenylated proteins.



Detection of protein oxidation

Reactive oxygen species act as important second messengers in cell signalling and homeostasis through the oxidation of protein thiols. However, the dynamic nature of protein oxidation and the lack of sensitivity of existing molecular probes have hindered the understanding of such reactions; therefore, new tools are required to address these challenges. Funding from the Medical Research Council was obtained to study the role of protein oxidation in the regulation of cell migration. In collaboration with Richard Hartley (Chemistry, University of Glasgow), a bifunctional variant of the strained bicyclo[6.1.0] nonyne (BCN-E-BCN) was designed that enables the tagging of intracellular protein sulfenic acids for biorthogonal copper-free click chemistry. In validation studies, BCN-E-BCN binds the sulfenylated form of the actin-severing protein cofilin, while mutation of the cognate cysteine residues abrogates its binding. BCN-E-BCN is cell permeable and reacts rapidly with cysteine sulfenic acids in cultured cells. Using different azide-tagged conjugates, we demonstrated that BCN-E-BCN can be used in various applications for the detection of sulfenylated proteins. Remarkably, cycloaddition of an azide-tagged fluorophore to BCN-E-BCN labelled proteins produced *in vivo* can be visualised by fluorescence microscopy to reveal their localisation. These findings demonstrate a novel and multifaceted approach to the detection and trapping of sulfenic acids.

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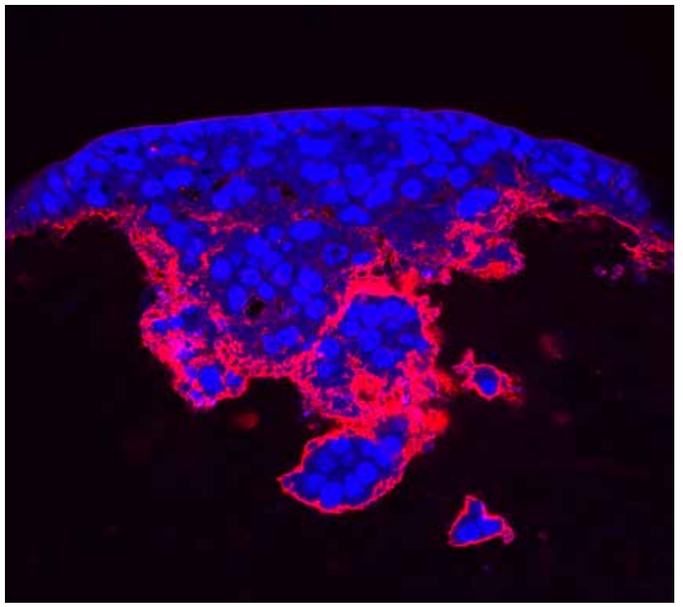
Figure 2
ROCK signalling contributes to the progression of pancreatic ductal adenocarcinoma by increasing the production and release of collagenases that induce extracellular collagen remodelling (red) to facilitate invasive tumour cell growth.



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COLORECTAL CANCER AND WNT SIGNALLING

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

Elucidating the cell of origin for colorectal cancer

Understanding the differences between *APC* and β-catenin mutations in terms of signalling provided mechanistic insights but one question that still puzzled us was that if an activating mutation of β-catenin was within an intestinal stem cell, then this would be long lived and therefore cells should have ample time to accumulate β-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 β-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 β-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 β-catenin mutations but if you include in 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.

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.

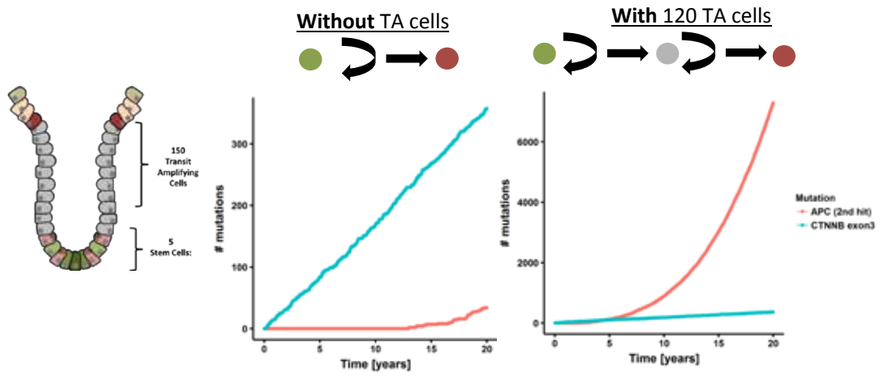

Figure 1
Simulation suggests that transit amplifying (TA) cells can act as the cell-of-origin in colon cancer. Left hand panel, if just the stem cell is considered then β-catenin mutations are more likely to occur (blue line) rather than two APC mutations (red line). If the TA cells are included then two APC mutations accumulate.

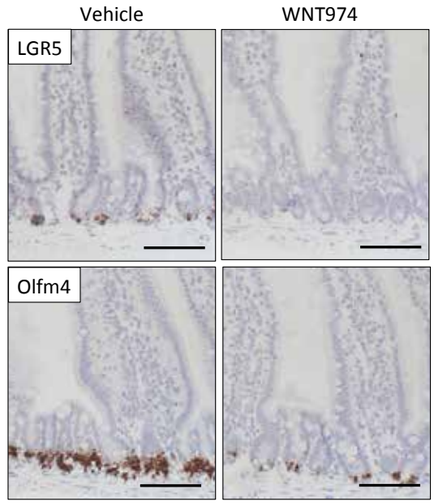

Figure 2
WNT974 treatment reduces intestinal stem cell markers. Porcupine inhibition reduces the expression of the stem cell markers Lgr5 and Olfm4 using RNASCOPE in situ hybridisation (performed by Colin Nixon and his group).

Demonstrating that HUWE1, which is mutated in up to 10% of colorectal cancer (CRC), is a bona fide tumour suppressor gene

In human cancers, many genes are mutated often at relatively low frequencies. Assigning these as functional modifiers of cancer development or treatment is therefore very important. In CRC, mutations of HUWE1 (an E3 ubiquitin ligase) occur in approximately 10% of cases and as they are on the X chromosome, a single mutation should result in loss of function. To investigate whether HUWE1 can act as tumour suppressor gene, we deleted this in combination with APC loss *in vivo* and found a dramatic increase in tumour initiation. Mechanistically, this was associated with a marked increase in the DNA damage marker gamma, H2AX, which is a target of HUWE1 ligase activity. Other HUWE1 targets, c-Myc and MCL-1 were also upregulated. Importantly, this increase in DNA damage correlated with a sensitivity to cytotoxic agents and patients who carry HUWE1 mutations respond well to chemotherapy. Together, our results therefore indicate HUWE1 mutations are driver mutations in CRC and may alter the response to therapy.

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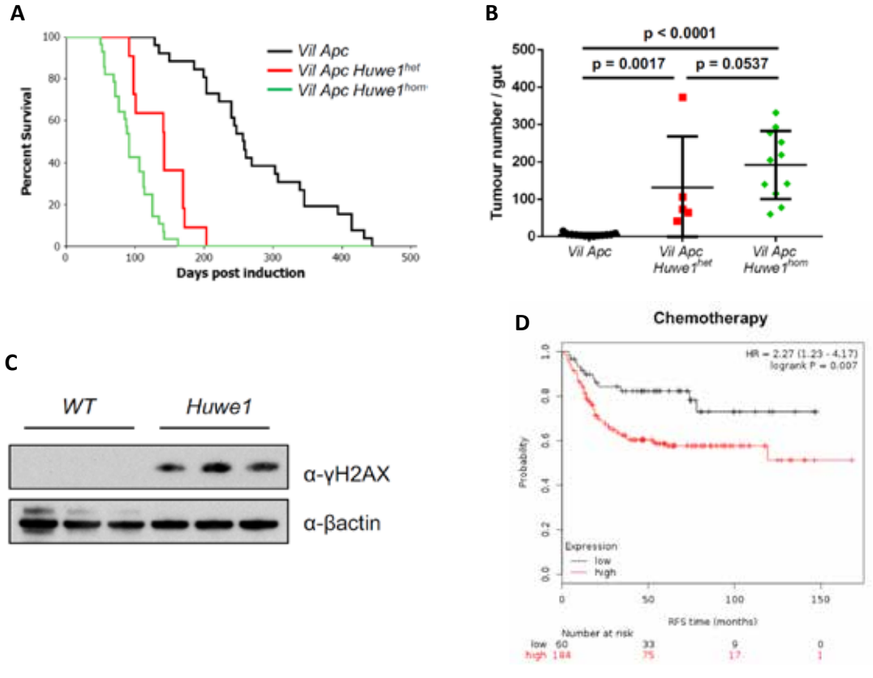

Figure 3
HUWE1 is a bona fide tumour suppressor gene. A) Huwe1 los accelerates time to intestinal tumourigenesis (green versus black line). B) Huwe1 loss increases numbers of intestina tumours. C) HUWE1 loss increases levels of gamma h2a D) Patients that have tumours with low levels of HUWE1 (blac line) have improved survival following chemotherapy compared to those with high levels of HUWE1 (red line)

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 metastasis. Cancer associated fibroblasts (CAFs), the most abundant non-neoplastic cells in tumours, are characterised by their ability to secrete a plethora of factors that contribute to the formation of an aberrant tumour microenvironment and alter the behaviour of the surrounding cells. For this reason, CAFs play a key role in cancer pathology. Our group aims to identify molecular mechanisms that control the ability of CAFs to create a pro-invasive tumour microenvironment and to investigate the possibility of targeting these cells for therapy.

In particular, we study: 1) factors secreted by CAFs that can modify the tumour microenvironment and affect the behaviour of and communicate with the surrounding cells; and 2) how CAFs influence endothelial cell (EC) behaviour. ECs line the inner layer of the vessel wall and play a crucial role in regulating the function and growth of the vessel. In many solid tumours, the vasculature is responsible for disease progression. Initially, tumours recruit blood vessels to obtain nutrients and oxygen to sustain cancer cell growth. Late, the tumour vasculature becomes leaky and provides a route for the cancer cells to escape and form distant metastases.

Our group exploits its experience in high resolution mass spectrometry (MS)-based proteomics 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 new light on the complexity of the tumour microenvironment.

CAFs can originate from normal fibroblasts resident at the site where the primary tumour develops. Under stress conditions, such as chronic stimulation by factors secreted by cancer cells, normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels such that CAFs are characterised by being highly contractile with many secreting soluble factors and ECM components that promote cancer progression. This highlights the importance of a better understanding of how CAFs alter the tumour microenvironment. To do this, we have used unbiased MS-proteomic approaches, which we have previously shown to be a powerful tool to investigate cellular secretomes and molecular mechanisms regulating EC functions, together with functional *in vitro* and *in vivo* assays. We have discovered and characterised previously unknown mechanisms through which CAFs drive invasion.

Unravelling CAF-induced paracrine mechanisms of cell invasion

We have developed methods to perform in-depth quantitative proteomic analysis of secreted proteins and ECM components, and used these to identify proteins aberrantly secreted by fibroblasts when activated by cancer cells. Since CAFs are highly abundant in the stroma of breast cancers, we have investigated the process in this tumour type. We have identified more than 1000 proteins secreted by fibroblasts, of which around 300 had altered levels in CAFs. These included ECM components and growth factors, such as collagens, fibronectin and transforming growth factor β, which are well-known CAF markers. Intriguingly, we identified that the chloride intracellular channel protein 3 (CLIC3) was secreted by CAFs and deposited in the ECM. We have now shown that extracellular CLIC3 is a pro-invasive redox enzyme able to promote vessel growth and tumour invasion by activating the extracellular transglutaminase, TGM2 (Fig. 1) (in collaboration with Jim Norman's group).

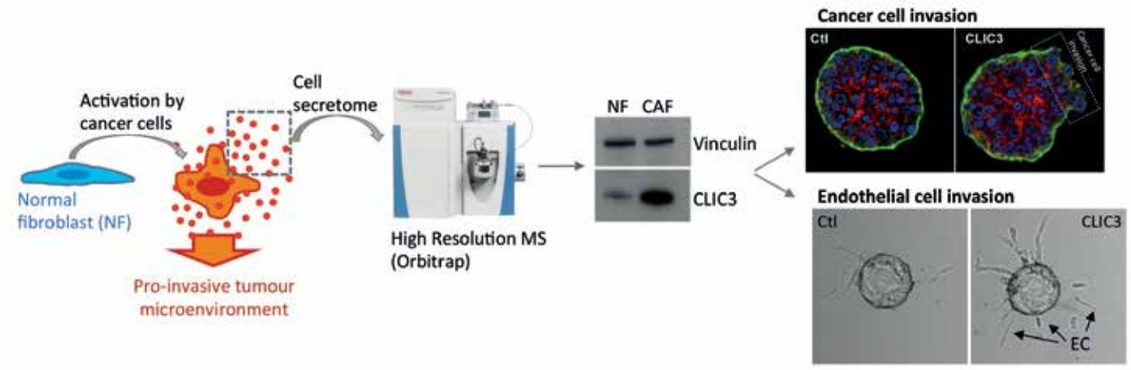

Figure 1
Scheme showing that breast cancer cells induce the transformation of normal mammary fibroblasts (NF) into CAFs. CAFs secrete factors that are pro-invasive. By using mass spectrometry (MS)-based proteomics, we have discovered that CLIC3 is highly secreted by CAFs (as shown by western blot) and that CLIC3 is pro-invasive. Top right: MCF10DCIS.com breast cancer cells (red) embedded in Matrigel and treated with soluble recombinant CLIC3 (CLIC3) breach the basement membrane (green) and invade the surrounding matrix. Bottom right: Human umbilical vein endothelial cells (HUVEC) embedded in fibrin gel and treated with soluble recombinant CLIC3 (CLIC3) sprout and invade the surrounding matrix (arrows highlight some invading cells).

Detailed analysis of CLIC3 expression in different cancer types revealed that it is highly expressed in the stroma of aggressive breast and ovarian cancers, and that high levels of CLIC3 in these tumours predict poor patient outcome. We are now investigating the function of CLIC3 in the progression of tumours and the possibility of targeting it to block the pro-invasive function of CAFs in tumours.

Tumour stiffness favours tumour cell invasion
CAFs secrete a number of ECM components and ECM modifiers, such as cross-linking enzymes, which are responsible for the increased stiffness of tumours, a phenomenon that has been observed in many cancer types during progression. Blood vessels play a key role in the formation of metastasis, because cancer cells must intravasate into the blood stream to spread to distant sites (Fig. 2). It has previously been shown that high matrix stiffness enhances tumour invasion by promoting the invasive behaviour of cancer cells. However, it is not known if/how high stiffness can directly affect endothelial cell function related to tumour invasion.

Using MS proteomics, we have measured proteomic changes occurring in ECs when adhering to fibronectin-coated polyacrylamide gels of physiological and 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 were upregulated by tumour stiffness. We have characterised one mechanism through which high stiffness induces increased levels of a member of the CCN protein family that in turn enhances the expression and exposure to the plasma membrane of a transmembrane receptor of the cadherin family. We show that this mechanism is key for the binding of cancer cells to ECs, which is the first step in cancer cell intravasation into the blood stream, and favours the formation of metastasis. Hence, we have identified a new function of tumour stiffness on the vasculature and discovered a pathway that can be targeted to reduce and possibly block stiffness-induced intravasation of cancer cells.

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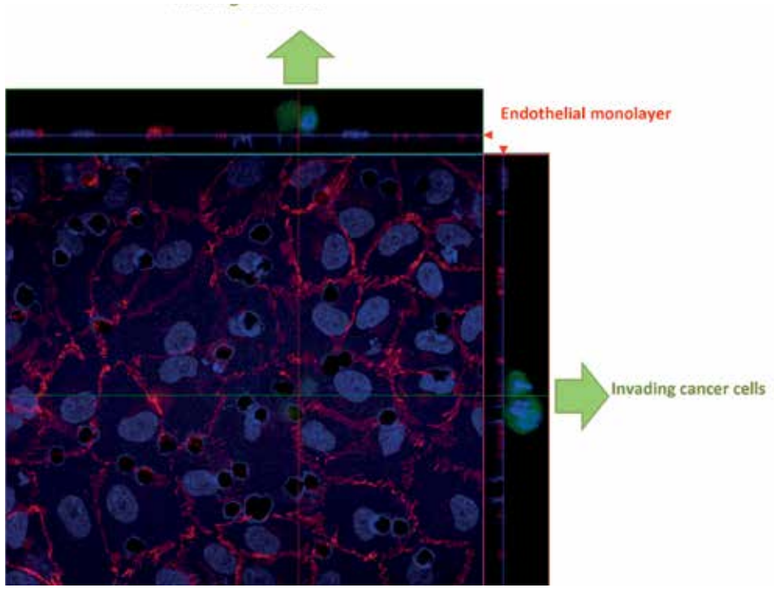

Figure 2
Example of an *in vitro* model that mimics the intravasation of the cancer cells in the blood. In red are HUVECs (human umbilical vein endothelial cells) that have been cultured to form a monolayer (centre panel: seen from above; top and left panel: seen from the side). In green are the cancer cells that have been plated on top of the endothelial monolayer and been able to pass through it.

DRUG DISCOVERY

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Drug Discovery continues to utilise structure-based methodologies to advance our projects against some of the most important and challenging targets in cancer today. In particular, our progress targeting KRAS is hugely encouraging. In addition, our expertise in fragment-based hit identification has been recognised through participation in a CRUK Centre Network Accelerator Award that enables us to collaborate more broadly with the wider CRUK community to utilise these techniques to advance their drug discovery efforts.

KRAS

One of the most fundamental and devastating causes of cancer initiation and progression arises from mutations of the membrane signalling protein KRAS. In normal cells, KRAS activation is responsible for a cascade of signalling events through multiple pathways leading to growth and survival of cells. For this reason this 'switch-like' GTPase is tightly controlled through activation of cell surface receptors and on/off regulators of its activity. In cancer, mutations of KRAS result in this master growth control protein being constantly switched on, as the deactivating hydrolysis enzymes are unable to access their binding site and switch off its activity. The result is an excess of growth signals that drive cancer progression in an uncontrollable manner.

A number of drugs on the market and in clinical trials today, target molecules in the signalling pathways downstream of KRAS such as the PI3K/AKT/mTOR and the RAF/MEK/ERK pathways. However, these approaches have been plagued with issues of redundancy on the one hand and toxicity on the other, when treatment combinations have been used. KRAS mutations are so widespread in cancer, particularly in some of the most difficult to treat cancers such as pancreatic, colorectal and lung (98%, 45% and 31% of patients, respectively, have a KRAS mutation), that they represent a huge unmet clinical need (Cox *et al.*, Nature Reviews Drug Discovery 2014; 13: 828-51).

For over 30 years, scientists have tried to block the activity of KRAS without success but importantly they have made crucial discoveries along the way. The new era of fragment-based drug design, which is a cornerstone of our expertise, has raised exciting new possibilities to inhibit this most intransigent target. Together with the application of medicinal and computational chemistry, crystallography, structural biology and state-of-the-art biophysical techniques in surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC), we have made substantial progress over the last few years.

Following the initial screen of our fragment collection against GDP loaded (inactive form) G12D mutant KRAS using high field NMR, we

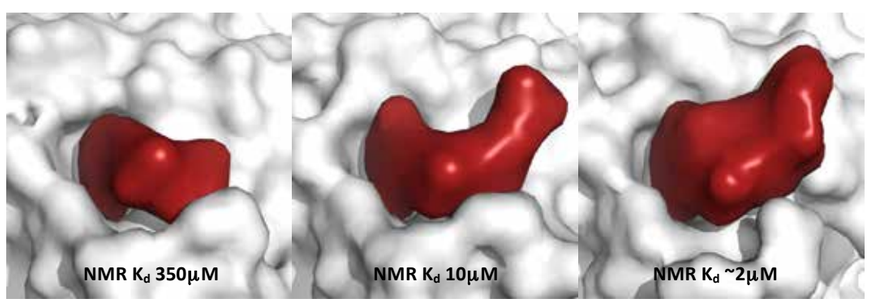
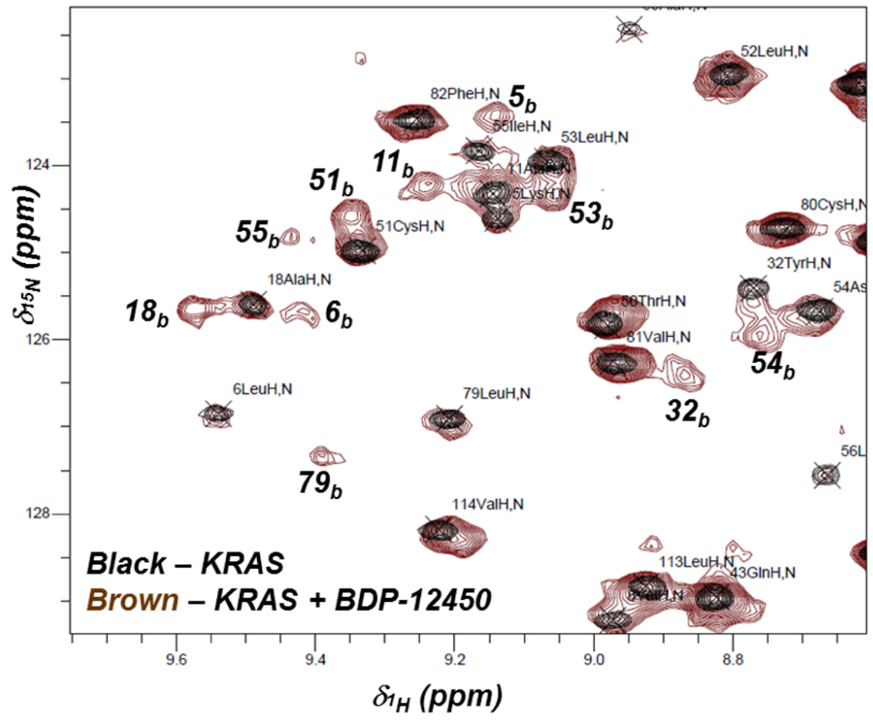


Figure 1
Optimisation of KRAS inhibitors.

Figure 2
^{1}H-^{15}N HSQC of KRAS-G12D.GDP with BDP-00012450.



have been able to optimise these weakly binding starting points into substantially increased affinity compounds. Key to this success has been the ability to generate high quality proteins and their crystallisation to reveal ligand-bound structures at high resolution. The structural insight this has afforded, has allowed us to further develop an optimisation strategy resulting in the design and synthesis of KRAS inhibitors that demonstrate binding affinities in the low µM range (Fig. 1).

The binding affinity of compounds has been determined using NMR against KRAS-G12D.GDP, which has been the primary screen for the project. The K_d values for our best compounds against KRAS-G12D.GDP is now K_d ~2µM, which is a substantial increase from the original µM binding fragment hits. (Fig. 2, showing cross peaks for both free and bound protein, BDP-00012450 estimated K_d ~2µM). This success means that the compounds are now too high affinity for the Kd to be accurately determined using NMR, as a result of a too slow exchange on the NMR timescale. To address this, additional assays have been developed including SPR, ITC and HTRF. We have established two additional biophysical assay formats that allow us to measure K_d values below the 10 µM range, namely SPR and ITC (Fig. 3 overleaf).

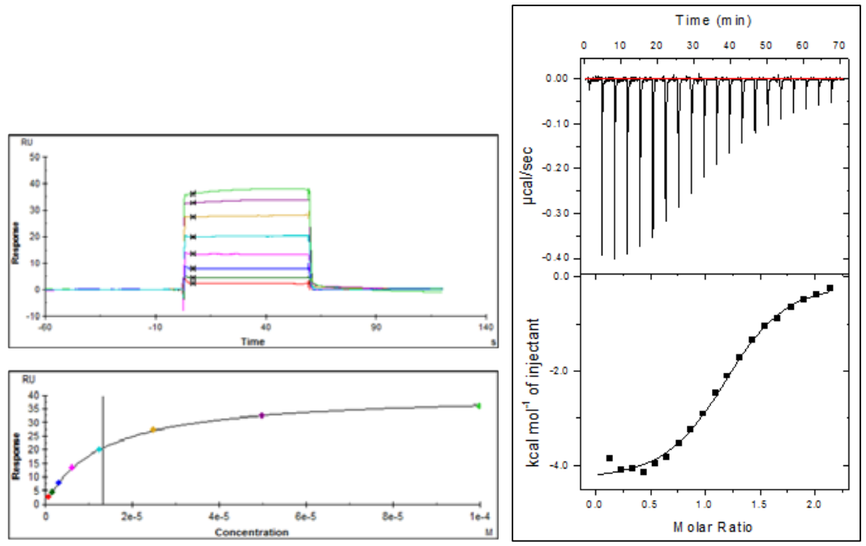
During the last year we have expanded our capability to use the more challenging but more clinically relevant active form of KRAS loaded with the stable GTP analogue GMPPnP. Use of this protein has been implemented across all of the assays including crystallography, NMR, SPR and biochemical assays. Importantly, we have directly addressed one of the most challenging aspects of the project and developed a robust biochemical assay for measuring the potency of our inhibitor compounds against KRAS-effector binding using a homogeneous time resolved fluorescence (HTRF) system. Comparison of data from the HTRF assay with the previously established nucleotide exchange assay (NEA), which measures inhibition of inactive GDP KRAS, has enabled us to assess the ability of compounds to inhibit these two important functions of RAS (Fig. 4 overleaf).

Future strategies will build on the progress made above, leveraging a combination of chemistry, biology and structural biology to develop sub-µM mutant KRAS inhibitors that are effective in cell assays and ultimately be taken into clinical trials. In order to maximise the opportunity for success with this project, the group has initiated key collaborations to boost resource and expertise in this challenging but potentially highly rewarding project.

CRUK Centre Network Accelerator Award: fragment-based drug discovery

In October 2015, CRUK awarded a significant five-year Centre Network Accelerator Award to establish a collaborative network across its drug discovery programmes, enabling challenging target identification and early stage drug discovery through the provision of specialised structural biology resource. Its purpose is to stand

Figure 3
SPR and ITC data for BDP-00013092 (Kd values 13.8mM and 3.7mM respectively).



as a networked, flexible resource that aims to deliver more oncogenic targets leading to rapid development of targeted therapies.

The resource is focused on the following areas (participating centres): Protein production and characterisation (Newcastle and Leicester); Biophysics and fragment screening (CRUK Beatson Institute); Protein structure determination (Leicester); and Computational chemistry (ICR) (Fig. 5 shown right). Participating groups within the network (CRUK Beatson Institute, Leicester, Newcastle, ICR, CRUK Manchester Institute, Cancer Research Technology Discovery Lab (CRT-DL), Belfast and Leeds) are encouraged to submit proposals for resource to undertake work in any of the above areas to a steering committee comprising members from each of the participating centres. The proposal will then be entered into a workflow that for successful projects will result in a fragment screen (Fig. 6 shown right).

As a recognised centre of excellence for fragment screening, biophysics and protein structure determination our group has committed to deliver fragment screens against challenging targets for participating Accelerator Award groups. Fragment screening offers the possibility to discover new potential drugs starting from the binding of a low molecular weight 'fragment' molecule to the target, which is then evolved stepwise increasing its affinity by chemical modification into the final drug. An example of a drug developed by this method is Vemurafenib (Fig. 7 shown right, which targets mutant BRAF malignant melanoma. The simple, low complexity molecular architecture of fragments enables them to access small molecule binding sites on proteins that can, through appropriate choice of assays and structures, be developed further, even against the most challenging, novel drug targets. With this approach we have designed robust and reproducible biophysical/structural biology binding assays using SPR and NMR screening protocols, and identified validated fragment hits for multiple CRUK-funded projects, providing a list of validated hits and a summary report (Fig. 6).

Figure 4
HTRF and NEA dose response curves for BDP 13646, EC50 values 36mM and 6mM respectively.

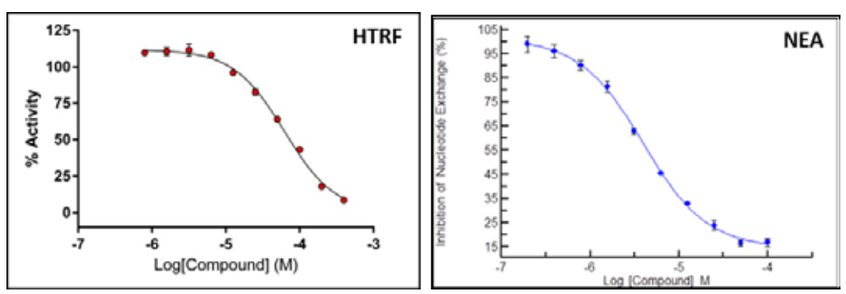
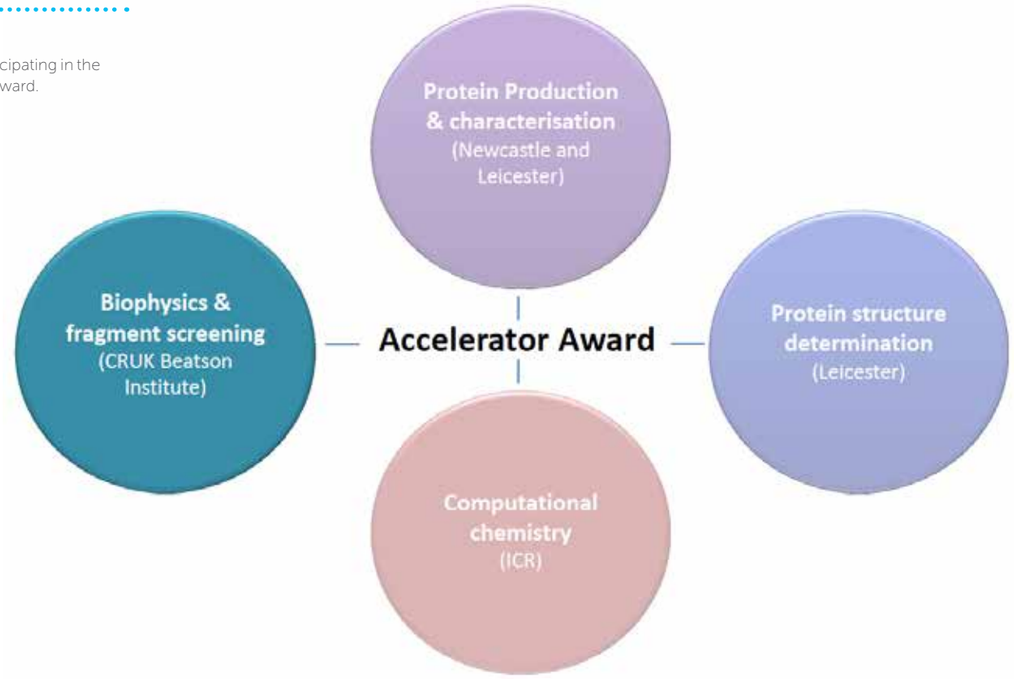


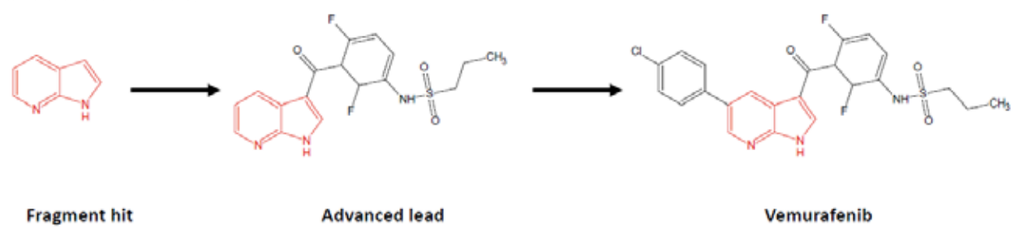
Figure 5
Facilities participating in the Accelerator Award.



The setup of a fragment screen consists of an initial quality control of the protein target followed by a primary screen of ~ 1000 fragments against the target using SPR or NMR. The resulting hits are then validated by a secondary screen (NMR or SPR, respectively) to increase confidence in target engagement. Throughout the screen an ongoing consultation is provided, starting from protein purification up to the further evolvement of the fragment hits after the screen. We also undertake virtual screens in order to provide potential additional start points and enrichment for the fragment screening hits (upon generation of structural information). So far, we have completed three fragment screens within this award, all of which have provided promising starting points for the development of new anti-cancer drugs. For the upcoming year, we are planning to continue to provide our service as a fragment screening facility to members of the Accelerator Award but also to other CRUK-funded projects increasing collaboration across the CRUK network and enabling new project starting points.

Figure 6
Process of fragment screening within the Accelerator Award.

Figure 7
Vemurafenib: Drug created by fragment-based screening (Proc Natl Acad Sci USA 2008; 105: 3041-6, Nature 2010; 467: 596-9).



ADVANCED TECHNOLOGIES

BEATSON ADVANCED IMAGING RESOURCE (BAIR)

Head
Leo Carlin

Scientific Officers
Ewan McGhee
Margaret O'Prey
David Strachan

Modern light microscopy allows us to gather information about important regulatory mechanisms in tumours and key cells of the microenvironment whilst preserving spatial and temporal information. No other technique allows us to simultaneously record multiple important molecules and cells with subcellular sensitivity and resolution in living samples whilst maintaining the context of the microenvironment, be that model substrate or living organism.

Beatson Advanced Imaging Resource (BAIR) scientists work closely with the Institute's researchers to uncover and interrogate important molecular pathways in cancer. The BAIR is thus involved at some stage in nearly every paper from researchers at the Beatson that contains a light micrograph. We train scientists in all stages of modern microscopical research from advice on sample preparation, basic and advanced microscope operation and data acquisition through to quantitative image analysis and interpretation. At the start of a new project or application we are keen to help researchers identify how light microscopy can be used to test key hypotheses and to help them design experiments that make the most of the resource we have. We also help to identify new technology and methodology that allow our researchers to take the most elegant approaches.

This year was marked by our inaugural BAIR image competition, eliciting some stunning images that exhibit the excellent imaging performed here. The winning image by Jean-Philipe Parvy can be seen on pages 86 and 87. It shows a symmetric montage of Drosophila fat body. Random recombination has been used to generate a mosaic tissue overexpressing a HA-tagged protein. Nuclei are stained with DAPI (white), recombinant cell expressing the GFP and the HA-tagged protein are stained with an anti-GFP (green) and anti-HA (red) and the F-actin is stained with phalloidin (blue). Microscope: Zeiss 710 confocal; objective: 20X, zoom 0.6.

Core themes and new methodologies

Although basic transmitted light and epifluorescence microscopy may not appear to have advanced much in recent years, the way that we use them has substantially. We have stable, long-term time lapse systems capable of automated, unattended imaging and we also have increased our numbers of IncuCyte incubator-based imaging systems to four this year. These can multiplex up to six imaging plate-based experiments and perform week-long recordings, making the most of the stable incubator environment and, for example, allowing researchers to look at the effects of several mutations or perturbations at once. Our new 'PhaseFocus' microscope allows us to perform long-term, label-free live cell imaging with automated tracking, potentially drastically increasing the number of cells that can be tracked in each experiment. We continue to work with our colleagues in Functional Screening and Histology to make sure that researchers are using the best combination of high-content/throughput methodologies and extract as much meaningful data as possible from each experiment.

Confocal microscopy is a mainstay of the BAIR and we have three confocal laser-scanning instruments, two spinning disc microscopes and two multiphoton systems (one of which is embedded in the Biological Services Unit, BSU). This means that we can capture highly resolved fluorescence data in several ways that optimise speed (spinning disk), resolution and number of channels (laser-scanning), depth (multiphoton) and other readouts, e.g. fluorescence lifetime (spinning disk FLIM; multiphoton FLIM). Excitingly, we have made more use than ever of our Zeiss LSM 880 'Airyscan', which uses a spatially arrayed multi-GaAsp detector to increase signal and resolution beyond the usual diffraction limit to 140 nm laterally and 400 nm axially. The addition of beam shaping optics to the excitation pathway essentially allow us to scan four lines at once increasing imaging speed by fourfold with a small decrease in resolution.

As an excellent start to the new year, the latest instrument to be added to the BAIR arrives in January 2017. This will be a second Zeiss LSM 880 'Airyscan FAST' with the addition of multiphoton excitation embedded in the BSU. This will massively increase our ability to address cancer processes by intravital microscopy. It will be an upright system, capable of fast super resolution imaging and synergise exceptionally with the existing BSU inverted multiphoton FLIM system. Second harmonic generation imaging will also be possible on this microscope, as well our other multiphoton systems, allowing us to perform label-free imaging of the extracellular matrix, an important component of the tumour microenvironment (as exemplified in Rath *et al*, 2016. We are particularly excited about using this new microscope to study tumour cell biology and immunology of primary and metastatic lung, liver, brain and pancreatic tumours that will benefit from increased speed, resolution or simply being able to address the tissue from above rather than below.

BAIR team: from left, David Strachan, Leo Carlin, Ewan McGhee and Margaret O'Prey.

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

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

Our team focuses on exploratory data analysis, with an ultimate goal of providing insights that enhance our understanding of cancer biology. We offer routine processing of RNA and DNA sequencing data, differential expression analysis and splicing, and 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 Toolbox and the Statistics Toolbox). 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. We have been developing a new search strategy to utilise publicly archived RNASeq data and search for evidence of differential alternative splicing patterns between normal and tumor samples. We aim to quantify the occurrence of both known and novel splicing events in genes of specific interest to our research groups.

Matt Neilson has been analysing how a single cell breaks down chemical attractants. He uses PDE-based simulations that couple a 1D representation of the cell edge with a 2D representation of the attractant distribution. This fairly simple and obvious process becomes very unpredictable and complex when you consider the cell moving in response to the attractants while it breaks them down. The result is a number of complex changes, in particular a cell's ability to convert tiny random changes in the attractant into prolonged migration away from its original location. Another complex issue is how changes in the cell's shape, while it breaks down attractants, affect the distribution of the attractants. Again, the outcome is often unexpected persistent movement. This is of course very important for cancer, in which persistent movement away from the original location is a key part of metastasis.

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 designs, statistical techniques and data presentation. We are also involved in teaching MSc in Cancer Sciences students, and provide personalised training in the use of specific methods, programming in R and Bioconductor.

Publications listed on page 97

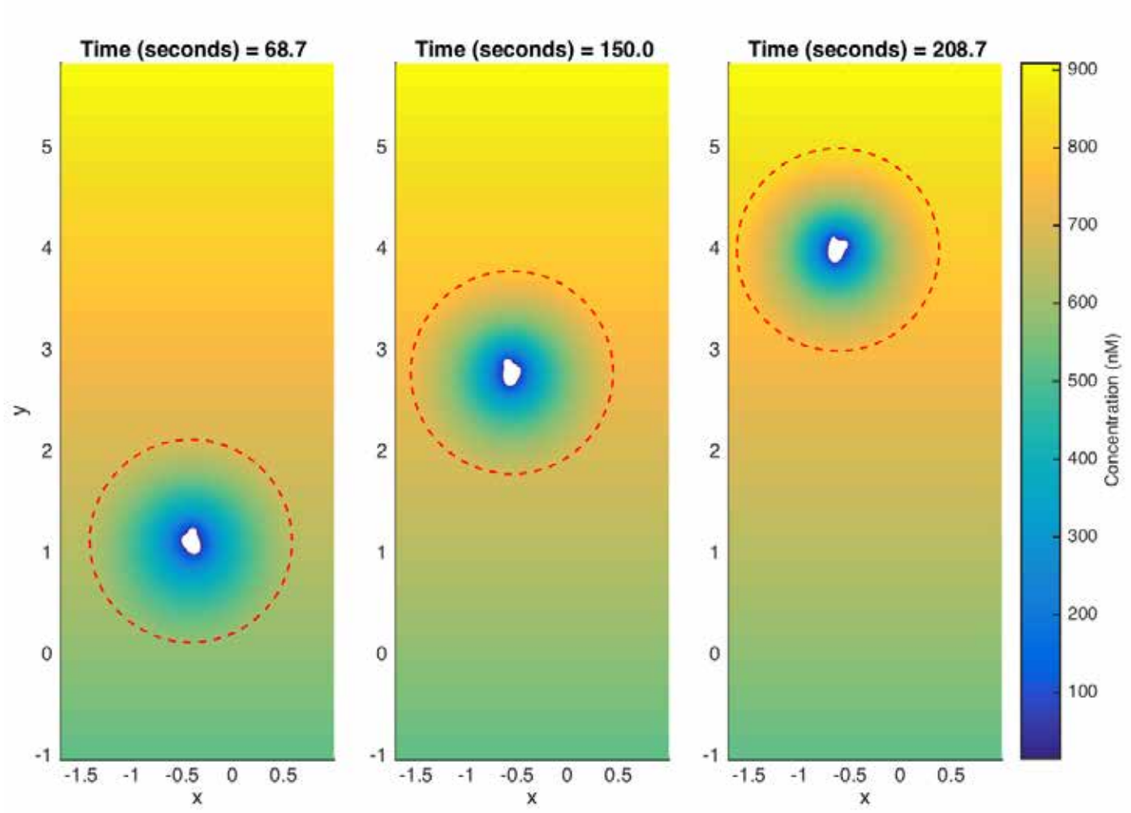


Figure 1

Three 'snapshots' from a simulation, in which a cell (the white area within the red-dashed circle) is chemotaxing in a field containing a gradient of attractant. The local concentration of the attractant is denoted by the background colour and a colour map is provided on the right hand side of the image. The far-field gradient is acting 'up' the page, while a more localised gradient is created around the cell due to the cell itself breaking down the attractant. This results in very strong chemotaxis, as seen from the three snapshots.

METABOLOMICS

Head
Gillian Mackay

Senior Scientific Officer
Niels van den Broek /
David Sumpton

To demonstrate changes in metabolic pathways in cancer cells, the Metabolomics facility uses mainly LC-MS technology for both targeted and untargeted approaches. We have well established targeted LC-MS methods, where we measure approximately 100 metabolites per sample. For untargeted analysis, we have now developed and validated a robust workflow using LC-MS methods and Nonlinear Dynamics' Progenesis QI software, which can be used for biomarker identification, hypothesis generation and as a discovery tool. In 2016, we were involved in organising and delivering a practical metabolomics course at Cold Spring Harbor Laboratory in the USA.

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. We are continuing to develop new methods for improved metabolite coverage using different LC columns.

With our targeted approach to metabolomics, we analyse a range of samples including cell extracts, 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). Experiments using stable isotope tracers (often labelled with ^{13}C 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.

Metabolomics can be used to look for novel metabolic changes, by identifying compounds showing different abundances in cancer cells, using an untargeted approach. We have developed a LC-MS/MS workflow, which includes a longer analysis time to include both high resolution full scan MS and ddMS2 (fragmentation of the 10 most abundant compounds throughout the analysis), as well as the removal of background ions. With Progenesis QI and the additional EZinfo statistical software, metabolites that have changed due to experimental conditions can be examined using various statistical approaches, such as PCA and OPLS-DA, resulting in S-Plots showing those that have changed the most. These altered metabolites can be selected and examined in more detail in Progenesis QI. 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. Our fragmentation spectra are compared with an *in silico* fragmentation database, to try to increase the confidence of the metabolite identification. Unique identifications are often not found and comparing fragmentation patterns with Thermo's mzcloud database has proved a valuable additional tool. We are planning to build our own fragmentation library and have purchased a mass spectrometry metabolite library of 600 standards from Sigma. We have also started to use the online MetaboAnalyst, for pathway analysis for our altered metabolites. This untargeted 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 derivatised to allow them to be volatile in the GC. We have methods in place for fatty acids, amino acids and acetate.

We work closely with the groups of Eyal Gottlieb, Karen Vousden, Jurre Kamphorst and Alexei Vazquez and also support several other research groups within the Beatson with specific interests in cancer metabolism. We train researchers in data analysis using Thermo Scientific TraceFinder software and work together with scientists for untargeted data analysis with Progensis QI software. External collaborators have visited us to learn practical metabolomics with LC-MS.

Publications listed on page 99

NUCLEAR IMAGING

Head
Gaurav Malviya

Scientific Officer
Agata Mrowinska

Multi-modal imaging is now well established, especially in the field of nuclear imaging. Magnetic resonance imaging (MRI) provides high contrast among soft tissues and the combination of this anatomical information with the functional information offered by the ever-increasing number of tracers available for positron emission tomography (PET), has incredible potential. Specific tracers used in PET imaging allow non-invasive longitudinal assessment of biological processes, such as angiogenesis, hypoxia, metabolic heterogeneity, cell proliferation, receptor dynamics and monitoring response to cancer therapy at the molecular level.

PET and MRI are complementary imaging methods for better understanding of tumourigenesis and evaluation of novel treatments in small animal cancer models.

PET imaging involves the administration of tiny amounts of radioactive tracers followed by a non-invasive scan, after this a sequentially acquired MRI scan helps localise radioactive tracer uptake within the body. MRI provides superior quality images due to its high intrinsic soft tissue contrast. Consequently, we are able to have 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.

Through collaboration with Anthony Chalmers and Andrew Sutherland (University of Glasgow) and Sally Pimlott, Jonathan Owens and Gerry Gillen (NHS Greater Glasgow and Clyde), we have increased our PET imaging armamentarium. Now, we have ^{18}F-FLT for cellular proliferation, ^{18}F-FDG for glucose metabolism, ^{18}F-FAC for lipid synthesis, ^{18}F-NaF for bone lesion imaging, ^{18}F- AB5186 for translocator protein imaging and ^{18}F-FZ236 for poly(ADP-ribose) polymerase-1 (PARP-1) imaging.

Changes this year

This was a year of great advancements in the facility. The UK's first fully integrated preclinical dual modality PET/MR scanner was successfully installed at the Institute. This cutting-edge imaging modality is equipped with a 1 Tesla permanent magnate MRI, with 100 µm spatial resolution, and a LYSO crystal PET detector, with 700 µm spatial resolution.

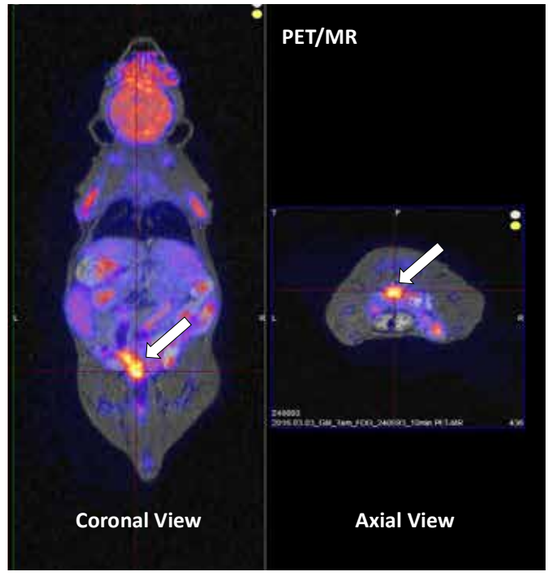
In order to facilitate the use of this newly installed PET/MR scanner for the investigation of transgenic mouse models of cancer, David Lewis has been recruited to a junior group leader post at the Institute. David will bring considerable expertise in PET imaging with carbon-11 labelled tracers, and will lead a team for novel tracer development and imaging of metabolic heterogeneity.

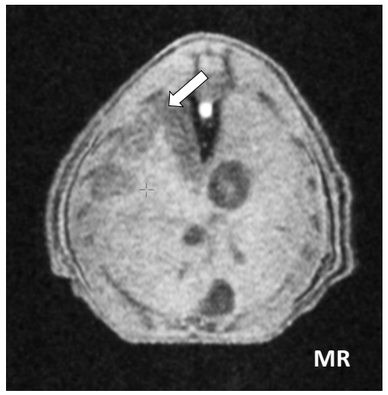
Furthermore, in collaboration with Sally Pimlott and Hing Leung, we have begun an imaging study with a new PET radiotracer, ^{18}F-FACBC, a synthetic L-leucine analogue (anti-1-amino-3-18F-fluorocyclobutane-1-carboxylic acid) for imaging expression of ASCT2/SLAC1A5 and LAT1 transporters. This novel radiotracer is being used in clinical trials in patients with prostate cancer. This year, we also included Gadolinium (Magnevist®) and Manganese ion (Mn^{2+}) contrast agents for contrast-enhanced MRI imaging.

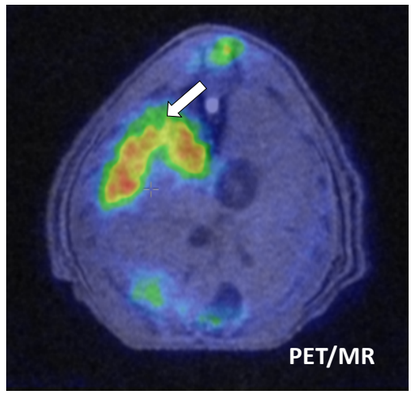
Publications listed on page 99

Figure 1
^{18}F-FDG PET/MR image shows uptake in a colon cancer mouse model (arrow).

Figure 2
^{18}F-FDG PET/MR images in a liver metastasis mouse model (arrow).







PROTEOMICS

Head
Sara Zanivan
(see page 46)

Scientific Officers
Sergio Lilla
David Sumpton

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

The facility is actively working to use and develop MS-based proteomic platforms to help researchers better understand the mechanisms that regulate various aspects of cancer disease. To do this, the facility is well equipped with three LC-MS systems, Q-Exactive HF, which was installed early this year, LTQ Orbitrap Elite and LTQ Orbitrap Velos, which are coupled online to Easy-nLC systems.

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

During 2016, we worked with many of the groups at the Institute to address a wide variety of questions, including single protein identification, identification/quantification of post-translational modifications, protein-protein interactions and quantitative global proteomics of 2D and 3D cell culture, tumour tissue samples and cell secretomes, including soluble factors and vesicles.

We are continuously striving to develop methods to answer more complex biological questions using proteomics and improve the methods currently in place to enrich the quality of the data that the facility can provide. We have recently established methods to quantitatively measure cysteine oxidation and optimised high pH reverse phase LC fractionation for in-depth proteomic analysis for low sample amount.

Publications listed on page 103

FUNCTIONAL SCREENING

Head
Emma Shanks

Scientific Officers
Kay Hewit[1]
Lynn McGarry

Informatics Manager
Daniel James

Graduate Student
Matthew Davidson

[1]CRUK Glasgow Centre

The Screening facility couples high throughput functional genomics 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 for elucidating novel drug targets and/or drug partners to improve existing cancer therapeutic approaches.

In 2016, the facility invested in the expansion of the resources it can provide for researchers. This has seen the development and implementation of workflows to support new functional genomics screening tools such as the CRISPR-Cas9 system in both pooled and arrayed formats. Moreover, we have significantly advanced our image analysis toolbox to provide machine learned, phenotype-led image analysis in an automated manner: this has been applied in both 2D and 3D environments with great success.

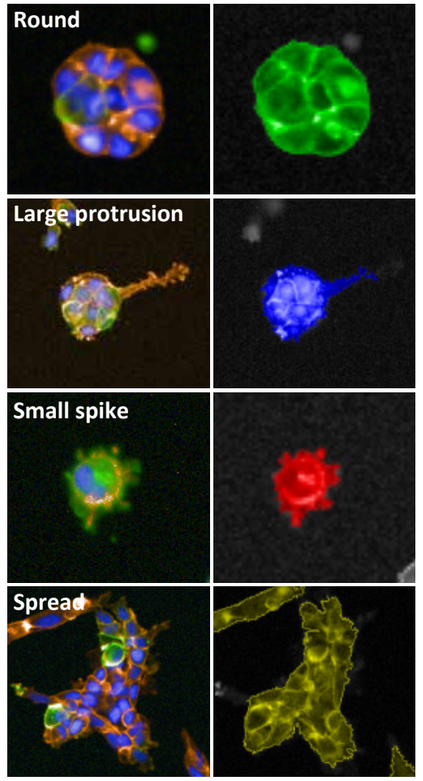
During 2016, we ran a total of 9 screening campaigns in collaboration with both the Beatson and CRUK Glasgow Centre groups. To date, we have generated over 4.5 million data points across 30K screening plates: we have screened more data points in 2016 than all previous years combined, exemplifying the substantial increase in the demand of the facility's resources.

Over the past year, the majority of our projects have been target validation-led. This approach uses custom-built genomics libraries and/or drugs to validate a shortlist of candidates identified from complementary approaches, i.e. bioinformatics, genomic characterisation. This reflects a shift away from previous years, with the volume of our work now focusing on screening smaller libraries across a greater number of cell lines, and testing more conditions. Technical demand is therefore greater for the facility, and has represented a substantial increase in our undertakings.

In 2016, we have addressed the following questions in multiple cancer backgrounds:

- The role of ARF GTPases, regulators (GAPs, GEFs) and effectors in regulation of prostate spheroid polarity. This shRNA screen was supported by quantification of cellular polarity and formation of dynamic protrusions in 3D. Following development of a robust shRNA screening platform, spheroids were imaged and phenotypes of interest were taught to the analysis software using machine learning image analysis tools (Fig. 1)
- Identification of radiosensitising partners as therapeutic candidates for glioblastoma (an externally funded collaboration between CRUK and Bayer)
- Factors shaping the aggressive ESC/iPSC-like prostate cancer subtype
- Optimising taxane therapy for prostate cancer (part of the OptiChem initiative)
- Identifying innovative therapeutic avenues for metabolomic and immunogenic subclasses of pancreatic cancer using drug repurposing
- Synthetic dosage lethal partners of ACSS2 in colorectal cancer
- Identifying metabolomic biomarkers of fascin function

Figure 1
Example quantification of machine-learned phenotypes: ARF GTPases, regulators and effectors modulating polarity in prostate cancer spheroids. A total of four phenotypes were identified and taught to machine learning software to generate analysis algorithms: roundness, long protrusions, small spikes and spread.



- Exploring combinatorial partners for the mTOR inhibitor AZD8055 using drug repurposing in nasopharyngeal cancer
- Identification of genes supporting proliferation of radio-resistant oral cancer cells under hypoxic conditions (pooled CRISPR screen)

Furthermore, our image analysis capabilities are being increasingly being used by researchers as stand alone tools, incorporating lower density plates and slides. Machine-learned image analysis is a powerful tool, bringing robustness and throughput to the process, while alleviating researcher-dependent analysis. During the last quarter of 2016, demand for this toolset escalated dramatically, demonstrating its extremely high value utility to research groups. We anticipate that this demand will further increase in 2017. To support anticipated image analysis queries, the facility has been considering options for enhanced instrumentation to meet the needs of researchers with efficiency and precision.

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www.beatson.gla.ac.uk/karen_blyth

TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Research Scientist
Nicholas Rooney

Scientific Officers
Dimitris Athineos
Sandeep Dhayade
Susan Mason

Graduate Student
Alessandra Riggio

The focus of our lab is in the use of preclinical models to understand cancer processes in a physiological setting. We study how alterations in signalling and metabolic pathways contribute to cancer and how these tumour-associated disturbances might be exploited to improve therapies in the clinic. We have a particular interest in the role of the RUNX/CBFβ co-factor complex in breast and other epithelial cancers.

Preclinical models at the Institute

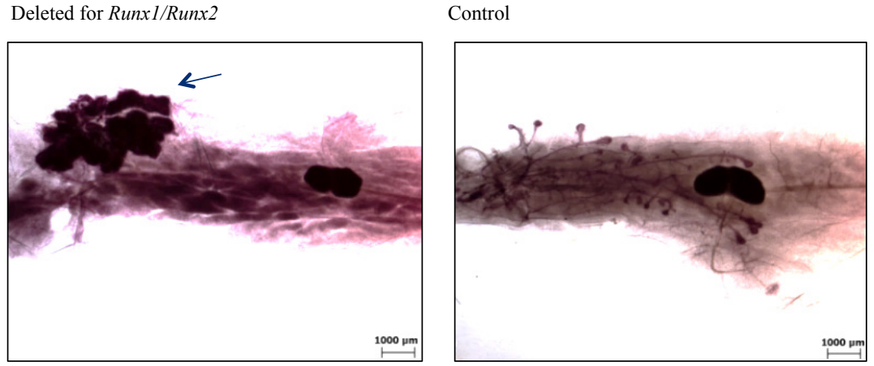
Epithelial cancers exist as a complex milieu of tumour cells co-evolving with other cell types (e.g. stromal fibroblasts, blood vessels, immune cells) and ultimately outgrow the organ of origin to invade surrounding tissue and metastasise to distant sites. Thus, investigating aspects of the cancer journey in a tissue culture dish has clear limitations, and an integrated understanding of the process demands physiologically relevant *in vivo* models. To this end, our lab develops and utilises xenograft, allograft and sophisticated genetic models of various tumour types such as breast, pancreatic and prostate cancers, and melanoma. Many groups at the Institute use these preclinical tools to study cancer development and metastasis, and to investigate novel approaches to cancer therapy. For example, it has been exciting to collaborate with Iain McNeish's lab (Glasgow) to develop better models of ovarian cancer (Walton *et al.*, 2016) in which we can probe how patient-specific mutations affect tumour growth and response to therapy. We have also been working closely with Sara Zanivan's group to show that CLIC3 promotes cell invasion *in vivo* (Hernandez-Fernaud *et al.*, 2017) and in the development of *in vivo* angiogenesis assays. Our ongoing collaborations with Karen Vousden's lab exploring the therapeutic potential of serine starvation using preclinical models have shown exciting results, while also with Karen's group we have demonstrated how loss of TIGAR affects reactive oxygen species and intestinal proliferation in genetic models (Cheung *et al.*, 2016).

The *RUNX* genes in epithelial cancer

The *RUNX* genes, together with their binding partner CBFβ, form a transcriptional complex that regulates transcription of several key signalling pathways associated with cancer. It is not surprising then that these genes are altered in different types of cancer. We previously showed that high levels of RUNX1 and RUNX2 in breast cancer patients correlated with poor patient survival in a particular subtype of the disease known as triple negative breast cancer. Conversely, however, *RUNX1* loss may be an important driver in other types of breast cancer that express the oestrogen receptor (ER+). The lab has been investigating this paradox and has shown that while deleting RUNX1 in triple negative breast cancer cells results in reduced growth and decreased tumourigenic potential, deletion of *Runx1* in a genetic model of breast cancer can lead to increased tumour burden. This is the first *in vivo* evidence of RUNX1 as a tumour suppressor in mammary epithelial tissue. A particularly interesting result from these studies shows that deleting both *Runx1* and *Runx2* dramatically accelerates breast cancer progression (Fig. 1).

We have also uncovered a role for the *RUNX* genes in prostate cancer. This was the work of Anne McKillop, a CRUK Glasgow Centre funded clinical research fellow, who successfully defended her PhD thesis earlier this year. By assessing expression levels in patient samples (Fig. 2), Anne showed that high levels of *RUNX2* correlated with more aggressive tumours and reduced patient survival. Substantiating a pro-oncogenic effect of RUNX2, we see that deleting *Runx2* in prostate cancer models

Figure 1
Whole mount images of mammary tissue. Deletion of *Runx1/Runx2* leads to early cancerous lesions as depicted by the arrow on left panel. Age-matched control tissue devoid of tumour is shown on the right for comparison.



results in smaller tumours, while increased levels precipitate early neoplastic changes. RUNX1 on the other hand acts in a tumour suppressor capacity with low expression levels in patient samples leading to poorer overall survival and more rapid tumour development in preclinical models.

Another interesting avenue where we believe there may be a role for the *Runx* genes is in the tumour microenvironment. We have observed that high *RUNX2* expression in the stromal compartment of breast cancer patients correlates with reduced disease-free survival. High levels of *RUNX* genes are also found in cancer associated fibroblasts. Preliminary results from the lab suggest that reducing *Runx2* gene dosage in mammary fibroblasts can retard the growth of mammary cancers thus demonstrating that changes in the surrounding tissue as well as in the tumour cells themselves affects cancer progression.

SEARCHBreast

In collaboration with colleagues in Leeds, Sheffield and London, we are founder members and contributors to the SEARCHBreast initiative, a network of researchers committed to sharing information, materials and samples to refine and accelerate breast cancer research through collaborative approaches (Blyth *et al.*, 2016; Speirs *et al.*, 2016). This year saw us launch our website (www.searchbreast.org) and host a very successful workshop at the Institute on *in vivo* models of breast cancer.

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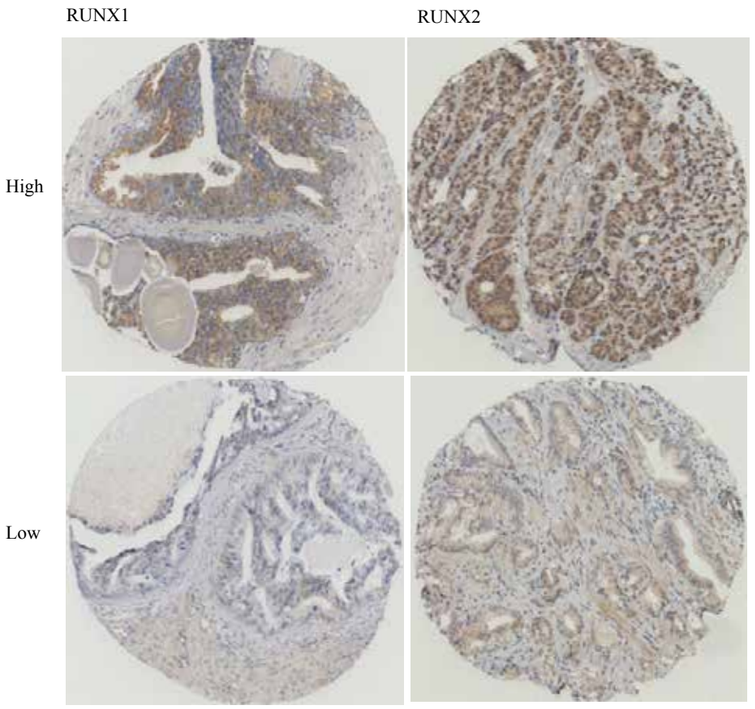
Figure 2
Representative images of prostate cancer biopsy samples with high (top) or low (bottom) expression for RUNX1 and RUNX2.



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TRANSGENIC TECHNOLOGY

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We use molecular genetic approaches to analyse the role genes play in contributing to the progression of cancer. By using techniques such as genome editing and gene targeting we introduce precise genetic changes into stem cells allowing us to accurately model the specific changes in genes detected in human cancers. By using specific combinations of these genetic changes we can generate more sophisticated models of human disease and understand how the genetic changes work together to contribute to the development of cancer.

Generating cancer models with stem cell technology

Embryonic stem (ES) cells have a number of properties that make them extremely useful tools for understanding the roles genes play in cancer development. Firstly ES cells have very high rates of genetic recombination, which allows us to rapidly make precise alterations in defined genes and sets of genes by gene targeting or genome editing. This can be introducing a mutation that completely disrupts the gene removing all of its function entirely or perhaps introducing a small point mutation analogous to that see in human cancers that may partially compromise or alter the function of the modified gene. The second property that makes ES cells useful for this kind of analysis is that they can be easily differentiated to form any adult cell type. Once a cell clone has been identified carrying the desired genetic alteration, the cells can then be allowed to develop into cell types from the tissue or tissues that are the subject of the study. Thus, the consequences of any set of genetic changes, which we introduce into the ES cells, can be analysed not in the stem cells themselves but in the cell types and tissues in which a particular cancer usually arises.

We are currently working in collaboration with a number of different groups at the Institute to introduce a variety of genetic alterations using the methods outlined above into different genes, including point mutations, conditional knockouts as well as using site-directed nucleases for targeted genome editing.

Developing better reagents for cancer modelling

In addition to making use of existing technologies, we are also continuing to update and improve the methodologies that we use routinely to allow experiments to work more efficiently and to allow the production of more sophisticated and accurate cancer models.

As well as loss-of-function or point mutations, genes in cancer are frequently amplified or overexpressed. In these cases an increase in the copy number or alterations in the chromatin microenvironment can alter the expression level of a gene, frequently resulting in a dominant phenotype. This type of event can also be modelled in stem cells using DNA constructs normally termed transgenes. The expression of these transgenes can be controlled by using the gene's endogenous promoter or by the addition of a heterologous promoter such as β-actin, which should allow widespread expression of the transgene.

Typically, transgenes such as this are introduced into cells by microinjection or transfection. The arbitrary nature of the integration following the use of these methods often results in unpredictable expression (Fig. 1A). Although this can still allow adequate expression of the gene of interest, the integration site is random, which can lead to unpredictable effects on transgene expression. Furthermore, there is a possibility that the transgene could integrate into an endogenous gene that could cause an unwanted mutation, complicating any

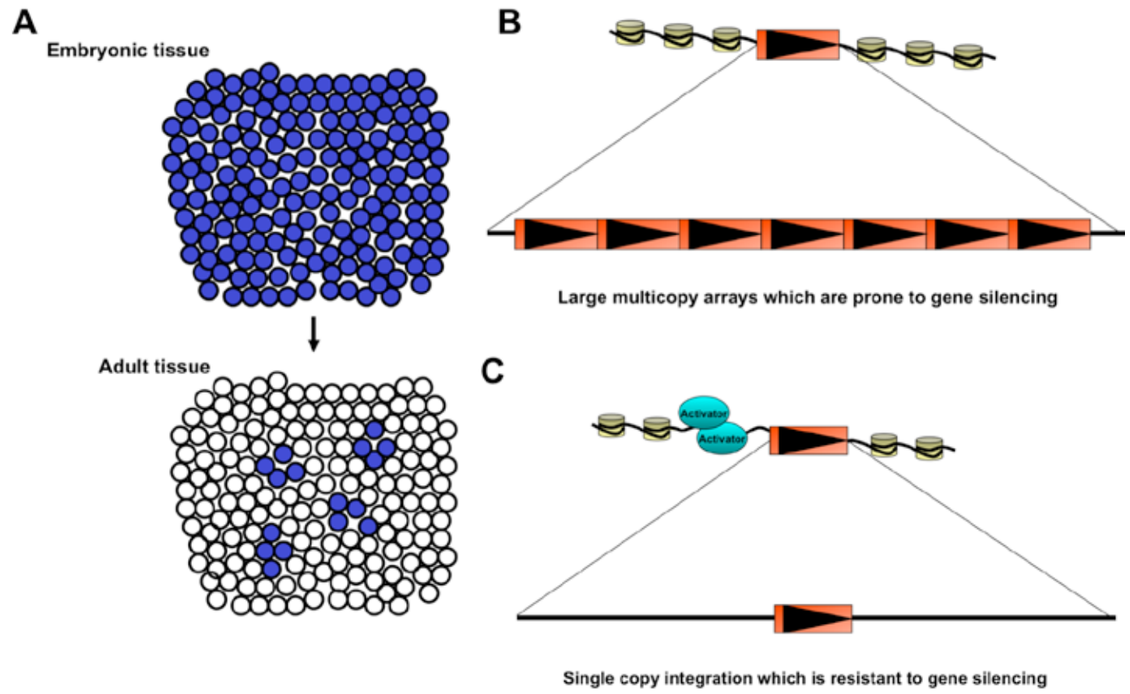

Figure 1
Improving transgene expression by gene targeting into a permissive locus. (A) An illustration of the consequences of transgene silencing. Frequently, transgene expression (illustrated in blue) can be silenced in adult cells leading to patches of expression where only a small proportion of cells express detectable levels of the transgene. (B) Typically, transgenes introduced by microinjection or transfection integrate randomly and most frequently in large multi-copy arrays that are targets for the epigenetic mechanisms that control gene silencing. (C) Using gene targeting to insert a transgene circumvents these problems allowing the integration site to be determined and insertion of a single transgene copy.

phenotyping. In addition, transgenes introduced in this manner often integrate in large multi-copy arrays (Fig. 1B) that are particularly susceptible to silencing. Consequently, this not only adds variability to the experiments but also means screening multiple separate integrations to identify one that expresses in a manner suitable for analysis.

To circumvent these problems, we routinely target transgenes to specific locations in the genome. This has a number of advantages when compared to the traditional approach. Firstly, it allows the integration site of the transgene to be chosen. This means that you can integrate the transgene into an area of the genome that is permissive for gene expression and where the chromatin microenvironment will not interfere with the transgene function. Secondly, as the transgene has a chosen integration site you can be confident that it will not interfere or mutate any endogenous genes. In addition, by targeting you can be certain that only a single copy of the transgene will be inserted into the appropriate locus (Fig. 1C). This helps reduce the chance that the transgene will be epigenetically silenced. The introduction of a single copy at the desired locus also ensures that the transgene can work effectively with other technologies such as Cre recombinase, to allow, for example, inducible expression of a transgene in a particular tissue or at a particular developmental stage following administration of an appropriate inducer.

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EPIGENETICS OF CANCER AND AGEING

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We investigate the impact of chromatin structure and epigenetics on cell proliferation, ageing and cancer. We hypothesise that age-associated changes in chromatin structure, function and regulation contribute to the dramatic age-associated increase in the incidence of cancer. While age is the biggest single risk factor for most cancers, the reason for this is current poorly understood. We are also exploiting epigenetics to develop novel drug combination therapies to combat cancer.

Research in the lab primarily focuses on the interface between ageing, epigenetics and cancer. We are particularly interested in age-associated epigenetic changes that lead to increased incidence of cancer 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 are also exploiting epigenetics to develop novel drug combination therapies to combat cancer.

Novel candidate therapies for acute myeloid leukaemia (AML)

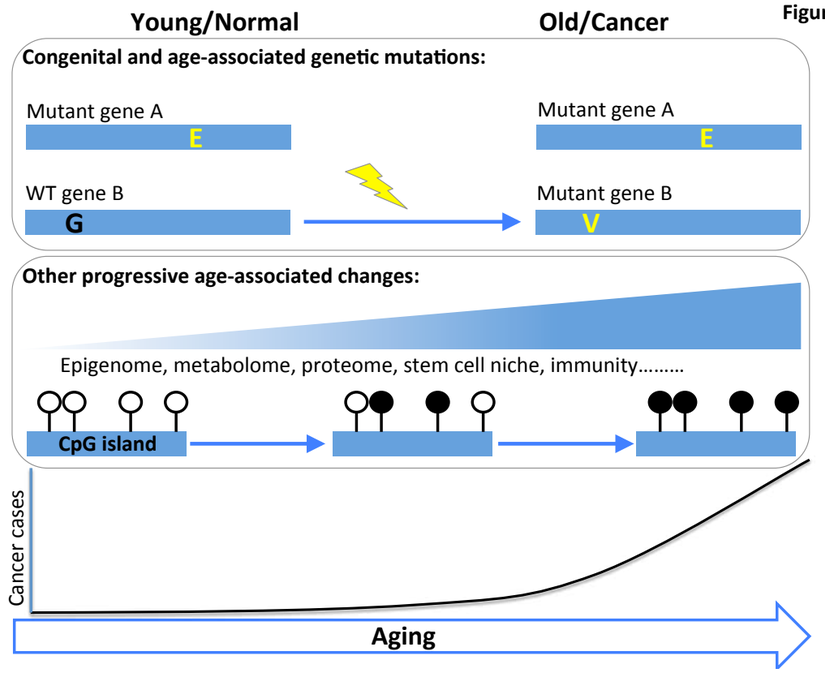
In 2015, there were approximately 2,400 new cases of AML in the UK. After diagnosis, five-year survival is currently ~15.5%. Therefore, there remains a critical requirement for novel therapies for AML. Bromodomain and extra-terminal domain (BET) inhibitors are emerging as exciting therapeutic agents for haematopoietic malignancies, including AML. Pharmacological inhibition of BET bromodomains targets malignant cells by preventing reading of acetylated lysine residues, thus disrupting chromatin-mediated signal transduction, which reduces transcription at oncogenic loci, such as c-Myc, Bcl-2 and Cdk4/6. BET inhibitors alone have shown promising pre-clinical activity against diverse AML subtypes, and are now in clinical trial in AML and other haematological malignancies. Most likely the benefits of BET inhibitors as anti-cancer therapeutics will be best realised in drug combination approaches that offer both mechanistic synergy and a decreased dose of the BET inhibitor. We are therefore developing a novel drug combination-based therapy to treat AML.

Why does the incidence of cancer 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.

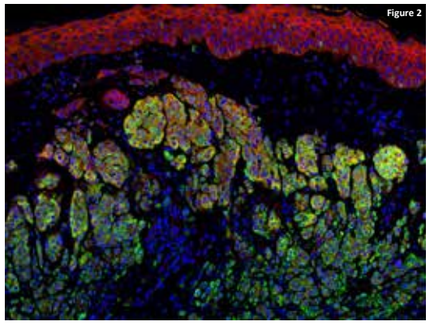
Figure 1
A model depicting the many factors proposed to contribute to age-associated increase in cancer incidence. These include congenital genetic mutations and mutations acquired in early growth and development; genetic mutations acquired during ageing; progressive age-associated changes to dynamic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



Epigenetic control of ageing and cancer

In collaboration with Ronen Marmorstein (Philadelphia) we have dissected the structure-function relationships between HIRA and its binding partners, UBN1, CABIN1 and ASF1a, and substrate histone H3.3. This included a crystal structure of the HIRA/ASF1a interaction surface and more recently the UBN1/histone H3.3 interaction surface. We were the first to describe the distribution of the HIRA complex across the mammalian epigenome. 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. 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. Of particular note, we have revealed a function for HIRA in promoting healthy ageing and suppression of cancer.

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



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. 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, 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. We have shown that the histone chaperone HIRA is one such factor that contributes to epigenetic stability in non-proliferating cells. Inactivation of HIRA predisposes mice to oncogene-induced neoplasia.

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MOLECULAR CONTROL OF EPITHELIAL POLARITY

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One of the universal features of solid tumour types is that a loss of tissue organisation is the strongest predictor of poor outcome. Our lab studies the molecular mechanisms that allow tumour cells to become disorganised and collectively invade. These are aimed at identifying potential targets for therapeutic intervention in patients.

Our group studies the gain and loss of collective cell polarity and invasion in prostate tumours. Our research is focused on two intersecting streams: 1) a highly interconnected network of ARF GTPases, and their regulatory and interacting molecules (which we term the 'ARFome') and 2) the function of the pro-metastatic transmembrane protein, Podocalyxin. Numerous ARFome proteins, and Podocalyxin, are highly overexpressed in metastatic prostate tumours. Our aim is to understand how these molecules regulate cancer cell invasion, to identify possible targets for future therapeutic interventions.

Developing tools for collective 3-dimensional (3D) invasion analysis

Traditionally, how cells move has been studied using single cells grown on glass or plastic. However, tumours are collections of many, not singular, cells. Dissecting how collective cell invasion is regulated requires developing methods to allow for 3D 'mini-tumours' (spheroids) to be grown, imaged and analysed *ex vivo*. Analysis methods for studying collective invasion have lagged far behind that for single cell analyses, primarily because of a lack of quantitative tools to do so. Our group aims to develop methods to overcome such limitations, including the dissemination of practical methods and the implementation of open-source, freeware software algorithms for distribution to the research community.

In collaboration with Functional Screening, our group is developing an integrated platform for high-throughput, high-content, live imaging-based analysis of spheroid invasion. We have developed lentiviral shRNA arrays to manipulate gene expression in spheroids in massive parallel. We have coupled this to semi-automated, multi-day imaging (both live and fixed) of spheroid invasion. Our analyses have been aided by the introduction of machine learning algorithms to classify different invasion phenotypes in a robust, quantitative method. This presents an exciting new possibility to examine, in a rapid fashion, the factors that regulate tissue formation and its disruption in cancer.

ARF GTPase circuits controlling cell invasion

The ARFome is a network of five GTPases, multiple regulatory proteins (GEFs, GAPs) and effectors that are involved in lipid signalling, cytoskeletal organisation and membrane trafficking. They form a highly overlapping network and are thought to share many of the same binding partners. This makes untangling specific functions for each GTPase difficult. We have performed a functional genomic screen to systematically interrogate each member of the ARFome family's influence on prostate cancer cell invasion.

In collaboration with the Ismail and Shanks groups, we are interrogating their functions in prostate cancer cell invasion from spheroids. We find that many ARFome family members assumed to be redundant have highly divergent and sometimes opposing roles in invasion, and show that there may indeed be specificity of signalling between family members. In addition, we are focusing on how alternate splicing of ARFome proteins can contribute to divergent functions of such alternately spliced isoforms. This is an important preamble to identify which, and how selectively, ARF GTPases may be targets for future therapeutic inhibition studies.

Podocalyxin function in collective cancer cell invasion

Podocalyxin is mutated in some families with congenital prostate cancer. Additionally, amplification of Podocalyxin expression is a predictor of poor outcome in several cancer types. We are characterising the molecular mechanisms by which Podocalyxin promotes collective cell invasion.

In collaboration with the Zanivan group, we are using in-depth quantitative mass spectrometry to identify the interacting partners of Podocalyxin ('Podxl interactome') that control its pro-invasive function. Additionally, we are mapping the proteomic changes required during cancer progression to promote Podocalyxin function. Furthermore, we have collaborated with Functional Screening to develop a functional genomic approach to systematically evaluate each member of the Podxl interactome for its role in invasion from spheroids. Our current aim is for a rigorous dissection of the exact cooperating protein modules that promote Podxl-driven invasion. Our future aim is to understand which of these *in vitro* modulators of invasion are consistently altered in prostate cancer patients, such that they may be potential therapeutic targets in the clinic in the future.

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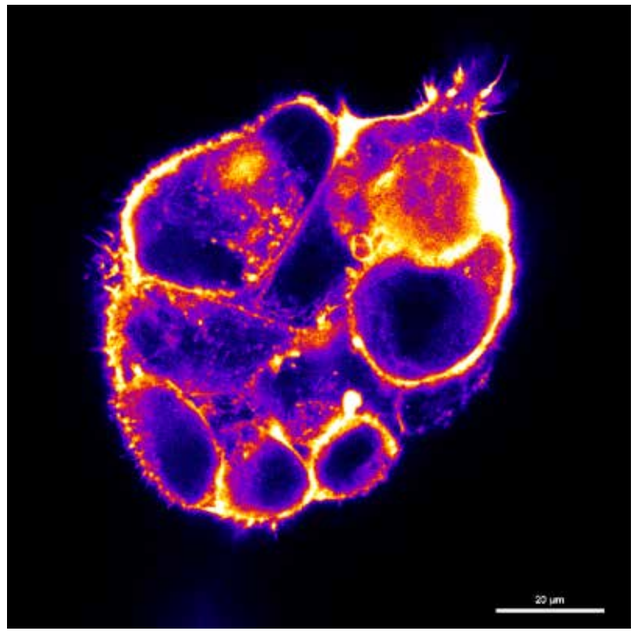

A prostate mini-tumour labelled for filamentous actin.

IMMUNE CELLS AND METASTASIS

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Immune cell participation in the metastatic cascade is dualistic, as immune cells can both prevent and promote secondary tumour formation. The goal of our lab is to understand the mechanisms underlying this dualism, in order to develop new strategies to dampen the functions of pro-metastatic immune cells and boost the anti-metastatic activities of other immune cells.

Our group was established in June, so we have been setting up the lab and recruiting people. Our research is focused on γδ T cells, which are a rare population of T cell receptor-expressing cells that function like innate immune cells. γδ T cells participate in the maintenance of tissue integrity by surveying for pathogens, toxins and epithelial cell stress at body surfaces. They influence the outcome of immune responses via the secretion of cytokines. Like other immune cells, γδ T cells can be divided into functionally distinct subsets based on the cytokines they secrete: IL-17 or IFNγ. In the cancer arena, a new appreciation is growing for γδ T cells and their unique contributions to cancer progression and metastasis. IL-17-producing γδ T cells are largely pro-tumourigenic, whereas IFNγ-producing ones are critical players in immunosurveillance of transformed cells and oppose the growth of established tumours.

Recent advances in γδ T cell biology have shown that IL-17-producing γδ T cells are present in human breast, pancreatic, colon and bladder cancers where they correlate with poor prognosis. These cells drive tumour progression through angiogenesis and immunosuppression. My postdoctoral work at the Netherlands Cancer Institute uncovered a mechanism by which γδ T cells dampen anti-tumour immunity and promote breast cancer metastasis (Fig. 1). In this process, mammary tumours secreted IL-1β, instructing γδ T cells to produce IL-17. The upregulation of IL-17 led to G-CSF-dependent neutrophil expansion and neutrophil polarisation, so that neutrophils acquired the ability to inhibit anti-metastatic CD8+ T cells.

These data provided a mechanistic explanation for earlier clinical observations in breast cancer patients, where a higher neutrophil-to-lymphocyte ratio was associated with worsened metastasis-specific survival, and accumulation of γδ T cells in primary tumours correlated with advanced tumour staging, increased metastasis and decreased overall survival.

One outstanding question about γδ T cells in the metastatic setting is how these cells are regulated. We previously showed that IL-1β released by macrophages in mammary tumours induces IL-17 expression in γδ T cells, but very little information exists about other molecules involved in stimulating or suppressing IL-17. As such, we will tackle this question over the next year with particular focus on T cell-specific receptor molecules. Acquisition of this knowledge may lead to new immunotherapeutic approaches that control IL-17-producing γδ T cells, prevent neutrophils from acquiring an immunosuppressive phenotype and unleash the full potential anti-metastatic CD8+ T cells. Additionally, we will determine whether IL-17-producing γδ T cells play a role in promoting metastasis of other tumour types, such as pancreatic and colorectal cancers. Since the role of IFNγ-producing γδ T cells in metastasis biology is entirely unknown, we will also explore their regulation and function during the metastatic process.

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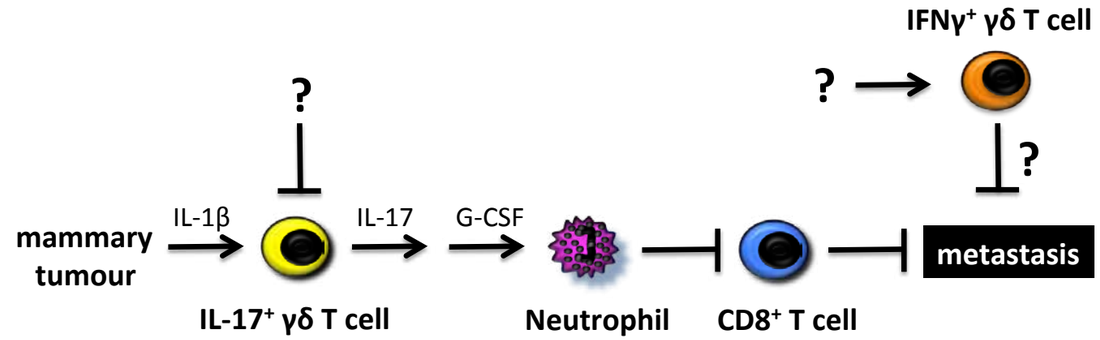

Figure 1
The gd T cell – IL-17 – neutrophil axis in breast cancer metastasis. IL-1b released into the circulation by mammary tumours activates gd T cells to produce IL-17. An unknown cell type responds to IL-17 by upregulating G-CSF, causing neutrophil expansion and polarization towards an immunosuppressive phenotype. Through an iNOS-dependent mechanism, neutrophils dampen $CD8^+$ T cell killer functions so that disseminated cancer cells are protected from attack. Our current focus is on determining how IL-17-producing gd T cells can be blocked and understanding the role of IFNgamma-producing gd T cells in metastasis.

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CANCER METABOLOMICS

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Lipids are a diverse class of biomolecules that are involved in tumour onset and progression. From a metabolic perspective, they are amongst the most abundant cellular 'building blocks' that cells need for growth. They are also important signal transducers. In fact, the most frequently deregulated pathway in cancer, the PI3K-AKT pathway, features a PIP_3 lipid as its central messenger. Despite their clear relevance, technical limitations have until now prohibited the elucidation of fundamental aspects of lipid metabolism and signalling. To address this, we harness novel lipidomics and stable isotope tracing strategies to better understand the role of lipids in cancer, with an emphasis on studying the effect of the tumour microenvironment on cancer cells.

Acetate consumption and metabolism by tumour cells

Fatty acids are the bulk components of cellular membranes and are therefore in high demand by growing cancer cells. The precursor for fatty acids is acetyl-CoA, of which the acetyl group is mainly derived from glucose-derived carbon. However, in hypoxic (i.e. low oxygen) conditions - a common occurrence in solid tumours - most glucose carbon is shunted towards lactate, causing a reduction in the available carbon for acetyl-CoA, and hence fatty acid production. Exactly how cells cope with this has remained unclear. Previously, we reported a drastic increase in the apparent production of nuclear-cytosolic acetyl-CoA from acetate in hypoxic conditions. 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. Acetate was therefore considered to be an alternative substrate for acetyl-CoA production during oxygen limitation.

While acetate is considered a 'backup' nutrient in hypoxic cancer cells, how much acetate cells actually consume and how it is dispersed among downstream pathways (fatty acid synthesis, mitochondrial oxidation, protein acetylation) remained unstudied, although it might reveal cancer cell vulnerabilities. To address this, we developed a method to measure both free and bound acetate directly, based on derivatisation by alkylation and gas chromatography – mass spectrometry (Tumanov *et al.*, 2016). This method is fast and sufficiently sensitive to permit analysis of physiologically relevant acetate levels. We next investigated heavy (^{13}C) acetate uptake and its incorporation into fatty acids in a panel of cancer cell lines, both in atmospheric and low oxygen conditions. We found that there is a direct relationship between acetate uptake and usage with ACSS2 expression levels. In other words, the ACSS2 expression level directly determines how much exogenous acetate is used for fatty acid synthesis. Further quantitative analysis revealed that the majority of exogenous acetate is used for the high acetyl-CoA demanding flux of fatty acid biosynthesis, and to a lesser extend for mitochondrial oxidation in cells that express the mitochondrial ACSS isoform (ACSS1). Measurements of labelled acetate bound to histones revealed that exogenous acetate is only very sparingly used for histone acetylation.

Although exogenous acetate activated by ACSS2 does not appear to be an efficient substrate for histone acetylation, ACSS2 has previously been reported to be both expressed in the cytosol and nuclei of cells. To investigate ACSS2 localisation in cells exposed to various conditions, we performed immunofluorescence experiments. Interestingly, not only did total ACSS2 expression go up when cancer cells were exposed to hypoxic and low serum conditions, so did the nuclear localisation of ACSS2. Thus, although exogenous acetate does not substantially contribute to histone acetylation,

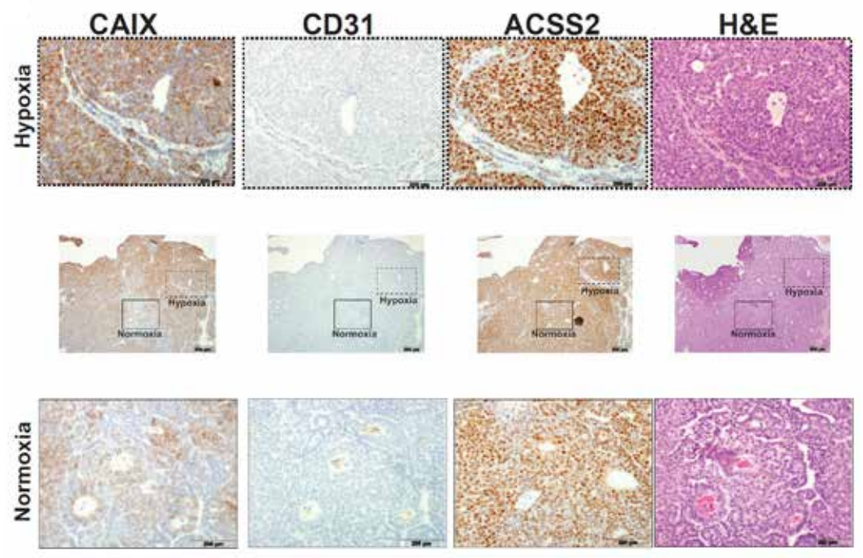

Figure 1
ACSS2 is prominently nuclear in hypoxic tumour regions. Immunohistochemical staining of serial sections from a representative tumour of the MMTV-PyMT mouse model for carbonic anhydrase 9 (CAIX, a hypoxic marker), CD31 (a marker for blood vessels), ACSS2 and H&E staining. ACSS2 is especially prominent in the nuclei of hypoxic and nutrient-deprived tumour cells, where it prevents loss of carbon and maintains histone acetylation to promote survival.

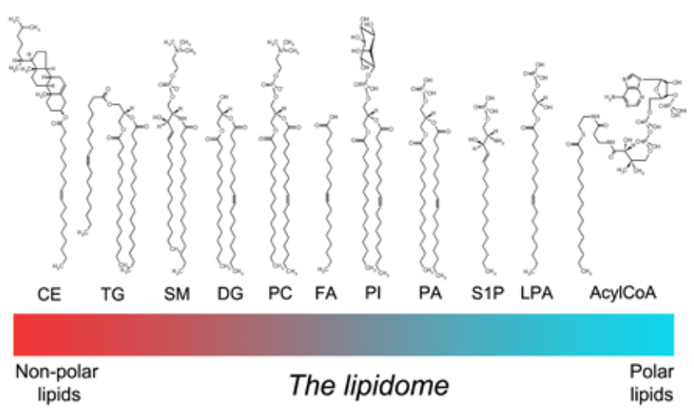

Figure 2
Lipids are structurally diverse. Examples of very apolar lipids (cholesterol esters, triglycerides) to relatively polar lipids (lysophosphatidic acid lipids, acyl-CoAs). We are developing a platform for their comprehensive analysis based on two extraction procedures and separation methods. Abbreviations: CE, cholesterol ester; TG, triglyceride; SM, sphingomyelin; DG, diglyceride; PC, phosphatidylcholine; FA, fatty acid; PI, phosphatidylinositol; S1P, sphingosine-1-phosphate; LPA, lysophosphatidic acid.

the acetate activating enzyme ACSS2 becomes more nuclear. We solved this apparent conundrum by showing that ACSS2 not only captures exogenous acetate, but that it also plays an important role in recapturing acetate that is released by cells. In the nucleus, histone deacetylases continuously remove histone acetyl marks and ACSS2 reactivates the resultant acetate to acetyl-CoA so that it can be reused for histone acetylation, and maintenance of histone acetylation promotes cell survival. With help from Karen Blyth we were able to show that nuclear ACSS2 expression is also especially prominent in the poorly perfused, hypoxic regions of mouse breast cancer tumours (Fig. 1). In short, nuclear ACSS2 retains endogenously produced acetate to maintain histone acetylation (Bulusu *et al.*, Cell Reports, 2017). We suggest that this is especially important in hypoxic, nutrient-deprived tumour regions, to retain as much carbon as possible and to promote cancer cell survival and growth. As a next step we plan to study acetate metabolism in pancreatic cancer.

Developing a comprehensive lipidomics strategy

It has been estimated that mammalian cells contain up to 10,000 individual lipids. Arguably more important is that, even in the most comprehensive recent lipidomics studies, only half of all measured lipids could be assigned a molecular structure and function. It is therefore apparent that much lipid biology remains to be discovered, and given the important roles known lipids play in cancer, we hypothesise that many lipids that currently remain uncharacterised contribute to tumour progression. Unfortunately, the ability to explore these unknown lipids is limited when using standard mass spectrometry-based lipid profiling approaches, as they are unable to cope with the structural diversity of lipids (Fig. 2). Recognising this issue, we are working towards a practical approach using two separate extraction procedures and two modes of separation, enabling the extraction and analysis of lipids over the entire polarity range (Tumanov & Kamphorst, 2016). Preliminary experiments using this strategy have revealed the presence of bioactive lipids in the tumour microenvironment of pancreatic tumours. A major focus of the lab is to characterise their involvement in tumour progression and to study the effect of modulating their synthesis and degradation.

Publications listed on page 105

ONCOGENE-INDUCED VULNERABILITIES

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[1]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 β-catenin. In a number of *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumourigenesis and, moreover, is typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC overexpression would likely have a tremendous impact on human health.

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. Importantly, 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.

Additionally, we have now found that NUAK1 plays a key role in protecting cells from toxic levels of reactive oxygen species (ROS). ROS are naturally produced as by-products of mitochondrial electron transport chain activity and the elevated metabolic demand of cancer cells can thus increase ROS production. Paradoxically, hypoxia can also elevate ROS production and is moreover a common feature of most cancers. Tumours cells cope with the threat posed by ROS in part by diverting glucose away from the mitochondria but also by increasing pathways that detoxify ROS. We have found that suppression of NUAK1 impairs this latter response, thereby exposing an intrinsic vulnerability in cancer cells. We have determined that acute inhibition of the antioxidant response pathway, via targeted suppression of NUAK1, eradicates MYC-driven adenomas in a GEMM of colorectal cancer. As well as providing strong evidence to support targeting NUAK1 in human colorectal cancer, this observation challenges dietary advice commonly given to patients who already have cancer, in that popular consumption of antioxidant supplements may actually benefit the cancer cells more than the patient.

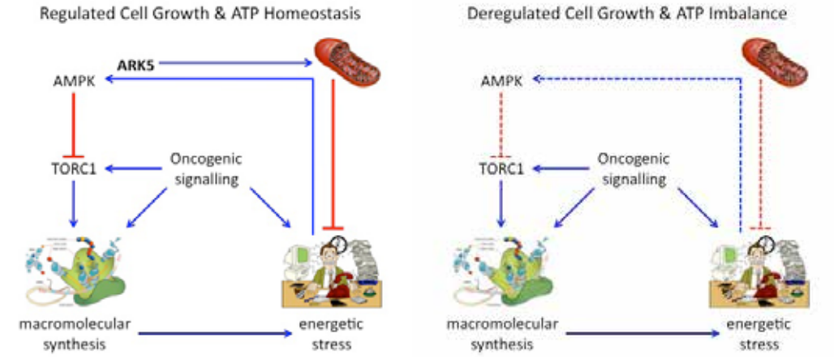

Figure 1
Induced dependencies need not reflect direct molecular interactions. Oncogene-induced cell growth, typically requiring signal transduction via the mechanistic target of rapamycin (mTOR) pathway, drives rampant ATP consumption, which must be compensated for through increased cellular intake of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

Exploiting MYC sensitisation to apoptosis for cancer therapy

Apoptosis plays a key role in shaping the body and eliminating damaged or potentially harmful cells (e.g. autoreactive T lymphocytes) during development. Accordingly, the apoptotic machinery is widely expressed during development and this expression persists throughout childhood but begins to taper off during adolescence as bodies reach their mature size. One consequence of this differential expression is that children are far more sensitive to radiation than adults. In collaboration with the Letai lab (Dana Farber Cancer Institute), we have found that MYC expression is a major determinant of radiosensitivity, *via* transcriptional regulation of both pro and anti-apoptotic BCL2 family (BH3) proteins. We showed that acute activation of MYC expression in adult tissues restores radiosensitivity to levels approaching that normally observed in immature tissues, by increasing expression of multiple BH3 proteins, in particular BAX and BIM. MYC is widely overexpressed in human cancer and its regulation of the apoptotic machinery thus goes a long way to explain the differential sensitivity of cancer cells over normal ones in adults. However, because MYC is also widely expressed in healthy cells during early life, it is also responsible for much of the undesirable toxic side effects of standard radio- and chemotherapy in young patients. Greater efforts are thus needed to protect normal tissues in young cancer patients receiving such treatment.

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 the incidence of lung cancer is on the rise especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have found that modestly elevating MYC levels in a KRAS-driven model of lung cancer is sufficient to drive progression to metastatic disease. This progression arises in part through increased transcription of promiscuous ERBB family ligands. We have identified an unexpected requirement for signal transduction through the ERBB receptor tyrosine kinase network for both establishment and maintenance of KRAS mutant lung cancer. Our data suggest that KRAS-driven tumours actively seek ways to amplify signalling through the RAS pathway in order to sustain the tumour phenotype. As there are presently no clinically proven small molecule inhibitors of KRAS, our observation raises the exciting possibility that simultaneously inhibiting signalling components upstream and downstream of KRAS with existing therapeutic agents may benefit the very large number of lung cancer patients whose disease is driven by mutant KRAS.

Major developments in 2016

We made excellent progress on two core projects over the past year, with both reaching a stage of maturity approaching suitability for publication: 1) the functional requirement for ERBB signalling in KRAS-driven lung cancer and 2) the role of NUAK1 in colorectal tumour maintenance. At the time of writing both manuscripts have been submitted for publication. Additional manuscripts detailing 1) the development of GEMMs for metastatic lung and pancreatic cancer and 2) non-canonical regulation of NUAK1 in cancer, are planned for early next year. As noted above, our fruitful collaboration with the Letai lab led to a major publication in *Cancer Cell* and our GEMM expertise was key to a second collaborative study published in *Oncogene* with the Gaubatz lab (University of Wuerzburg). In collaboration with Douglas Strathdee, we confirmed the generation of two new GEMMs expressing CreER in mesothelial cells, which will be used to develop new authochthonous models of malignant pleural mesothelioma. PhD student Sarah Neidler successfully defended her thesis and returned to work in Germany. Martina Brucoli and Amy Bryson (University of Glasgow BSc student) both had successful internships, contributing to our NUAK1 manuscript. We welcomed Katarina Gyuraszova as postdoc maternity cover for Nathiya and bade farewell to Jaqueline Tait-Mulder and Allan McVie. Finally, we received a very generous donation from the Miss M.J.M. Smith Trust to support the purchase of a new, state-of-the-art instrument for cell analysis.

Publications listed on page 106

MITOCHONDRIA AND CANCER CELL DEATH

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[1]CRUK programme grant
[2]Breast Cancer Now project grant
[3]EMBO Fellowship
[4]BBSRC project grant
[5]CRUK Glasgow Centre

Cell death inhibits cancer 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 anti-cancer therapies work. Our research focuses upon mitochondrial regulation of cell death and inflammation. 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. Our findings will be used 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 intermembrane 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 often inhibit apoptosis by preventing MOMP, often through upregulation of anti-apoptotic Bcl-2 proteins. Importantly, this can be exploited therapeutically and newly developed anti-cancer therapies target these apoptotic blocks.

Mito-priming - a method to enforce Bcl-2 addiction

Many methods to trigger mitochondrial apoptosis suffer from engaging this process in a slow, asynchronous manner that is often accompanied with unwanted, off-target effects. Driven by a desire to circumvent these issues, we developed a method called 'mito-priming'. In this method, pro- and anti-apoptotic Bcl-2 proteins are expressed at equal levels. Cells in this primed state are massively sensitive to drugs that target Bcl-2 proteins called BH3 mimetics, rapidly undergoing cell death within a matter of minutes following BH3 mimetic addition (Fig.s 1 and 2).

Applying mito-priming to define the potency and selectivity of Bcl-2 targeting drugs

Directly targeting Bcl-2 proteins to trigger or sensitise cancer cell death represents an exciting and effective new way to treat cancer. For example, the recently clinically approved BH3 mimetic compound, venetoclax exploits the Bcl-2 addicted state of chronic lymphocyte leukaemia (17p deleted CLL), triggering remarkable therapeutic responses. The Bcl-2 protein family is comprised of various different members (e.g. Bcl-2, BCL-xL and MCL-1) that can be targeted to varying degrees by different BH3 mimetics. Importantly, failure to neutralise specific Bcl-2 proteins represents a means of cancer cell resistance to BH3 mimetic therapy. We have surveyed a range of BH3 mimetic compounds using our mito-priming method to define the selectivity and potency of different Bcl-2 family members. Moreover, in combination with CRISPR-Cas9 genome editing to delete BAX and BAK (proteins essential for MOMP), we have applied mito-priming to address whether Bcl-2 targeting compounds kill in an on-target (MOMP) dependent manner. In sum, mito-priming provides a highly useful way to screen for specific and potent, new Bcl-2 targeting drugs.

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Figure 1
Mito-priming as a method to enforce Bcl-2 addiction. Cells express equimolar amounts of a pro-apoptotic BH3-only protein (in this case GFP tBID) and anti-apoptotic Bcl-2 protein (in this case BCL-xL) making use of a 2A-linker sequence. Co-expression of these two proteins renders cells highly sensitive to BH3 mimetic addition (ABT), which induces MOMP and mitochondrial apoptosis.

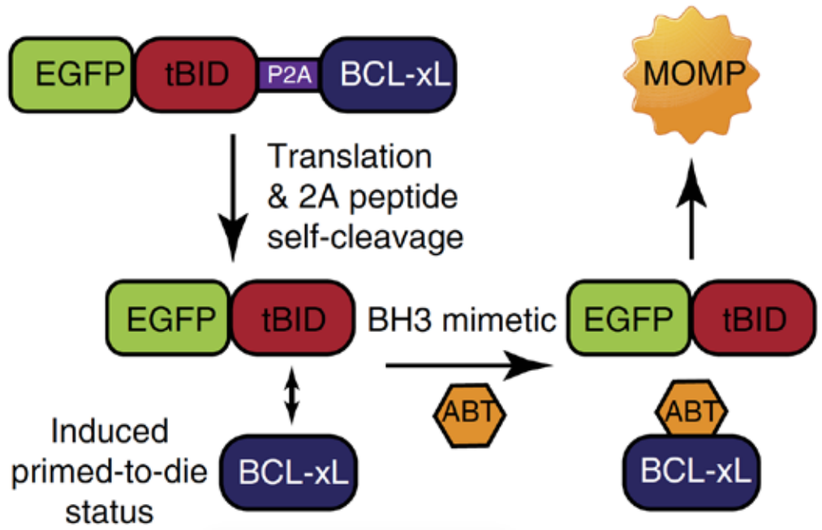
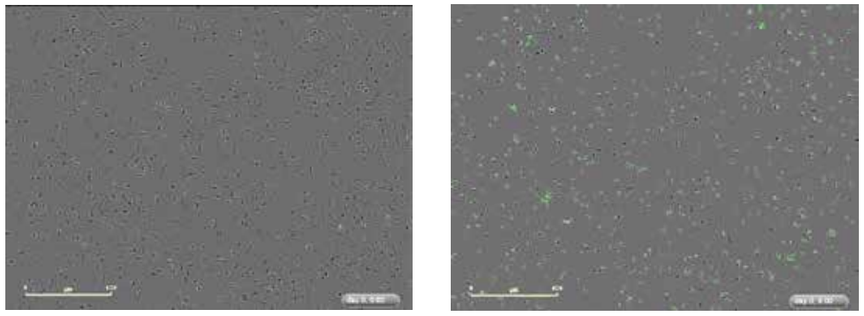
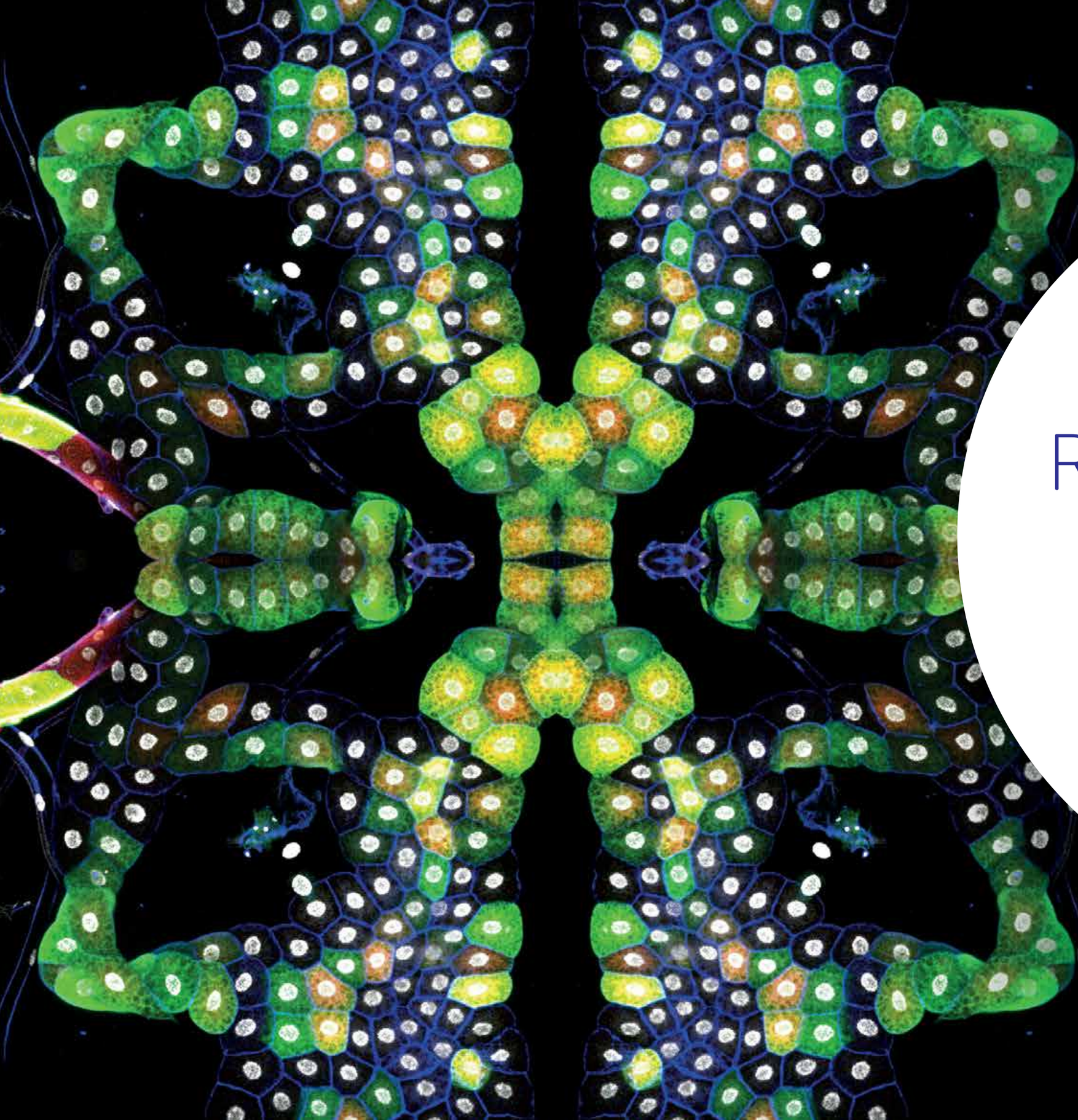


Figure 2
Mito-priming enables rapid and synchronous mitochondrial apoptosis. SVEC murine endothelial cells co-expressing GFP-tBID and BCL-xL were treated with ABT-737. Cell viability was measured by live-cell imaging and exclusion of the green dye, SYTOX Green. Left: Viable cells at the point of BH3 mimetic (ABT-737) addition Right: Cells at 8 hours following BH3 mimetic addition, at which point most have died (SYTOX Green positive).





CANCER RESEARCH UK BEATSON INSTITUTE

RESEARCH FACILITIES PUBLICATIONS AND OPERATIONS

RESEARCH FACILITIES

Head of Research Facilities
Sue Fowler

Research Facilities support Beatson Institute research groups and University of Glasgow groups based on the Beatson site. This year, there was investment in major new equipment for the Flow Cytometry facility with the acquisition of a BD Fortessa 5 laser analyser, while the Histology facility purchased a Leica Bond Rx autostainer for fully automated *in situ* hybridisations and Information Services significantly extended the capacity of the new main storage server. In addition, Laboratory Management replaced several main pieces of core equipment and Building Facilities were active with a number of projects to refit laboratory areas.

Building Facilities

Alistair Wilson, Alex Kernahan, Michael Daly (until July), John Trivett (from October)

Building Facilities manage the outsourced provision of catering and janitorial services. We provide maintenance support for the Institute buildings, manage alterations and refurbishments, and ensure that all statutory compliance issues with respect to buildings and systems are up-to-date. An online helpdesk is used as an effective means to log and react to calls for maintenance and repair.

This year, new close control cooling was installed in the mass spectrometry laboratory and the server room was reconfigured to extend space, and add new cooling and a larger uninterruptable power supply. There were also a number of laboratory refits to facilitate the move and installation of new equipment.

Central Services

Margaret Laing (Supervisor), Elizabeth Cheetham, Dilhani Kahawela, Kirstie McPherson, Jonny Sawers, Lauren Ure, Linda Scott, Sarah Neill, Tracy Shields, Jack Sleight (until September), Rose Steel (until November), Robert Storey

Central Services perform a wide range of duties, including 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 cleans and checks equipment such as centrifuge rotors, X-ray processors, water baths and pH meters. On a daily basis, it also stocks the tissue culture suites, and collects and autoclaves laboratory waste to make it safe.

Flow Cytometry

Tom Gilbey, Tim Harvey

Flow Cytometry combines the flow facilities of the Beatson Institute and the neighbouring Wolfson Wohl building to create a comprehensive service. We provide advice, assistance and education to researchers on all aspects of flow cytometry.
Pre-acquisition, users discuss their needs with facility staff to get advice on which instrument to use, the best combination of fluorochromes and the correct controls and gating strategy. Before working on their own, researchers are trained to use the analysers and templates are created to enable data acquisition. Due to their complexity, only facility staff members operate the cell sorters. Post-acquisition, facility staff can help researchers analyse their data using various software options.

The service has a number of cell analysers and sorters: BD FACSCalibur, for simple flow experiments such as cell cycle and proliferation studies; BD FACSVerse, for most routine flow experiments, including analysis on 6-96 well plates; Attune NxT (Applied BioSystems), for complex flow analysis, including analysis of rare

events and red fluorescent proteins; BD Fortessa, recently acquired for complex flow analysis; BD FACSAria, for sorting red fluorescent proteins and identifying side populations in a sample; and BD FACSAria Fusion, enclosed in a class II safety cabinet for safely sorting samples 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 processes a wide range of tissue samples and cellular material fixed in an array of different fixatives. Tissue samples are trimmed, processed and orientated in paraffin wax blocks for sectioning and staining. Three large capacity automated tissue processors allow large-scale, consistent processing, although specialised processing cycles can also be designed. Other material such as organotypic slice cultures, cell pellets, spheroids and agar plugs can also be processed to provide a wax block for sectioning and further investigation. All sections are stained with haematoxylin and eosin for a general analysis of cell morphology and structure before more specialised histology stains are used to investigate specific tissue structures.

When fixation is not required, we also offer a frozen section resource, allowing frozen tissue, embryos or cells to be sectioned and stained using routine histological, immunohistochemical or immunofluorescence methods. Both paraffin-embedded and frozen tissue can be sectioned for DNA/RNA investigation, PCR analysis and immunofluorescence staining.

We also offer a comprehensive immunohistochemistry service that includes access to a large repertoire of previously validated antibodies, three autostainers that allow consistent, high quality staining, and training for researchers. New antibodies can be optimised to produce a working protocol that allows the antibody to be used on an autostainer or by a researcher. In addition, we provide *in situ* hybridisation using a reagent system designed to visualise cellular RNA targets in formalin fixed paraffin embedded tissue sections and bright-field microscopy. Staining for this is performed on a Leica Bond Rx autostainer and specific probes can be purchased or designed to exact specifications by external companies such as Advanced Cell Diagnostics.

The facility has a Leica LMD6500 laser microdissection system that allows subpopulations of tissue cells to be obtained from histological slides under microscopic visualisation. Both DNA and RNA material can thus be retrieved from the tissue sections for downstream analysis. It also provides a fully automate, large capacity Leica SCN400F slide scanner capable of capturing bright-field or fluorescent images. This enables high quality digital images to be scanned, stored and, if required, quantification performed. The image analysis software allows staining techniques to

be scored using specifically designed algorithms. Finally, if required, mouse tissue microarrays (TMA) can be constructed using paraffin embedded tissue blocks.

Information Services

Peter McHardy, Iain White

Information Services provides server support, hardware cover, an on-site helpdesk for both repair and software support as well as help with 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 store and network printing. The servers have in excess of 750 TB of online storage with frequent backups, to provide support for microscopy, DNA sequencing and mass spectrometry data. Our central data store is a multi-headed Isilon storage system, offering fast 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, with 10 planned. All e-mail services run on Microsoft Exchange, which allows local client-based access and web access to email as well as delivering email, diaries and address books to mobile devices including iPhones, iPads and other smart phones.

Migration from physical servers to virtual servers 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 currently provide virtual desktops for OS X users requiring access to Windows-based packages. We have rolled out vApps for specific imaging, 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 is 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 analysis, in some cases reducing data analysis runs from days to minutes. A network upgrade was completed, allowing desktop computers to access central data file stores at 10Gb. Backbone network speeds are being moved up to 40Gb. This will allow us to provide users higher speed access to research data.

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 Institute and other CRUK 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 theatre.

Laboratory Management

Laura Bence, Richard Selkirk, Michael McTaggart, George Monteith, Michael Kilday, Karen Thomas (from April)

Laboratory Management provides 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 with regard to fire risk is also managed. Health and safety processes are reviewed and monitored regularly as are training needs in order 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.

We also oversee equipment servicing, replacement and purchase. This year, we welcomed new start, Karen Thomas to the team. She will be focusing on the maintenance and curatorship of core equipment, incubators and freezers, and the equipment inventory. Servicing and maintenance of core equipment is essential and 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 annually 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. We also have a good relationship with suppliers to ensure we achieve best prices and discounts for goods. All outgoing orders are monitored to ensure compliance with Institute safety procedures, particularly those relating to COSHH. In addition, assistance is given to users to enable smooth processing of their orders and to ensure best prices are used and orders comply with any requirements for import or regulatory requirements.

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. A porter service is run to deliver external orders to the researchers, while stores processes outgoing samples or materials for courier collection. We also negotiate free samples from suppliers to enable the scientific staff to assess new or alternative products. By maintaining a good relationship with suppliers, preferential pricing is obtained, especially for bulk orders. 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 sequencing of plasmids and PCR products on an Applied Biosystems 3130xl (16 capillary) Sequencer that has good sample throughput, long read lengths and a sample turnaround time of 24 hours. Post-PCR products can now be purified for sequencing by the addition of Cleansweep (Applied Biosystems). In recent years, DNA sequencing has been revolutionised by the introduction of next generation technologies offering large-scale sequencing in a matter of hours. An Illumina NextSeq500 platform has enabled us to sequence libraries at a lower cost with increased data output and a faster turnaround time. Protocols currently used are ChIP-seq and RNA-seq. We also offer library production for NGS. QC of libraries is done using a Qubit fluorometic quantification assay and an Agilent Tapestation 2200.

Small-scale DNA purification is performed on a Qiagen 8000 Biorobot. Researchers provide overnight bacterial cultures that are processed by the facility. We continue to provide a very popular large-scale DNA purification (maxiprep) service from bacterial cultures.

Human cell line authentication using the Promega Geneprint 10 Kit is available as an internal service. 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 to confirm data integrity and is increasingly requested by journals.

The Reagent Service ensures the servicing and fumigation of biological safety cabinets is coordinated every six months with the engineer. The mycoplasma screening service offers testing of cells every three to four months. Newly imported cell lines are tested as soon as possible after arrival using a luciferase assay that detects mycoplasmal enzymes. Hoechst staining to detect the presence of mycoplasma DNA, enzyme immunoassay against the four most common species of mycoplasma or a colorimetric microplate assay to detect 16S ribosomal mycoplasma RNA can also be used.

The facility prepares cell-derived matrices from Tiff 5 cells to order, stocks of orders commonly used tissue culture medium and coordinates batch testing of serum. It 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.

PUBLICATIONS

Kurt Anderson

Primary Research Papers

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Tom Bird (page 28)

Liver Disease and Regeneration

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Karen Blyth (page 68)

Transgenic Models of Cancer

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Jeff Evans (page 32)

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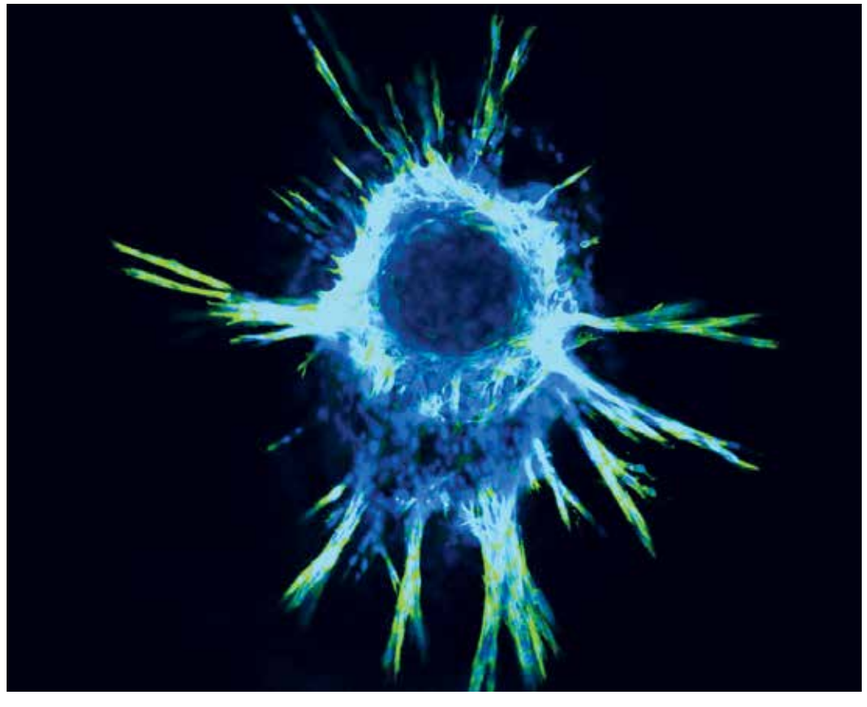
This image by Amelie Juin, which came second in the BAIR image competition, shows pancreatic cancer cells invading Matrigel with phalloidin in green and DAPI in blue. Microscope: Nikon A1R.



Eyal Gottlieb (page 12)

Tumour Metabolism

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Danny Huang (page 14)

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Gabriela Kalna (page 58)

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Shehab Ismail (page 36)

Structural Biology of Cilia

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Hing Leung (page 16)
Prostate Cancer Biology

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Laura Machesky (page 38)
Migration, Invasion and Metastasis

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Gillian Mackay (page 60)
Metabolomics

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Gaurav Malviya (page 62)
Nuclear Imaging

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Michael Olson (page 42)
Molecular Cell Biology

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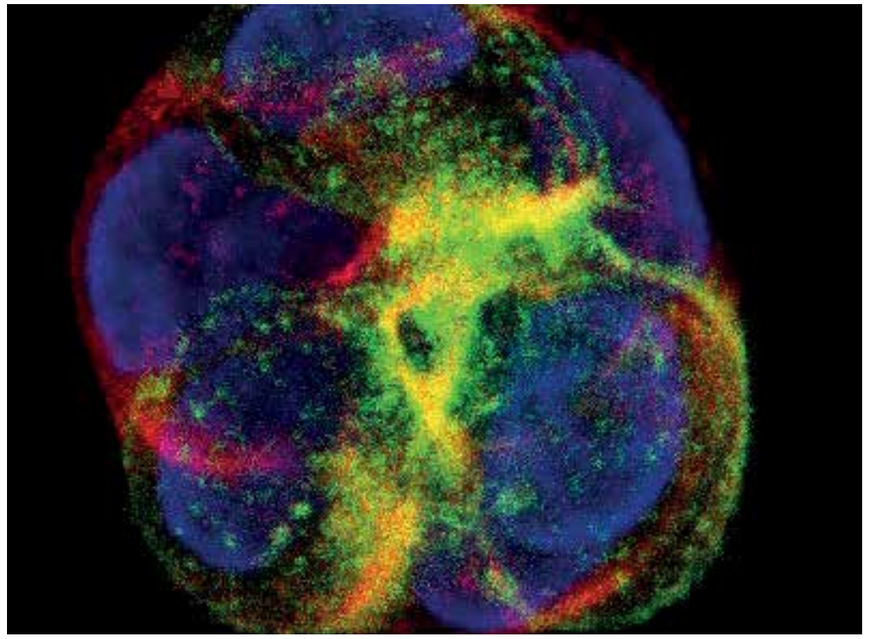

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Jurre Kamphorst (page 80)

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Stephen Tait (page 84)
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Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, Adachi H, Adams CM, Adams PD, Adeli K, Adhihetty PJ, Adler SG, Agam G, Agarwal R, Aghi MK, Agnello M, Agostinis P, Aguilar PV, Aguirre-Ghiso J, Airoldi EM *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). ***Autophagy*** 2016; 12: 1-222

Oberst A, Ichim G, Tait SWG. Mitochondrial Permeabilization: From Lethality to Vitality. In: ***Cell Death in Biology and Diseases*** pp 213-26, 2016 (Ed Hockenbery DM)

Riley JS, Tait SWG. Mechanisms of mitophagy: putting the powerhouse into the doghouse. ***Biol Chem*** 2016; 397: 617-35

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 Christin Bauer from Marcos Vidal's group. She has been studying the role of Bursicon/dLGR2 signalling in enteroendocrine control of systemic metabolism in the fruit fly Drosophila.

Theses
Batista, Jose (2016) FAM49 – a new candidate regulator of membrane protrusion dynamics and cell motility [PhD thesis, University of Glasgow, Beatson Institute]

Brown, Jennifer (2016) The actin cytoskeleton in cancer [PhD thesis, University of Glasgow, Beatson Institute]

Giampazolias, Evangelos (2016) Investigating non-apoptotic cell death in cancer [PhD thesis, University of Glasgow, Beatson Institute]

Huels, David (2016) Investigating the impact of WNT deregulation upon intestinal stem cells and cancer [PhD thesis, University of Glasgow, Beatson Institute]

McKillop, Anne (2016) Investigating Runx2 in prostate cancer [PhD thesis, University of Glasgow, Beatson Institute]

Morris, Hayley (2016) Investigating the role of N-WASP in the development and metastasis of colorectal cancer [PhD thesis, University of Glasgow, Beatson Institute]

Mrschtik, Michaela (2016) Characterisation of the role of DRAM-related TMEMISO proteins in cancer cell survival, cell death and autophagy [PhD thesis, University of Glasgow, Beatson Institute]

Nowicki, Stefan (2016) The metabolic and epigenetic effects of isocitrate dehydrogenase (IDH) mutations in glioma [PhD thesis, University of Glasgow, Beatson Institute]

Pajak, Malgorzata (2016) Investigation of 18F-fluoro-L-thymidine to monitor treatment response in murine models of pancreatic cancer: Development of tools and validation [PhD thesis, University of Glasgow, Beatson Institute]

Reid, Steven (2016) A proteomic approach to study how the extracellular matrix stiffness affects angiogenesis [PhD thesis, University of Glasgow, Beatson Institute]

Ruengeler, Elena (2016) A study into the role of secreted CLIC3 in tumour cell invasion [PhD thesis, University of Glasgow, Beatson Institute]

Slater, Sarah (2016) Does Brf1, a component of the transcription factor (TFIIIB), have a role in prostate carcinogenesis? [PhD thesis, University of Glasgow, Beatson Institute]

Woodham, Emma (2016) Investigating the role of the Rho GTPase Cdc42 in the migration and invasion of the melanocyte lineage [PhD thesis, University of Glasgow, Beatson Institute]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Modelling the Mechanisms of Malignancy - *In Vivo* Veritas

3 - 6 July 2016
Bute Hall, University of Glasgow
Scientific Committee: Jim Norman, Jeff Evans, Jurre Kamphorst, Jennifer Morton, Owen Sansom

This year's conference focused on the latest developments in the generation of sophisticated animal models of cancer, and emphasised how diverse aspects of the human disease can now be faithfully recapitulated in the laboratory. During the opening session, Richard Marais (CRUK Manchester Institute) gave the Colin Thomson Memorial Keynote Lecture, sponsored by Worldwide Cancer Research (WCR), describing the substantial challenges to be faced before the full promise of precision medicine for cancer patients can be realised.

There was a range of excellent speakers throughout the meeting, including Gareth Inman, Toby Phesse, Pierre Close, Adrianna Fumagalli, Emmanuel Dornier, Daniel Schramek, Daniel Murphy, Andy Finch and Jurre Kamphorst who gave selected short talks sponsored by WCR. We were also grateful to Advanced Cell Diagnostics for sponsoring the opening session talk by Owen Sansom as well as the 'stem' session, and to Transnetyx for supporting the 'microenvironment' session.

Following the poster session, Michael Hodder (CRUK Beatson Institute), whose work demonstrated anti-apoptotic Mcl-1 as a novel regulator of proliferation in the intestine, was awarded the AMSBIO-Trevigen sponsored prize. The meeting was generously co-sponsored by Cancer Research UK and Worldwide Cancer Research.

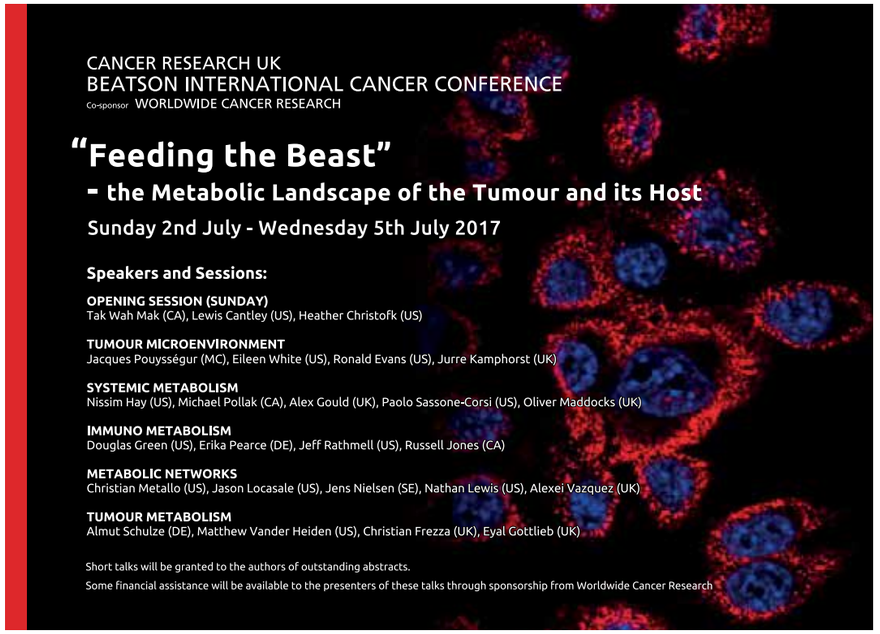
The 2017 meeting (Feeding the Beast - The Metabolic Landscape of the Tumour and its Host, 2-5 July) will provide a topical view of the metabolic vulnerabilities of cancer cells and how the interplay between tumour, stroma and systemic metabolism contributes to cancer progression. It will also highlight opportunities for cancer treatment and prevention (see www.beatson.gla.ac.uk/conf for more details and to register).

SEARCHBreast Workshop

In vivo models of breast cancer – how to choose the right model for your research
We were delighted to host a SEARCHBreast workshop at the Institute on 3-4 March, which focused on the contributions complex models have made to breast cancer research. Speakers included Mohamed Bentires-Alj, Jos Jonkers, Matt Smalley, Val Brunton, Ingunn Holen, Rob Clarke and Will Brackenbury. SEARCHBreast is a resource to facilitate sharing of archived material derived from *in vivo* breast cancer models (see searchbreast.org for more details)

FLIM-FRET-FSC Workshop

This event was held on 7-8 September and hosted by the Beatson Advanced Imaging Resource (BAIR), now headed by new junior group leader Leo Carlin. The meeting consisted of a series lectures, including guest speaker Kurt Anderson, and hands-on sessions from experts in probing molecular dynamics with FLIM, FRET and FCS. We were grateful to Nikon UK, Carl Zeiss UK and LaVision for their input and sponsorship.



CRUK Glasgow Centre meetings

The Institute was the venue for two Centre meetings this year. The first on 7 October was a symposium highlighting progress in cancer research across the whole of Glasgow, while the second on 28 November focused on interactions between researchers working in Bioengineering and Cancer.

Beatson Retreat

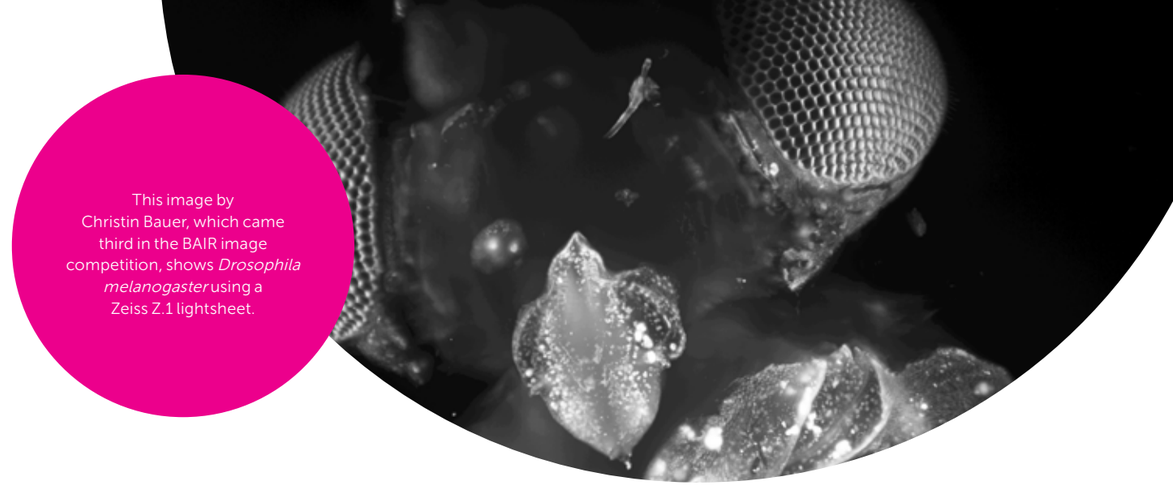
An Institute retreat was held in our lecture theatre on 6 May, giving everyone a chance to hear about the latest, exciting work being done by researchers here. This included talks by new starts Leo Carlin and David Lewis; elevator talks from Amelie Juin, Louise Stephen, Jiska van der Reest, Sergey Tumanov, Iqbal Rather, Joel Riley, Ming Yang, Nicola Rath and Charles Parry; highlight talks by Patrizia Cammareri and Rachana Patel, a 'Grand Challenge' event with groups of students and postdocs coming together 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.

Open Evenings

Given their popularity particularly with school students, teachers and Cancer Research UK supporters, we held two open evenings again this year. One was on 16 March (during National Science and Engineering Week) and included speakers David Bryant, David Vincent, Evangelos Giampazolias and Mark Salji, and the other was on 14 September. Our enthusiastic volunteers provided a series of engaging talks, lab tours and demos for the visitors.

SEMINARS

The following seminars were held at the Cancer Research UK Beatson Institute during 2016.



This image by Christin Bauer, which came third in the BAIR image competition, shows *Drosophila melanogaster* using a Zeiss Z.1 lightsheet.

January

Werner Kovacs, Institute of Molecular Health Sciences, ETH Zurich, Switzerland

Michael Pacold, Dana-Farber Cancer Institute, Boston, USA

Paul Gissen, University College London

Richard Tavare, Crump Institute for Molecular Imaging, University of California, Los Angeles, USA

James Knight, Department of Oncology, University of Oxford

Tony Letai, Dana-Farber Cancer Institute, Boston, USA

David Lewis, CRUK Cambridge Institute, University of Cambridge

February

Karin de Visser, Division of Immunology, The Netherlands Cancer Institute, Amsterdam

Daniel Marston, Department of Pharmacology, University of North Carolina, Chapel Hill, USA

Tiziana Bonaldi, Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

April

Anne Simonsen, Institute of Basic Medical Sciences, University of Oslo, Norway

May

Distinguished Seminar Speaker: Hermann Steller, Strang Professor and Investigator, Howard Hughes Medical Institute, The Rockefeller University, New York, USA

Parthive Patel, German Cancer Research Center (DKFZ) - Center for Molecular Biology, University of Heidelberg (ZMBH) Alliance, Germany

Distinguished Seminar Speaker: *Morag Park*, Professor, Department of Biochemistry and Director, Rosalind and Morris Goodman Cancer Centre, Montreal, Canada

June

Lorna Young, Dartmouth College, Hanover, USA

Jerry Chipuk, The Icahn School of Medicine at Mount Sinai, New York, USA

Jean-Ehrland Ricci, INSERM U1065, University of Nice, France

Stella Valenzuela, School of Life Sciences, University of Technology Sydney, Australia

Mads Daugaard, Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, Canada

July

Connie Eaves, Terry Fox Laboratory, British Columbia Cancer Agency and University of British Columbia, Vancouver, Canada

Distinguished Seminar Speaker: David E James, Leonard P Ullmann Chair in Metabolic Systems Biology, The Charles Perkins Centre, The University of Sydney, Australia

August

Richard Clarkson, European Cancer Stem Cell Research Institute, University of Cardiff

Distinguished Seminar Speaker: Alfred Wittinghofer, Max Planck Institute of Molecular Physiology, Germany

Ingunn Holen, University of Sheffield

September

Herbert Schiller, Comprehensive Pneumology Center, Helmholtz Zentrum München, Germany

Melchiorre Cervello, Consiglio Nazionale delle Ricerche, Palermo, Italy

October

Distinguished Seminar Speaker: Douglas Green, Department of Immunology, St Jude Children's Research Hospital, Memphis, USA

Pekka Katajisto, Institute of Biotechnology, University of Helsinki, Finland and Department of Biosciences and Nutrition, Karolinska Institute, Sweden

Simak Ali, Department of Surgery and Cancer, Imperial College London

Keith Brennan, Faculty of Life Sciences, University of Manchester

November

Viktor Korolchuk, University of Newcastle

Luke Gaughan, Northern Institute for Cancer Research, University of Newcastle

Distinguished Seminar Speaker: Bill Sellers, Boston, USA

Bernard Thienpont, Center for Cancer Biology, VIB-KU Leuven, Belgium

Jacco van Rheenen, Hubrecht Institute and University Medical Center Utrecht, The Netherlands

Eduard Batlle, Institute for Research in Biomedicine, Barcelona, Spain

December

Victor Peperzak, University Medical Center Utrecht, The Netherlands

Joern Dengjel, University of Fribourg, Switzerland

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.

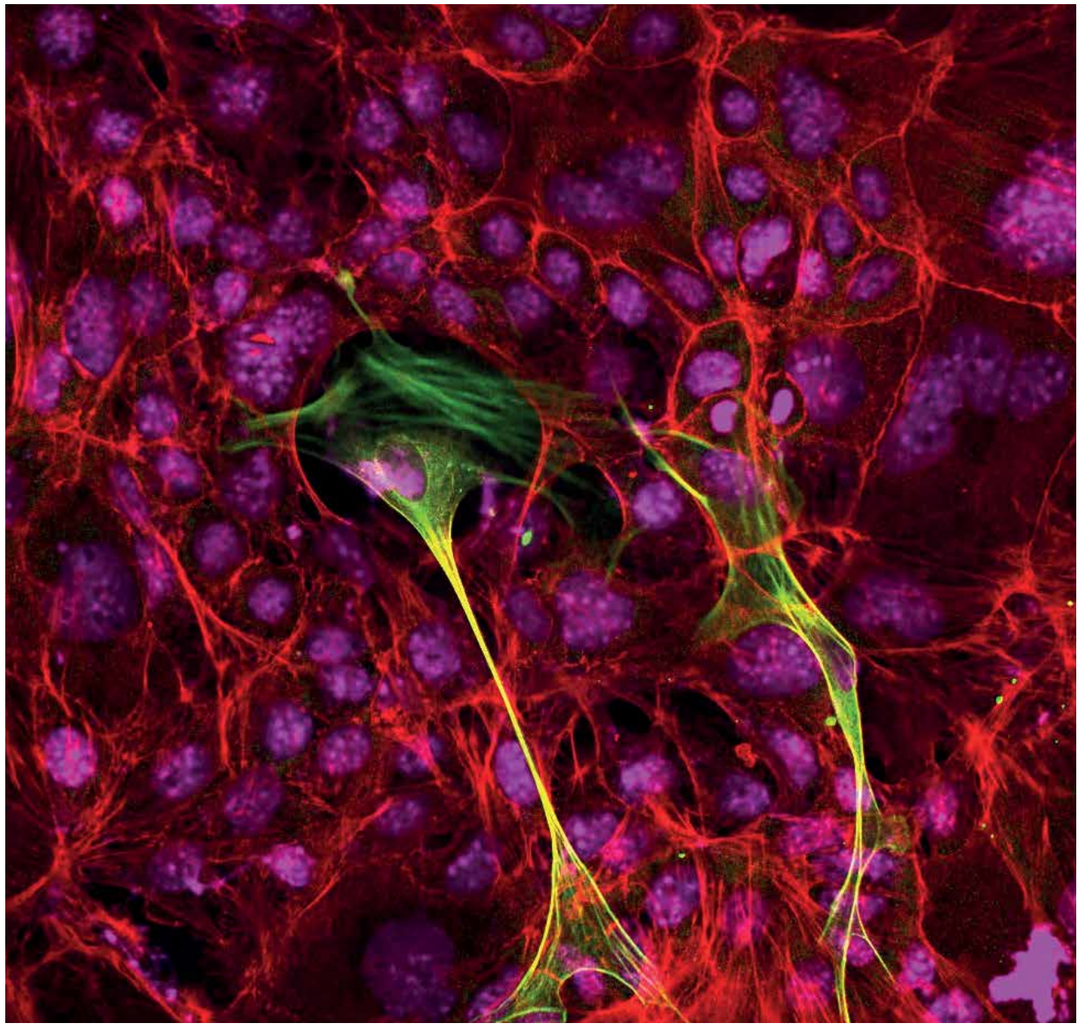
Our students are registered at the University of Glasgow and are allocated a supervisor and an advisor who are jointly responsible for supporting and monitoring their performance and progress. The supervisor is responsible for developing the student's abilities, providing all practical support required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

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



Cancer-associated fibroblasts in contact with pancreatic cancer cells. Staining alpha-SMA in green, phallaoidin in red and DAPI in magenta. Microscope: Nikon A1R (image from Amelie Juin).

OPERATIONAL SERVICES

Finance and Human Resources

Director and Company Secretary:
Peter Winckles ACA DChA

Finance

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 mission is to enable cancer discovery for patient benefit by providing a professional finance and human resources service to our stakeholders to allow them to efficiently and effectively manage the Institute's resources. Our vision is to be a Finance and Human Resources team that is professional, open, inclusive and collaborative.

The focus of the last year has been team development. Working with Joe Lafferty from LifeTree (www.lifetree.co.uk) the team has worked on establishing a clear vision underpinned by values and acceptable behaviours. This project provided an added energy and focus to the team. We now understand each other better and there is a real desire to work more effectively with our scientific colleagues at all levels. In September 2016, we relaunched the team as Finance and Human Resources to highlight the significant role that our Human Resources team play in the success of the Institute.

The Finance team is 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.

Building on the successful implementation in 2015 of the first new finance system for over 15 years, the team has focused on continuing to improve the quality and timeliness of management information for all its business partners. Key projects started during the year include the development of a project budgeting and forecasting model as well as detailed costing models for our support services. The significant changes in accounting regulations and reporting introduced in 2016 were very successfully implemented during our year end statutory accounts production.

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 2016, the team commenced the Institute's first line management development programme. Through investing in and developing our managers we aim to further improve their performance and that of individuals in their teams.

In addition, the Finance and Human Resources team is 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 the Director), 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. This has 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.

Finance and Human Resources team.

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

Tom Bird
Wellcome Trust

Martin Drysdale
Daphne Jackson Trust, Medical Research Council

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

Eyal Gottlieb
Metabomed

Danny Huang
European Community, Nuevolution

Hing Leung
Medical Research Council

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

Michael Olson
Medical Research Council, Worldwide Cancer Research

Kevin Ryan
Astellas Pharma Inc, Worldwide Cancer Research

Owen Sansom
AstraZeneca, European Community, Gilead, Janssen Pharmaceutica NV, Intrexon, Novartis, Royal Commission for the Exhibition of 1851, Worldwide Cancer Research, Wellcoem Trust

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

Beatson Associates

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

Jurre Kamphorst
CRUK, Rosetrees Trust

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

Stephen Tait
BBSRC, Breast Cancer Now, CRUK, EMBO, 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 that 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.

Claire Hughes, Lindsey MacDonald and Lauren McLeod from Enterprise Holding Foundation, who are partnered with Clyde Travel, present a cheque for £1,500 to our interim director and other Institute staff.

Florence Adams
Olanshile Akintola
Legacy from the Estate of Miss Agnes Calder Galbraith
Margaret G Brown
Clyde Travel
A D'Apice
Senga Dempster
Thomas Donaldson and his mother Mrs Agnes Donaldson, in memory of many friends who have died from cancer
Dowling
Ian Downie, Kilmarnock Football Club Mascot
Employess of Edrington
Edrington
Employees of Diageo
Enterprise Foundation Fund
Elaine Fanning, in memory of her sister and brother-in-law Helen and David
Hazel Gardiner
George Watson's College Girls' Cricket Club
Eleanor Gibb, in memory of her father Mr Thomas Hay
Glasgow Caledonian University
May Gow
In memory of Mrs Eleanor Robina Kellas
Mr & Mrs Olav Kerr's Charitable Trust
Kilmarnock Football Club Supporters Association
Kirk of the Holy Rood, Women's Association
Kirkhill Primary School
Grace Lang, in memory of her husband Arthur H Low
Janet Lyke
Christine MacCormick
Hector MacLean
John Macpherson
K G McCafferty
Ann McDonald
Christina McDougall, in memory of her husband John and son David
Legacy from Catherine Anne McIntyre
Fiona McNeill and family
McQuarrie family, in memory of their daughter Rhoda
Miller Beckett & Jackson Solicitors
Mosshead Primary School
Allan M Mowat, in memory of Stuart Harvey Lynn Murray
Ivy Nicolson, in memory of Kevin Flynn
Order Of The Eastern Star - Lily of the Valley Paisley Patchers
Sarah Perry
Polaroid Eyewear Ltd
Rikbar Ltd
Jim Rogers, in memory of his father James Rogers
In memory of the Late Jean Sanders
Rotary Club of Strathendrick
Ann Soutar, in memory of Walter Soutar
St Andrew of Glasgow Royal Arch Chapter No.69
St Rollox Bowling Club
John Teevan, in memory of their late mother
The Robertson Trust
M Thomas, in memory of Mrs Mary Thomas
Jacqueline Thomson, in memory of her father Mr Eddie Doyle
Thornhill Gardening Society
J Walker
West of Scotland Women's Bowling Association
G H Whitaker, in memory of James D Craig
Legacy from William Cluggie Wilson
Yorkshire Building Society, Bishopbriggs Branch

PATRONS AND BOARD OF GOVERNORS

Patrons

His Grace the Duke of Hamilton
The Rt Hon. Lord Mackay of Clashfern
The Viscountess Weir

Board of Directors

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

Prof Nic Jones (Chair)
Chief Scientist, Cancer Research UK

Mr Craig Anderson
Senior Partner, KPMG

Mr Kirk Murdoch
Chairman, Pinsent Masons, Scotland & Northern Ireland

Prof Anton Muscatelli
Principal of the University of Glasgow

Dr Iain Foulkes
Executive Director, Strategy and Research Funding, Cancer Research UK

Mr Ian Kenyon
Chief Financial Officer, Cancer Research UK

Company Secretary
Mr Peter Winckles
Cancer Research UK Beatson Institute

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Registered as a company limited by guarantee in Scotland No. 84170
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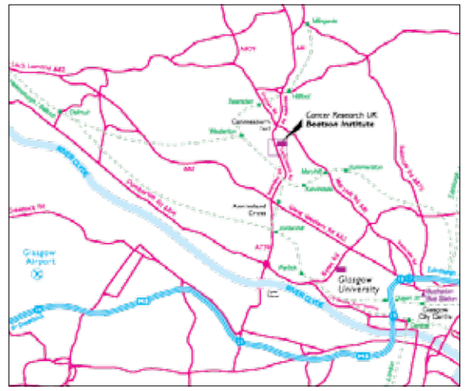
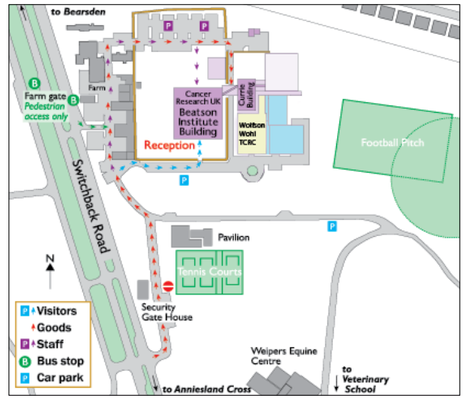
Electronic version of this report can be found at:
www.beatson.gla.ac.uk/annual_report

Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).
Registered address: Angel Building, 407 St John Street, London EC1V 4AD

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





NOTES

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