SCIENTIFIC REPORT 2013

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Cover image
Graduate student Loic Fort from Laura Machesky's group.

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The Beatson Institute for Cancer
Research building

CONTENTS

INTRODUCTION

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

This year was an extremely important and busy one for us with quinquennial reviews of both the Institute and the Drug Discovery Programme being held in June. We also took a lead role in the renewal of the CRUK Glasgow Centre.

Our Institute review was a great success and a fantastic endorsement of everyone's hard work over the past few years. The review panel was very positive about our progress to date and future plans – especially our move to develop cancer metabolism. An increasing number of our research groups are doing excellent work in this area as can be seen from our research highlights, which include a number of metabolism-focused papers. We are also putting considerable effort into establishing our Cancer Metabolism Research Unit (CaMeRU), headed by Eyal Gottlieb, and we have been actively engaged in recruiting the people and building the resources in support of this. Our first group leader appointment will be Jurre Kamphorst, whose expertise is in lipid metabolism and who will join us as a Beatson Associate in early 2014.

The review of the Drug Discovery Programme, the first since its inception, was also encouraging about what the unit has achieved so far and its future plans. There is a growing integration between the work of the unit and the rest of the Institute, an initiative that is being led by the head of the DDP, Martin Drysdale.

We were also co-applicants, along with colleagues from the Institute of Cancer Sciences at the University of Glasgow, on a successful application to renew our CRUK Centre status. The new award includes an important training component in support of students and clinical fellows but particularly focuses on providing infrastructure in the areas of co-clinical trials, informatics, biomarkers, biospecimens and functional screens. The goal is to provide a platform of technical expertise and technological capabilities to support members of the Centre across all disciplines and tumour types and we were delighted to be awarded a substantial increase in the funding available to support these areas. The Centre also had the pleasure of welcoming Stan Kaye as Chair of its Governance Board. Stan, who has worked at the Institute of Cancer Research in London for many years, brings considerable expertise to this role as well as extensive knowledge of cancer research in Glasgow from his time here as Chair of Medical Oncology.

This year saw the departure of a long-standing member of the Institute **David Gillespie**, who secured a position at the University of Tenerife where he will continue his research programme on checkpoints and cell cycle control. Dave joined the Beatson in 1989 and made important contributions to the work of the Institute as well as acting as a mentor and overseeing our postdoctoral training programme. We wish Dave all the best in sunnier climes.

We also said farewell to our Laboratory Manager **Robert MacFarlane**, who retired from the Institute at the end of August after 42 years of service. His contribution to the Institute was extraordinary and we wish him the very best for his retirement. Meanwhile, **Laura Bence** joined us to take on this key role.

I was delighted to be elected to the American Association for the Advancement of Science, while one of our junior group leaders **Marcos Vidal** was recognised by being elected to the Young Academy of the Royal Society of Edinburgh.

The Institute review panel highlighted the excellent training environment we provide for students and postdoctoral scientists and this was reflected again this year with several of our departing postdocs winning fellowships and taking up independent group leader positions elsewhere, including **Celia Berkers** (Utrecht), **Julia Cordero** (Glasgow), **Jason King** (Sheffield), **Patricia Muller** (MRC Toxicology Unit, Leicester) and **Tobias Zech** (Liverpool).

We also saw a large number of students successfully graduate this year and we look forward to seeing their careers develop in the coming years. Our students had the great pleasure of playing host to Sir Paul Nurse who visited this year in his role as President of the Royal Society.

Finally, as every year we would like to extend our heartfelt thanks to all the donors and supporters whose generosity makes our work possible.

Members of the Beatson Cancer Metabolism Research Unit at work (CaMeRU)

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.

Cheung EC, Athineos D, Lee P, Ridgway RA, Lambie W, Nixon C, Strathdee D, Blyth K, Sansom OJ, Vousden KH.
TIGAR is required for efficient intestinal regeneration and tumorigenesis. ***Dev Cell*** 2013; 25: 463-77

In this study, the authors generate a TIGAR-deficient mouse and demonstrate the importance of this fructose-2,6-bisphosphatase in regulating glucose metabolism during intestinal regeneration and adenoma development. They conclude that TIGAR contributes to both tissue regeneration and tumour development and thus could be a potential therapeutic target in diseases such as ulcerative colitis and intestinal cancer.

Dou H, Buetow L, Sibbet GJ, Cameron K, Huang DT.
Essentiality of a non-RING element in priming donor ubiquitin for catalysis by a monomeric E3. ***Nat Struct Mol Biol*** 2013; 20: 982-6

This paper describes the crystal structure of a monomeric RING E3 ligase. RING E3 ligases transfer ubiquitin from E2 to substrate during ubiquitination, a process that regulates protein degradation and which is important for many cellular activities. Based on their analysis of the structure, the authors suggest that interactions outside the canonical RING domain are crucial for optimising ubiquitin transfer in both monomeric and dimeric RING E3s.

Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G, van der Burg SH, Verdegaal EM, Cascante M, Shlomi T, Gottlieb E*, Peeper DS*.
A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. ***Nature*** 2013; 498: 109-12
*corresponding authors

Oncogene-induced senescence (OIS) blocks cancer development by acting as a brake and stopping cell proliferation. However, little is known about the metabolism involved in this process. This study uses metabolic profiling and functional perturbations to show that pyruvate dehydrogenase (PDH), an enzyme that ties glucose metabolism to oxidative phosphorylation, is a crucial mediator of OIS in melanoma induced by the oncogene BRAF(V600E). Furthermore, induction of PDH activity blocks melanoma growth and progression *in vivo*, leading the authors to suggest that the relationship between OIS and PDH might be exploited therapeutically.

Klejnot M, Gabrielsen M, Cameron J, Mleczak A, Talapatra SK, Kozielski F, Pannifer A, Olson MF.
Analysis of the human cofilin 1 structure reveals conformational changes required for actin binding. ***Acta Crystallogr D Biol Crystallogr*** 2013; 69: 1780-8

In this paper, the authors determine the crystal structure of a C147A point mutant of human cofilin 1, a protein that disassembles actin filaments and contributes to remodelling of the actin cytoskeleton. Actin remodelling is crucial to several processes within the cell, including some such as motility and division that have a role in cancer. This study is the first to describe the crystal structure of an animal cofilin and reveals how its actin-binding helix undergoes a conformational change that increases the number of potential hydrogen bonds available for substrate binding.

Long JS, Crighton D, O'Prey J, Mackay G, Zheng L, Palmer TM, Gottlieb E, Ryan KM.
Extracellular adenosine sensing - a metabolic cell death priming mechanism downstream of p53. ***Mol Cell*** 2013; 50: 394-406

It is known that adenosine accumulates under conditions of cellular stress including during tumour development and this study provides a mechanism that links this change in metabolism to the induction of cell death via the tumour suppressor p53. The authors use microarray data to identify A2B, the adenosine receptor, as a p53 target gene. They go on to show how A2B is upregulated by p53 and then activates apoptosis upon adenosine binding.

Lourenco FC, Munro J, Brown J, Cordero J, Stefanatos R, Strathdee K, Orange C, Feller SM, Sansom OJ, Vidal M, Murray GI, Olson MF.
Reduced LIMK2 expression in colorectal cancer reflects its role in limiting stem cell proliferation. ***Gut*** 2014; 63: 480-93

In this work, the authors demonstrate that a reduction in LIM kinase 2 expression in colorectal cancer is associated with shorter patient survival. They also show, using genetically modified *Drosophila* and mouse models, that LIM kinase 2 can constrain gastrointestinal stem cell proliferation and thus colon tumour development.

Ma Y, Li A, Faller WJ, Libertini S, Fiorito F, Gillespie DA, Sansom OJ, Yamashiro S, Machesky LM.
Fascin 1 is transiently expressed in mouse melanoblasts during development and promotes migration and proliferation. ***Development*** 2013; 140: 2203-11

This study investigates the role of fascin 1, an actin-bundling protein, in melanocytes and melanoma cells *in vivo* and in skin explants. The authors show that fascin 1 knockout leads to both migration and proliferation defects in melanoblasts, the melanocyte precursors, and in melanoma cells. They conclude that fascin 1 has a role in cell cycle progression as well as migration and that its expression may be advantageous in tumours.

Myant KB, Cammareri P, McGhee EJ, Ridgway RA, Huels DJ, Cordero JB, Schwitalla S, Kalna G, Ogg EL, Athineos D, Timpson P, Vidal M, Murray GI, Greten FR, Anderson KI, Sansom OJ.

ROS production and NF-kappaB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. ***Cell Stem Cell*** 2013; 12: 761-73

In this paper, the authors demonstrate that the GTPase RAC1 is a critical mediator of tumourigenesis following APC loss in colorectal cancer. RAC1 is shown to be responsible for the expansion of intestinal stem cells and progenitor hyperproliferation and thus intestinal transformation. Crucially, it does this by triggering ROS production and NF-κB signalling.

Park L, Thomason PA, Zech T, King JS, Veltman DM, Carnell M, Ura S, Machesky LM, Insall RH.
Cyclical action of the WASH complex: FAM21 and capping protein drive WASH recycling, not initial recruitment. ***Dev Cell*** 2013; 24: 169-81

In this study, the authors use *Dictyostelium* to generate mutants of each of the five subunits of the regulatory WASH complex. This allows them

to investigate the functions of each subunit during actin polymerisation, lyosomal trafficking and exocytosis, and highlights a distinct role for FAM21 in this latter step as well as in the recycling of the WASH complex itself.

Patel R, Gao M, Ahmad I, Fleming J, Singh LB, Rai TS, McKie AB, Seywright M, Barnetson RJ, Edwards J, Sansom OJ, Leung HY.
Sprouty2, PTEN, and PP2A interact to regulate prostate cancer progression. ***J Clin Invest*** 2013; 123: 1157-75

This integrative study uses preclinical and clinical resources to address the biology underlying collaborative tumour suppressor functions. The authors identify SPRY2, PTEN and PP2A deficiencies as prognostic biomarkers that cooperate to drive aggressive prostate cancer. This biomarker trio may facilitate patient stratification for targeted therapies and chemopreventive interventions.

Rajan P, Sudbery IM, Villasevil ME, Mui E, Fleming J, Davis M, Ahmad I, Edwards J, Sansom OJ, Sims D, Ponting CP, Heger A, McMenemin RM, Pedley ID, Leung HY.
Next-generation Sequencing of Advanced Prostate Cancer Treated with Androgen-deprivation Therapy. ***Eur Urol*** published online 14 Aug 2013, doi: 10.1016/j.eururo.2013.08.011

This work focuses on castration-resistant prostate cancer, the mechanisms underlying which are not fully understood. It is the first report on the RNA sequencing of matched prostate cancer tissue from patients before and after androgen-deprivation therapy (ADT) and highlights some ADT regulated signalling pathways that may be targets for the treatment of castration-resistant prostate cancer.

Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, Mackay G, Mrowinska A, Au A, Rai TS, Zheng L, Ridgway R, Adams PD, Anderson KI, Gottlieb E, Sansom OJ, Ryan KM.
p53 status determines the role of autophagy in pancreatic tumour development. ***Nature*** 2013; 504: 296-300

In this paper, the authors provide important insights into the role of autophagy, a key regulator of cellular homeostasis, in cancer. Using a mouse model of pancreatic cancer, the authors show that while loss of autophagy blocks tumour formation in mice containing oncogenic Kras, this is not the case in mice containing oncogenic Kras but also lacking p53. In fact, in this scenario loss of autophagy actually accelerates tumour formation, fuelled by increased glucose uptake and metabolism. The authors note that their findings have potential implications for autophagy inhibition in cancer therapy.

Tang H, Li A, Bi J, Veltman DM, Zech T, Spence HJ, Yu X, Timpson P, Insall RH, Frame MC, Machesky LM.
Loss of Scar/WAVE complex promotes N-WASP- and FAK-dependent invasion. ***Curr Biol*** 2013 ; 23: 107-17

This study describes the contrasting roles of the Scar/WAVE regulatory complex (WRC) and the Arp2/3 activator N-WASP in 3D cell migration. The authors show that N-WASP, in cooperation with FAK, is pro-invasive, while WRC inhibits invasive migration. This is contrary to its role in 2D migration. WRC loss also promotes FAK-dependent cell transformation and tumour growth *in vivo*. The authors conclude that more work is needed to determine the potential significance of WRC loss in cancer.

Trinidad AG, Muller PA, Cuellar J, Klejnot M, Nobis M, Valpuesta JM, Vousden KH.
Interaction of p53 with the CCT complex promotes protein folding and wild-type p53 activity. ***Mol Cell*** 2013; 50: 805-17

This paper investigates the control of p53 by the chaperonin CCT. The authors show that CCT binds wild-type p53 and is required for correct folding of the protein. Furthermore, CCT binding can regulate p53's growth suppressive and invasive behaviours, each of which the authors suggest may be differentially regulated.

Institute of Cancer Sciences

Cruickshanks HA, McBryan T, Nelson DM, Vanderkraats ND, Shah PP, van Tuyn J, Singh Rai T, Brock C, Donahue G, Dunican DS, Drotar ME, Meehan RR, Edwards JR, Berger SL, Adams PD.
Senescent cells harbour features of the cancer epigenome. ***Nat Cell Biol*** 2013; 15: 1495-506

In this paper, the authors use genome-wide sequencing to investigate the pattern of DNA methylation in senescent cells. They observe widespread hypomethylation and focal hypermethylation similar to that seen in cancer cells. These features are also largely retained when cells bypass senescence. The authors speculate that if these senescent cells escape the proliferative barrier, they might thus be already primed for malignancy.

Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, Shah PP, Hewitt G, Korolchuk VI, Passos JF, Wu H, Berger SL, Adams PD.
Lysosome-mediated processing of chromatin in senescence. ***J Cell Biol*** 2013; 202: 129-43

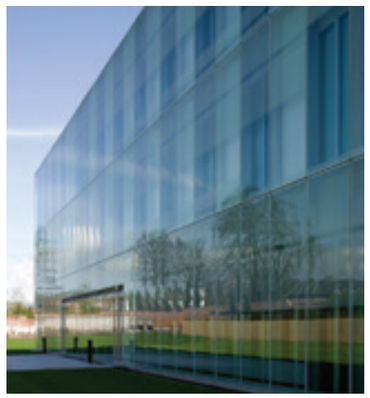
This study tracks cytoplasmic chromatin fragments to demonstrate that senescent cells process their chromatin via an autophagy/lysosomal pathway. This leads to a depletion of histones and is associated with tumour suppression. The authors suggest that processing chromatin in this way might contribute to the stability of cellular senescence and 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

The Beatson Institute for Cancer Research



REGULATION OF CANCER CELL GROWTH METABOLISM AND SURVIVAL

CANCER RESEARCH UK BEATSON INSTITUTE

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

APOPTOSIS AND TUMOUR METABOLISM

www.beatson.gla.ac.uk/eyal_gottlieb

Group Leader
Eyal Gottlieb

Associate Scientist
Zachary Schug[3]

Research Scientists
Simone Cardaci[1]
Nadja Pfetzer[2]
Saverio Tardito[4]
Liang (Leon) Zheng

Scientific Officer
Elaine MacKenzie

Clinical Research Fellow
Stefan Nowicki[5]

Graduate Students
Barbara Chaneton
Laura Galbraith

[1] FEBS
[2] Janssen Pharmaceutica
[3] CRUK Discovery Committee/Astra Zeneca
[4] Italian Association for Cancer Research
[5] CRUK Glasgow Centre

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 (the Warburg Effect). More recent research showed that metabolic alterations in cancer include many additional pathways, and this potentially increases the number of clinical targets. In fact, most, if not all tumour suppressors and oncogenes regulate metabolism. Our major interest is in metabolic enzymes that also function as tumour suppressors or oncogenes, or regulate the essential metabolic requirements of cancer cells. Over the past few years, my lab has generated state-of-the-art metabolomics capabilities for studying metabolic transformations and identifying metabolic vulnerabilities of cancer.

Role of oxidative metabolism in oncogene-induced senescence

Several tumour-suppressing mechanisms have evolved that avert the hazard of malignant transformation sparked by oncogenic events. One such programme is the cessation of cell proliferation, termed oncogene-induced senescence (OIS). In recent years, a large body of evidence has shown that OIS is a pathophysiologic mechanism that halts progression to cancer in model organisms and humans alike. Indeed, senescence biomarkers have been reported for a plethora of pre-cancerous lesions including melanocytic nevi, prostate intraepithelial neoplasia and lymphomas. Although it is generally assumed that senescent cells remain metabolically active, surprisingly few aspects of this process have been investigated in detail. To address this need we performed a comprehensive study of the role and regulation of metabolism in OIS. We used metabolic flux profiling to screen for metabolic changes in central carbon and energy metabolism in OIS evoked by mutant BRAF, a common driver of senescence in benign lesions (Fig. 1). We demonstrated that pyruvate oxidation in the mitochondria via pyruvate dehydrogenase (PDH) is necessary and sufficient for OIS. We also studied the functional consequences of perturbing the balance between glycolysis and oxidative phosphorylation on OIS and tumourigenesis, and further identified pyruvate dehydrogenase kinase 1 (PDK1), the inhibitor of PDH, as a vulnerable target in BRAF mutant melanoma cells. The inhibition of PDK1 (and the consequent activation of PDH) induced senescence in established BRAF-driven melanomas resulted in tumour regression *in vivo* (Kaplon *et al.*, Nature 2013; 498: 109).

Metabolic adaptations induced by loss of the mitochondrial tumour suppressor fumarate hydratase

Mutations in the tricarboxylic acid (TCA) cycle enzyme fumarate hydratase (FH) are associated with a highly malignant form of papillary and collecting duct renal cell cancer. Although the mechanisms of tumourigenesis related to FH mutations have been extensively investigated, little is known about the overall consequences that the loss of FH has on cellular metabolism. To address this, we utilised a previously characterised genetically engineered mouse model with a conditional gene ablation of FH (*Fh1*$^{fl/fl}$) and primary immortalised kidney cells in which *Fh1* was genetically deleted *ex vivo* by adenovirus-Cre infection. Initially, mice with

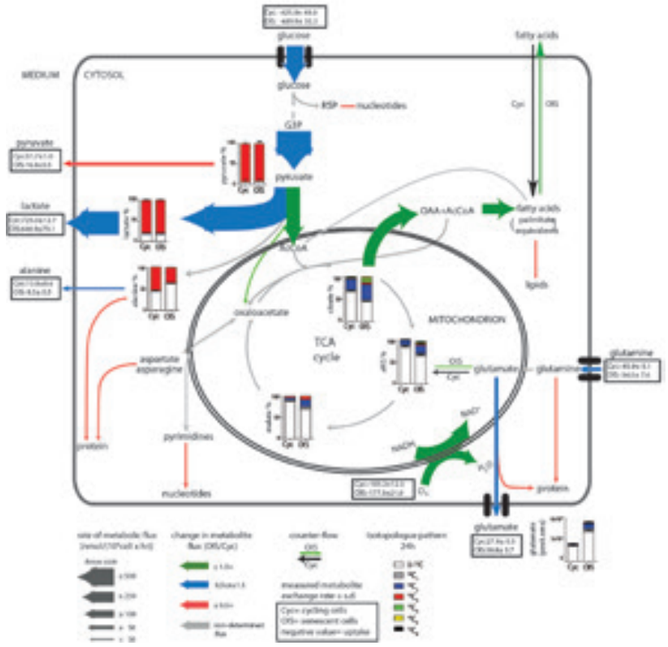


Figure 1
OIS is accompanied by enhanced TCA cycle flux mediated by PDH activation. Metabolic alterations in OIS versus cycling human diploid fibroblasts were studied using a mass-balance model. Measured metabolic fluxes were used for balancing the delineated metabolic network. The biosynthetic constraints and the rates of reactions that produce reducing equivalents NAD(P)H and FADH2 (glycolysis, pentose phosphate pathway, malic enzyme and TCA cycle) were balanced with those that oxidise them (oxygen consumption, lactate dehydrogenase and fatty acid biosynthesis). The calculated reaction rates are presented as coloured arrows where the thickness of the arrows represents the rate of reaction and the colour represents the ratio between OIS and cycling cells. All reactions are calculated as nmol/(10^6 cells x hour).

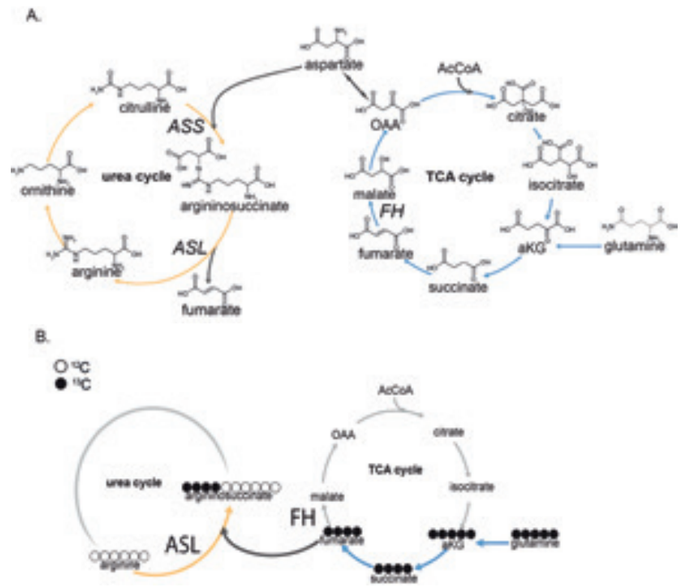


Figure 2
Argininosuccinate is produced from arginine and fumarate via reversed ASL activity. (A) Under normal conditions aspartate, which is derived from the TCA cycle metabolite oxaloacetate (OAA), reacts with citrulline to produce argininosuccinate - a reaction catalysed by ASS. Argininosuccinate is then converted to arginine and fumarate by ASL. (B) In light of the isotopomer distribution of glutamine-derived $^{13}C_4$-labelled argininosuccinate, this scheme represents the biochemical pathway that links TCA cycle metabolites in FH-deficient cells to the urea cycle and to argininosuccinate production.

deletion of kidney FH were generated by crossing the *Fh1*$^{fl/fl}$ mice with an appropriate Cre transgenic mouse. These mice developed multiple benign renal cysts. In order to investigate the metabolic signature associated with the loss of FH in these mice, urine was collected and analysed by LC-MS for metabolic profiling. The metabolites that consistently contributed to the urine metabolic signature of these mice were urobilin, fumarate and argininosuccinate. The presence of urobilin (the urine form of bilirubin) in the urine of mice with *Fh1*-deficient cysts strongly supports our previous report that heme degradation is upregulated in FH-deficient tumours (Frezza *et al.*, Nature 2011; 477: 225). The presence of fumarate in the urine was not unexpected considering the high levels of fumarate detected in FH-deficient cells and tumours. However, the detection of high levels of argininosuccinate in the urine of mice with FH-deficient kidney cysts was somewhat surprising as this is a urea cycle metabolite. Argininosuccinate was also detected at high levels in the spent media of patient-derived FH-deficient renal cancer cells and in the immortalised *Fh1*$^{-/-}$ mouse epithelial kidney cells.

Argininosuccinate is a urea cycle intermediate synthesised by argininosuccinate synthase (ASS) and is subsequently metabolised to fumarate and arginine by argininosuccinate lyase (ASL) (Fig. 2). Interestingly, argininosuccinate aciduria is observed in Inborn Error of Metabolism patients deficient in ASL. Therefore, it seemed plausible that the increase in argininosuccinate results from ASL inhibition by high levels of fumarate in FH-deficient cells. To test this possibility we incubated cells with uniformly labelled [$^{13}C_5$]-glutamine and using LC-MS traced heavy carbon (^{13}C) incorporation into TCA cycle and other intracellular metabolites. Unexpectedly, argininosuccinate was quickly labelled with four glutamine derived ^{13}C carbons, demonstrating that, in FH-deficient cells, argininosuccinate is not produced from citrulline and aspartate by ASS (as is the case in normal cells) but from arginine and fumarate via a reversed ASL reaction (Fig.2). These results were confirmed by the decrease in argininosuccinate secretion following *ASL* gene silencing, and by the increased sensitivity of FH-deficient cells to arginine depletion (Zheng *et al.*, Cancer Metab 2013; 1: 12).

Publications listed on page 78

UBIQUITIN SIGNALLING

www.beatson.gla.ac.uk/danny_huang

Group Leader

Danny Huang

Research Scientists
Lori Buetow
Mads Gabrielsen

Scientific Officer
Gary Sibbet

Graduate Students
Marta Klejnot
Gabriele Marcianò
Amrita Patel

Deregulation in the ubiquitin (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 in order to understand their regulation and function. We anticipate that the knowledge gained from these studies will assist in the development of selective therapeutic targets within the Ub pathway.

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three enzymes: Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3) (Fig. 1). E1 initiates the cascade by adenylating Ub's C-terminus, then forming of a covalent thioester intermediate with it. E1 then recruits E2 and transfers the thioesterified Ub to E2's catalytic cysteine. E3 plays a pivotal role in determining substrate fate. In general, E3 consists of an E2-binding module (HECT, RING or U-box domain) and a protein-protein interaction domain that confers substrate specificity. With this configuration, E3 recruits E2 thioesterified with Ub and substrate to promote Ub transfer from 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 ubiquitinate thousands of different substrates.

The Ub pathway has emerged as the target for therapeutic intervention. Velcade, a proteasome inhibitor, is currently used for treating patients with multiple myeloma and relapsed mantle cell lymphoma. Recent studies demonstrated that inhibitors of NEDD8 E1 (Soucy *et al.*, Nature 2009; 458: 732) and E2 CDC34 (Ceccarelli *et al.*, Cell 2011; 145: 1075) induced apoptosis and suppressed proliferation of human cancer cells, and are currently in clinical trials. In addition, several E3 inhibitors have been developed. Our group is interested in understanding the regulation and mechanistic functions of RING E3s with particular focus on those linked to cancer.

CBL proteins and receptor tyrosine kinase signalling

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

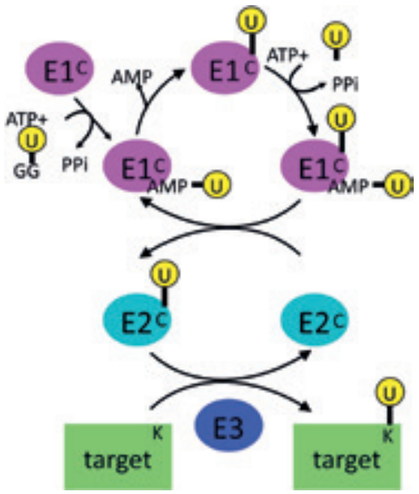


Figure 1
Enzymatic cascade for Ub modifications.

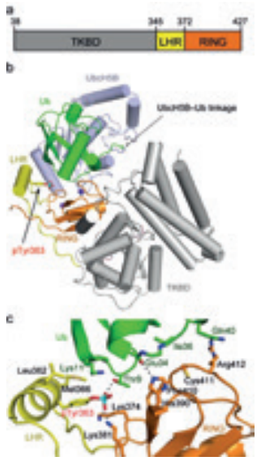


Figure 2
Phosphorylated LHR tyrosine participates in activation of E2~Ub. (a) Conserved CBL's N-terminal domain containing TKBD, LHR and RING domain. (b) Structure of Tyr363 phosphorylated CBLB bound to an E2~Ub and a TKBD substrate peptide. (c) Close-up view of pTyr363-Ub and RING-Ub interactions. All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

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

All CBLs share a highly conserved N-terminal SH2-containing tyrosine kinase-binding domain (TKBD), a linker helix region (LHR) and a RING domain (Fig. 2a) followed by a variable proline-rich region (PRR). The TKBD mediates substrate specificity by binding to proteins containing phosphotyrosine motifs commonly found in RTKs or tyrosine kinases, whereas the PRR recruits proteins containing an SH3 domain. The LHR and RING domain play central roles in recruiting E2s and in mediating target ubiquitination. To gain insights into the regulation of CBLs, we determined three new crystal structures of CBL: native CBL, CBL bound to a TKBD substrate peptide and CBL phosphorylated at Tyr371 in complex with an E2 and a TKBD substrate peptide. Our structures and the existing structure of CBL bound to an E2 and a TKBD substrate peptide (Zheng *et al.*, Cell 2000; 102: 533) reveal dramatic conformational changes in the LHR and RING domain. We showed that in the unphosphorylated state, 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. We found that Tyr371 phosphorylation enhances CBL's catalytic efficiency by 1400-fold. Tyr371 phosphorylation activates CBL ligase activity by inducing dramatic LHR conformational changes that (i) enhance overall E2 binding affinity by eliminating auto-inhibition and forming a new phosphoTyr371-induced E2-binding surface; and (ii) place the RING domain and E2 in proximity of the substrate-binding site. We showed that phosphoTyr371-induced conformational transition is required for EGFR ubiquitination. Together these results demonstrate how Tyr371 phosphorylation could transiently switch on CBL's ligase activity to attenuate RTK signalling (Dou *et al.*, Nat Struct Mol Biol 2012; 19: 184).

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

CBL's Ty371 is one of the mutational hot spots in patients with myeloid neoplasms. Our results explain why Tyr371 mutants cannot adopt an active CBL configuration and optimise E2~Ub for transfer. We are currently investigating on how *CBL* mutations could alter CBL's conformation transitions and contribute to oncogenicity.

Mechanism of Ub transfer by RING E3

RING E3s recruit E2 thioesterified with Ub (E2~Ub) and substrate to facilitate the Ub transfer from E2 to the amino group of a substrate lysine. How RING E3s promote Ub transfer is unclear. We have now determined two crystal structures of RING E3 bound to E2 UbcH5B covalently linked to Ub at its active site. Both structures reveal extensive non-covalent donor Ub interactions with UbcH5B and the RING domain. Notably an additional Ub-binding element outside the RING domain is crucial in stabilising the Ub's Ile36 surface (Fig. 3). In dimeric RING E3s such as BIRC7 and RNF4, the C-terminal tail of the second RING subunit acts as this additional Ub-binding component and, in CBLB, phosphorylation of LHR Tyr363 creates a phosphoTyr-induced element adjacent to the RING domain that functionally mimics the dimeric RING E3 tail in stabilising Ub. Both Ub-binding components optimise k_{cat} and K_m for Ub transfer. These results demonstrate that as well as Ub-E2, Ub-RING and E2-RING interactions, an additional Ub-binding component outside the RING domain is essential in stabilising the donor Ub configuration, restraining the globular Ub into a closed conformation and allowing optimal positioning of Ub's C-terminus for transfer (Dou et al., Nat Struct Mol Biol 2012; 19: 876; Dou et al., Nat Struct Mol Biol 2013; 20: 982).

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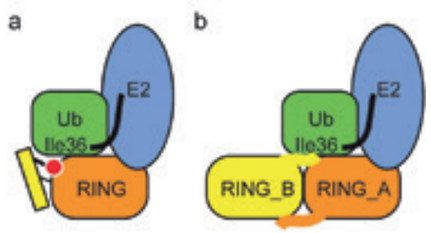

Figure 3
Requirements for E2~Ub activation for transfer by RING E3s. An additional component (highlighted in yellow) outside the RING domain (orange) is required for stabilisation of Ub by CBL (a) and by the dimeric RING E3s (b).

PROSTATE CANCER BIOLOGY

www.beatson.gla.ac.uk/hing_leung

Group Leader
Hing Leung

Clinical Lecturers
Imran Ahmad
Prabhakar Rajan[1]

Associate Scientist
Rachana Patel

Research Scientist
Yan Zhou

Scientific Officer
Janis Fleming

Technicians/Research Associates
Carolyn Loveridge
Jacqueline Stockley

Clinical Research Fellows
Lisa Bashford
Sarah Slater[2]

Graduate Student
Elspeth Brzezinska

[1] CRUK Clinician Scientist
[2] MRC

Prostate cancer remains a global health issue and a major cause of morbidity and mortality in men worldwide. We continue our translational research for developing new treatments for prostate cancer in the context of individualised medicine. Our research programme builds on ongoing work on aberrant cellular signalling (including androgen receptor, Sprouty2 and ERK5). In the past 12 months, we initiated new projects complimentary to existing pipelines to enrich our platform for targeted therapy. We will highlight some of our recent findings to illustrate our progress and direction of research.

Analysis of the transcriptome in lethal prostate cancer

Many men with prostate cancer have 'incurable' (locally advanced or metastatic) disease at diagnosis and a poor prognosis despite treatment. This observation may be due, in part, to the functional complexity of the cancer cell transcriptome primarily as a result of alternative mRNA isoforms, fusion transcripts and non-coding RNAs. In addition to expression changes, metastasis may be mediated by structurally or functionally differing protein isoforms produced by alternative pre-mRNA splicing (AS), controlled by RNA-binding proteins (RBPs). Our and others' work has implicated interacting RBPs Sam68 and hnRNP A2/B1 in prostate cancer, epithelial-mesenchymal transition (EMT) and metastasis (Fig. 1). We are examining the role of Sam68 and hnRNP A2/B1 in EMT and metastasis using prostate cancer cell lines, preclinical models, and primary tumours and metastases from patients with prostate cancer. In particular, we are interested to know how changes in expression of Sam68 and hnRNP A2/B1 proteins affect the cancer cell transcriptome during prostate cancer progression. Our work uses next generation sequencing (NGS) and will enumerate RBP target mRNA isoforms in prostate cancer metastasis, furthering our understanding of RBP-driven metastasis. We hope that mRNA targets identified in this study will help to delineate therapeutic strategies for 'incurable' prostate cancer in clinical trials.

In vivo sleeping beauty (SB) transposon screen to identify novel genes interacting with *Pten* loss in prostate tumourigenesis

Mutations in the tumour suppressor *PTEN* have been associated with the development and progression of clinical prostate cancer. Transgenic mice carrying homozygous deletion in *Pten* develop prostate cancer after a long latency (> 6 months) suggesting that additional genetic 'events' are required. We wish to discover the novel genetic events that cooperate with *Pten* loss to drive prostate cancer using a functional forward genetic screen in a transgenic mouse model.

In collaboration with Owen Sansom and Dave Adams (Sanger Centre, Cambridge), male mice with the appropriate SB genotype: *SB:Pten*^{Null} (*PB-Cre4:Pten*$^{fl/fl}$*T2Onc3/+Uren1/+*) and littermate controls [*Pten*^{Null} (*PB-Cre4:Pten*$^{fl/fl}$ and *SB*^{Control} (*PB-Cre4:T2Onc3/+Uren1/+*)] were generated and aged to study prostate tumourigenesis. *SB:Pten*^{Null} mice exhibited significantly accelerated prostate tumourigenesis, demonstrating larger tumours and enhanced metastasis compared to *Pten*^{Null} controls. We are now studying whether the identified transposon integration sites represent genes (or pathways) involved as 'driver events' in accelerating prostate carcinogenesis. Once these validation experiments are completed, we can then focus on genes with potential as novel therapeutic targets.

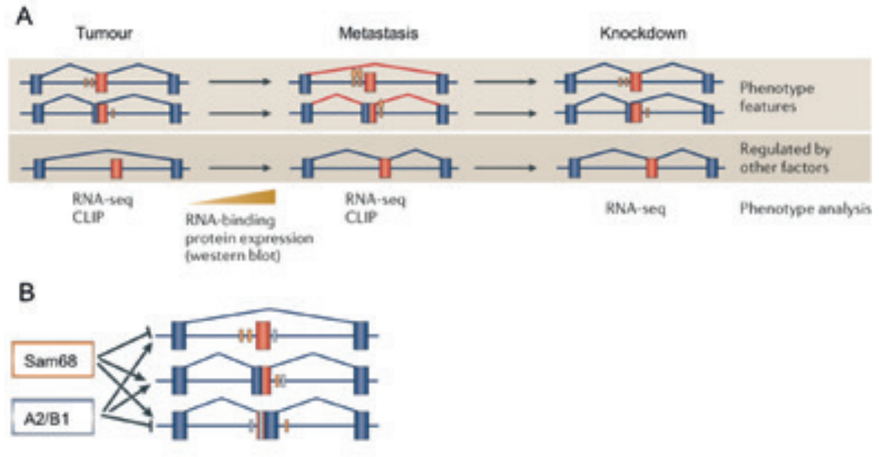

Figure 1
NGS approaches to identify RBP-regulated AS in metastasis (adapted from Kalsotra & Cooper, Nat Rev Gen 2011). (A) RBPs (orange ovals) are overexpressed in metastases and bind cis-elements (orange boxes) within a number pre-mRNAs resulting in metastasis-specific AS. Computational analysis of whole transcriptome CLIP (*in vivo* RBP/RNA UV crosslinking and RNA immunoprecipitation) and mRNA-Seq datasets differentiates directly regulated AS events from secondary effects. Knockdown of RBPs affect the metastatic phenotype. (B) Individual splicing events are regulated by the cooperative or antagonistic actions of RBPs, e.g. Sam68 and hnRNP A2/B1.

Understanding host-tumour interactions

Carcinomatosis, cancer-associated cachexia and multi-organ failure are common developments during prostate cancer progression (including treatment resistant disease). Tumour cells proliferate and spread by exploiting their tissue microenvironment. However, it remains to be tested whether tumours fuel their growth by modulating other (or distant) organs. Understanding such 'parasitic' modes of tumour growth may help the design of therapies that maximise anti-tumour effects while minimising host toxicity.

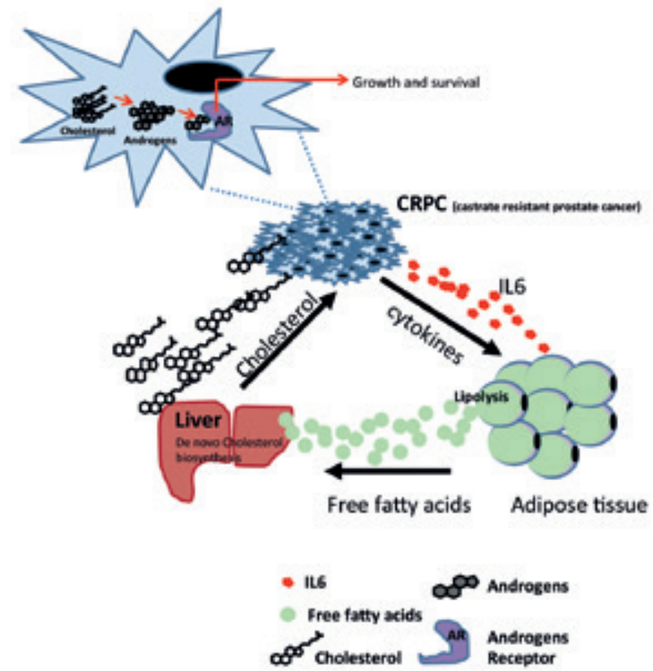

Figure 2
IL6 as a mediator for host-tumour interactions to drive tumour progression.

Androgen deprivation therapy (ADT) is the standard treatment for locally advanced and metastatic prostate cancer. We have established a range of *in vitro* and *in vivo* models for prostate cancer and studied ADT mediated effects. We found that prostate cancer responds to the therapeutic stress upon ADT by enhanced cytokine production, such as IL6, through HER2 receptor tyrosine kinase signalling. The paracrine action of IL6 renders the host cachectic by inducing lipolysis in adipose tissue, resulting in elevated levels of circulating cholesterol and free fatty acids. Intriguingly, despite ADT, sustained prostate cancer growth arises from self-sufficiency for androgens due to a combination of increased expression of steroid biosynthetic enzymes from IL6-mediated autocrine function and enhanced cholesterol uptake required for *de novo* intracellular synthesis of androgen (Fig. 2). In other words, tumours use cytokines to modulate distant adipose tissue to 'fuel' tumour growth and survival. Hence, our ongoing efforts focus on designing therapeutic strategies to target and disrupt this communication at multiple levels, which may ultimately (i) decrease treatment resistance and metastatic progression of the cancer and (ii) stall the detrimental effects, such as cachexia, on the host.

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

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TUMOUR CELL DEATH

www.beatson.gla.ac.uk/kevin_ryan

Group Leader

Kevin Ryan

Research Scientists

Alice Baudot[1]
Damian Graczyk[2]
Sanket Joshi[3]
Jaclyn Long[1]
Robin Macintosh[4]

Scientific Officer

James O'Prey

Clinical Research Fellow

Mathias Rosenfeldt [4]

Graduate Students

Yu Liu[5]
Michaela Mrschtik
Pablo Sierra Gonzalez[6]

Visitor

Pepijn Schoonen

[1] AICR
[2] EMBO
[3] until December
[4] until September
[5] until October
[6] from October

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

Metabolic cell death priming downstream of p53

The p53 tumour suppressor is a major cell death regulator. Recently, several studies have shown that p53 can also respond to and can modulate cellular metabolism. We reasoned therefore that the ability of a cell to respond to changes in metabolism might be connected via p53 to the eradication of stressed cells by programmed cell death. As a result, we searched microarray screens for genes that were activated by p53 and that may act as a bridge between tumour-associated metabolic changes and programmed cell death. This analysis resulted in the identification of the adenosine receptor, *ADORA2B* as a new p53 target gene.

ADORA2B encodes A2B, which is one of four human cell surface receptors that are activated by adenosine, the backbone of ATP. Extracellular adenosine accumulates under conditions of metabolic stress such as hypoxia and it is known that the extracellular space of many solid tumours contains unusually high levels of adenosine. In line with our idea that A2B might be a metabolic stress sensor, we found that treatment of A2B-expressing cells with adenosine analogues or exposure of these cells to hypoxia, resulted in a potent apoptotic response. This apoptotic response involved downregulation of anti-apoptotic members of the Bcl-2 family permitting release of apoptotic factors from mitochondria and programmed cell death.

We considered that chemotherapeutic drugs may cause activation of *ADORA2B* and this would cause cell death in situations of metabolic stress. To our surprise, however, we found that cisplatin not only causes activation of *ADORA2B* but also causes accumulation of extracellular adenosine. Importantly, further investigation revealed that a major component of p53-dependent cell death in response to cisplatin was actually mediated via A2B signalling (Fig. 1). These studies therefore identify a new cell death pathway downstream of p53 and provide considerable insight into the mechanism of action of this important chemotherapeutic drug.

E2F1 mediates drug resistance via ABCG2

The E2F1 transcription factor is a regulator of cell cycle progression and is frequently deregulated in human cancer. In addition to its role in cell cycle control, E2F1 has been reported to be a promoter of programmed cell death. To investigate E2F1 function further we performed microarrays screens to identify new E2F1 target genes. To our surprise, the gene that underwent the greatest fold induction upon E2F1 activation was the ABC family transporter, *ABCG2*. ABCG2 can efflux natural compounds and a broad spectrum of chemotherapeutic drugs. It is one of the three major drug transporters that has been implicated in human cancer and is considered to be a mediator of drug resistance in multiple settings.

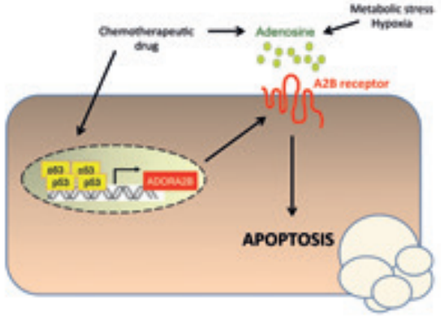

Figure 1
p53-mediated activation of A2B primes cells to die in response to accumulation of extracellular adenosine. Cellular stress and certain chemotherapeutic drugs cause accumulation of extracellular adenosine that results in cell death in the presence of the adenosine receptor, A2B. Since tumour cells often display altered metabolism and accumulate extracellular adenosine, our findings represents a novel link between cancer-related metabolic alterations and tumour cell death.

Subsequent analysis of the relationship between E2F1 and ABCG2 indicated that E2F1 expression causes elevation of ABCG2 in a variety of primary and tumour-derived cell lines from a variety of tissues. Moreover, we were able to identify an E2F1-responsive element within the *ABCG2* promoter and chromatin immunoprecipitations revealed that a site within the promoter was indeed bound by E2F1. Our analysis of tissue microarrays from human lung cancer also revealed that there was a highly significant correlation between high E2F1 and ABCG2 levels in this very common form of cancer.

Since ABCG2 is a multidrug transporter, we questioned whether E2F1 could induce drug efflux and if so, whether this effect was in a concentration dependent manner. Our studies revealed that E2F1 could induce drug efflux in a variety of cells and that this effect was completely reversed by either chemical inhibition or RNAi-mediated knockdown of ABCG2 (Fig. 2). Moreover, together with Christine Dufès (University of Strathclyde), we were able to show that activation of E2F1 in tumour xenografts caused drug efflux *in vivo* indicating that E2F1 activation in tumours could theoretically diminish the availability and efficacy of a systemically administered chemotherapeutic drug. Importantly, we also found that activation of E2F1 could markedly reduce the amount of cell death caused by the chemotherapeutic drug, mitoxantrone and that this effect could be reversed by inhibition of ABCG2 (Fig. 2).

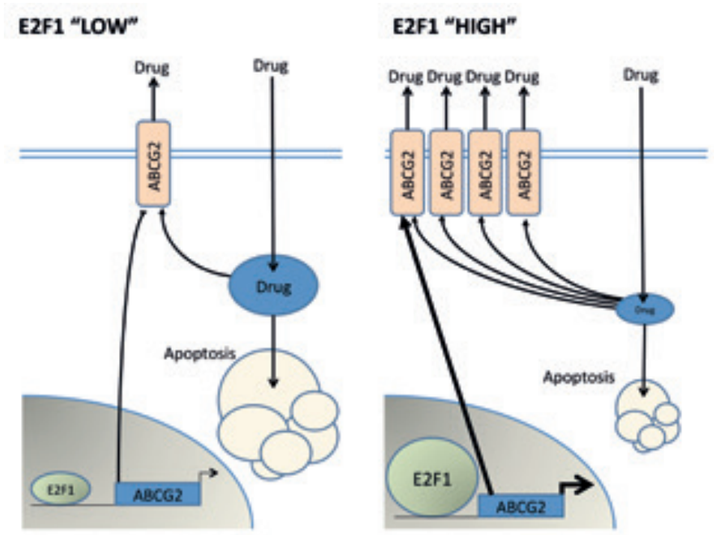

Figure 2
E2F1 activates ABCG2 leading to chemotherapaeutic drug efflux and decreased cell death. E2F1 activates expression of the multidrug transporter *ABCG2* via direct promoter binding. Higher levels of E2F1 result in higher levels of ABCG2, increased drug efflux and reduced cell death.

p53 status switches the role of autophagy in tumour development

Macroautophagy (hereafter autophagy) is a membrane trafficking process that delivers cellular constituents to lysosomes for degradation. It is a major homeostatic mechanism within cells and is a very adaptable process responding to the needs of the cell in response to various forms of cellular stress. Autophagy is a major regulator of cell viability and has been shown to have both oncogenic and tumour suppressive effects.

In order to target autophagy therapeutically, we considered that it is important to know where and when autophagy is either oncogenic or tumour suppressive in each tumour type. Therefore, together with Jen Morton and Owen Sansom, we chose to examine the role played by autophagy in a mouse model of pancreatic ductal adenocarcinoma (PDAC) that is characterised by activation of oncogenic *Kras*. In otherwise wild-type mice, the presence of oncogenic *Kras* in the pancreas led to development of PDAC over time. In the absence of autophagy, however, oncogenic *Kras* caused a marked increase in pre-cancerous lesions but progression to PDAC was blocked. By contrast, upon additional deletion of *p53* in the pancreas, tumours now not only formed in the absence of autophagy but they appeared at an earlier time when compared to autophagy proficient mice. The loss of p53 therefore uncovers a tumour suppressive role for autophagy and the tumours in these mice exhibited enhanced glucose uptake. Metabolic analysis of tumour-derived cell lines (undertaken with the help of Gillian Mackay and Eyal Gottlieb's group), revealed that this enhanced glucose uptake was accompanied by increased steady-state pools of glycolytic and pentose phosphate pathway intermediates that are known to fuel tumour growth. These findings therefore increase our understanding of the role of autophagy in cancer and provide important consideration for the use of autophagy modulators in cancer therapy.

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TUMOUR SUPPRESSION

www.beatson.gla.ac.uk/karen_vousden

Group Leader

Karen Vousden CBE, FRS, FRSE, FMedSci

Associate Scientist

Eric Cheung[1]

Research Scientists

Celia Berkers[2, 5]
Andreas Hock
Flore Kruiswijk[6]
Christiaan Labuschange[1, 7]
Oliver Maddocks[1]
Inbal Mor[1]
Patricia Muller[3, 8]
Antonio Trinidad[3, 9]

Associate Clinical Scientist

Patricia Roxburgh

Scientific Officer

Robert Ludwig

Graduate Student

Pearl Lee[4]

[1] ERC
[2] Rubicon
[3] AICR
[4] MRC
[5] until August
[6] from September
[7] from August
[8] until June
[9] until December

We have continued with our studies of how wild-type p53 helps cells to adapt to metabolic stress. These functions of p53, or p53-induced proteins such as TIGAR, make a contribution to the response of cells to stress such as nutrient or oxygen starvation, challenges that are faced by most developing tumour cells. We are beginning to translate these new insights into models of cancer growth *in vivo*, with the goal of testing new therapeutic strategies.

Survival and adaptation to metabolic stress

The pro-survival functions of p53 are likely to represent an important component of the p53 response, not only in the regulation of cancer development but also in other aspects of health and disease, e.g. metabolic homeostasis, ageing and diabetes. Previous work showed that the presence of wild-type p53 allows cells to survive under conditions of glucose limitation and we found over the past year that lack of p53 makes cells more vulnerable to death in response to serine depletion.

We showed that cancer cells rapidly utilise exogenous serine, significant amounts of which are used in the synthesis of glutathione and nucleotides. Serine deprivation triggered activation of the serine synthesis pathway (SSP) in both wild-type p53 expressing and p53 null cells. In collaboration with Eyal Gottlieb's group, we found that serine is an activator of PKM2, an effect that helps to maintain glycolytic flux. Reduction in intracellular serine levels resulted in rapid suppression of aerobic glycolysis and thus increased flux to the TCA cycle and SSP. These initial responses to serine starvation were not dependent on p53. However, serine depletion resulted in activation of the p53-p21 response, and the subsequent cell cycle arrest was found to be necessary for the promotion of cell survival, efficiently channelling depleted serine stores to glutathione synthesis and preserving cellular antioxidant capacity. Cells lacking p53 failed to complete the response to serine depletion, resulting in oxidative stress, reduced viability and severely impaired proliferation. The role of p53 in supporting cancer cell proliferation under serine starvation was translated to an *in vivo* model, where tumours lacking p53 developed significantly more slowly than p53-proficient tumours under conditions of serine limitation. Our studies also demonstrated that serine uptake supports the Warburg effect and that a serine deficient diet significantly enhanced the survival of tumour bearing mice. These results therefore suggest that serine depletion may have a potential role in anti-tumour therapy, particularly in cells lacking p53, and future studies will focus on examining the effects of serine starvation on the development of cancer in various genetically modified models.

Functions of TIGAR in supporting tissue regeneration and cancer development

To explore the importance of TIGAR activity in the context of a whole animal, we generated both constitutive and conditional deletions of *TIGAR* in mice. We showed that TIGAR is dispensable for normal growth and development in mice but plays a key role in allowing intestinal regeneration (Fig 1). Following ablation of the intestinal epithelium through whole body irradiation or genotoxic stress, mice deficient for TIGAR showed reduced regenerative capacity in their intestinal crypts. Similarly, in a model of ulcerative colitis in the colon, mice that were deficient for TIGAR showed poorer recovery. As seen in cultured cells, loss of TIGAR expression was accompanied by an increase in ROS. Further investigation using an *in vitro* intestinal crypt culture model showed that organoids lacking TIGAR were less able to form crypt structures in a three-dimensional tissue culture model.

These defects in *TIGAR*$^{-/-}$ cells could be rescued following the addition of nucleosides or the antioxidant N-acetyl L-cysteine (NAC), suggesting that TIGAR acts to provide antioxidants and nucleosides for intestinal growth. In an intestinal adenoma model where *Apc* is deleted in LGR5^{+} intestinal stem cells, mice deficient in TIGAR showed a reduction in total tumour burden and average tumour size in the small intestine compared to wild-type mice (Fig. 1). TIGAR is also highly expressed in these adenomas when compared to the surrounding normal tissue, supporting the importance of TIGAR in proliferating tissue. A similar contribution of TIGAR to tumour progression was also observed in the colon and importantly, the decrease in tumour burden observed in TIGAR deficient mice correlated with a greater survival in these animals. *In vitro*, the defective growth of TIGAR null tumour crypts could also be rescued with antioxidants and nucleosides. The pentose phosphate pathway (PPP) has been shown to be of particular importance in redox homeostasis under hypoxic conditions, and TIGAR deficient crypts were found to be more sensitive to hypoxia than wild-type ones. While the ability of TIGAR to promote tumour development might appear counterintuitive to its function in the p53 tumour suppressor pathway, it is important to note that in tumour cells overexpressing TIGAR, expression of TIGAR is uncoupled from p53 expression. Indeed, closer analysis in tumour cell lines showed that the basal expression of TIGAR is not dependent on the maintenance of wild-type p53. The ability of a p53 target protein to become oncogenic when no longer properly controlled has also been described for other mediators of the p53 survival response, such as carnitine palmitoyltransferase 1C. Understanding how these genes are regulated will be critical in determining their role in cancer development.

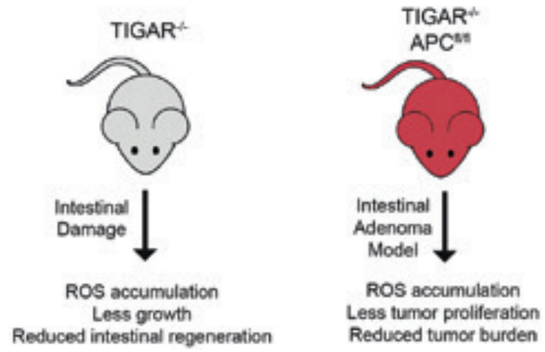

Figure 1
Loss of TIGAR leads to ROS accumulation and defects in proliferation that either reduce regeneration of damaged intestinal epithelium (LHS) or lower tumour burden in an intestinal adenoma model (RHS). Figure adapted from Cheung *et al.*, Dev Cell 2013.

Control of p53 conformation by CCT binding

The activity of p53 and whether it functions as a tumour suppressor or oncogene appears to be determined, to some extent, by the conformation adopted by the protein. Tumour derived point mutations in *p53* lead to the expression of a conformationally altered protein that has acquired invasive and metastatic function. We found that wild-type p53 binds to the chaperonin CCT and that this interaction promotes the folding of the p53 protein. Inhibition of this interaction, by either depletion of the CCT complex or mutation of p53 to prevent binding, resulted in the expression of misfolded p53, which adopts a conformation similar to that seen in tumour derived mutants. This misfolding was accompanied by a reduction in p53-dependent gene expression and the acquisition of an ability to promote invasion. These studies show that the interaction of p53 with the CCT complex is important to promote the tumour suppressor functions of p53 and that a failure to bind CCT promotes the oncogenic activity of wild-type p53. It therefore seems possible that one facet of the control of a normal p53 response is through regulation of protein conformation and that modifications that prevent p53/CCT interactions may allow the cell to toggle between the different activities of p53. It will be very interesting to determine whether the binding of p53 to CCT is a regulated step and which signals mediate this event.

Functions of mutant p53

In a continuation of our analysis of how mutant p53s function to drive invasion and metastasis, we have examined in more detail the importance of the interaction of mutant p53 with the p53 family member, TAp63. Our previous *in vitro* studies strongly suggested that the ability of mutant p53 to inhibit p63 was important in driving the gain of function, and so we carried out studies *in vivo* to assess the consequences of loss of TAp63 in the context of loss of p53. This work showed that loss of both TAp63 and p53 recapitulated the effect of mutant p53 expression in promoting the development of metastatic tumours. However, the incidence of metastasis remained lower in the TAp63/p53 null tumours than in wild-type p53 expressing ones, indicating that mutant p53 also provides other functions that drive an invasive phenotype. Further evidence for this model was provided by our identification of Dicer as a target for mutant p53. Dicer is a key component in the processing of microRNAs and previous studies have shown that reduction of its activity can promote metastasis. We found that the expression of mutant p53 downregulated Dicer expression through both direct inhibition of TAp53 but also through TAp63-independent mechanisms. Our studies reveal a further component of the pathway through which mutant p53 can function and also support previous evidence showing that mutant p53s act through TAp63-dependent and independent mechanisms.

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REGULATION OF CANCER CELL INVASION AND METASTASIS

CANCER RESEARCH UK BEATSON INSTITUTE

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

TUMOUR CELL MIGRATION

www.beatson.gla.ac.uk/research/kurt_anderson

Group Leader

Kurt Anderson

Research Scientists
Shereen Kadir
Ross McQueenie

Scientific Officer
Juliane Schwarz

Graduate Students
Orchi Anannya[2]
Zahra Erami Rud Majani
Max Nobis
Meg Pajak[1]

[1] Bruker
[2] from October

Our work is focused on development of imaging approaches to study the cellular and molecular dynamics of metastasis *in vitro* and *in vivo*. Metastasis is linked to mortality in most epithelial cancers. Metastatic invasion is challenging to study because it occurs randomly over large scales of time and space, and sensitively depends on features of the local tumour microenvironment.

Our goal is to develop mechanistic readouts of cell migration and apply them to mouse cancer models including pancreatic ductal adenocarcinoma (PDAC), melanoma and breast. We were the first group to apply fluorescence recovery after photo-bleaching (FRAP) in mice to study the dynamics of the cell adhesion tumour suppressor E-cadherin in tumour cell migration. More recently we have demonstrated the first *in vivo* use of fluorescence lifetime imaging (FLIM) to study the activation and response to therapy of the small GTPase Rho and the non-receptor tyrosine kinase Src during mutant p53-driven invasion of pancreatic cancer cells. We are also developing the preclinical use of PET, SPECT and CT.

Preclinical imaging

We recently acquired an Albira (Bruker) tri-modal scanner for PET, SPECT and CT imaging of mice, extending our imaging range from subcellular molecular dynamics to whole body molecular imaging. This new work is being undertaken in close collaboration with colleagues from NHS Greater Glasgow and Clyde, including Gerry Gillen and the clinical team from the Glasgow PET Centre, Jonathan Owen from the Gartnavel Cyclotron and Sally Pimlott from the Radiopharmaceutical Dispensary. Our goal is to develop the use of preclinical imaging, both as an investigative tool for the study of disease and response to therapy and as an operational tool for staging disease progression, in order to increase the translational utility of our mouse cancer models. Initial studies have focused on using FDG to measure glucose uptake and FLT to measure proliferation, primarily in pancreatic and prostate tumour models. A significant milestone was passed this year with the publication of our first PET data showing enhanced FDG uptake in pancreatic tumours deficient for p53 and ATG7 (Fig. 1).

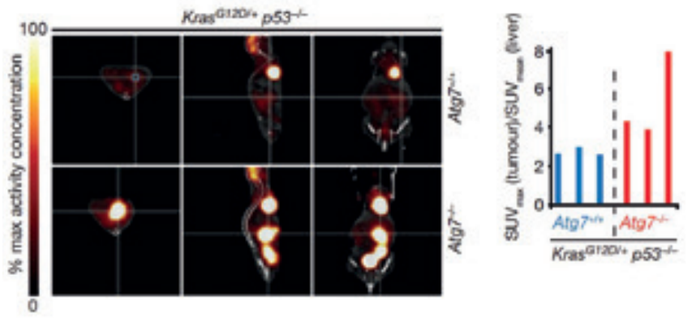

Figure 1
Merged PET and CT images showing enhanced FDG uptake in *Kras G12D/+ p53 -/-* mice in the absence of the essential autophagy gene *Atg7*. Quantification compares uptake in three mice of each genotype.

Intravital FLIM-FRET imaging of therapeutic Src inactivation

Pancreatic cancer is one of the most lethal forms of human cancer, with an overall 5-year survival rate of less than 5%. Initiating *KRAS* mutations occur in approximately 90% of human PDAC, while p53 mutations arise in 50-75% of human pancreatic cancer. Previous work has demonstrated that p53 mutation, rather than loss, can drive metastasis in a mouse model of pancreatic cancer. The metastatic effect of mutant p53 in this model can be substantially reversed by treatment with the clinically approved Src inhibitor dasatinib. We therefore investigated spatial and temporal aspects of Src inactivation by dasatinib using fluorescence lifetime imaging of fluorescence resonance energy transfer (FLIM-FRET). To do this we made use of a FRET reporter that changes conformation in response to phosphorylation by active Src.

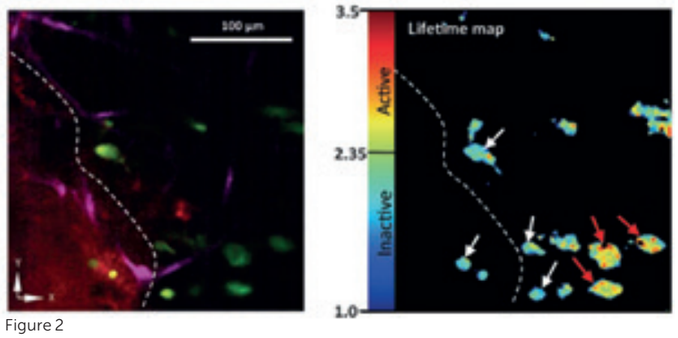

Figure 2

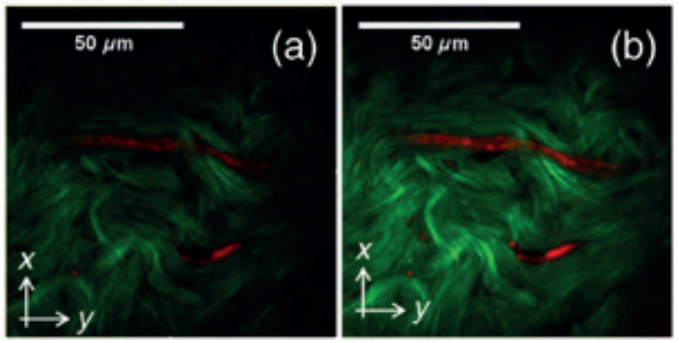

Figure 3

Figure 2
Intravital micrographs of PDAC cells expressing a Src FRET reporter grown as subcutaneous tumours. Intensity image (left) shows PDAC cells imaged using CFP (green), collagen I imaged using SHG (magenta), and vasculature imaged using Quantum Tracker 655 (red). Fluorescence lifetime image (right) shows that Src activity (colour coded according to intensity map on the left) increases with increasing distance from the edge of the nearest blood vessel (white dotted line).

Figure 3
Images of mouse skin showing ~2x gain in signal resulting from the use of adaptive optics. SHG from collagen I (green) and fluorescence from Quantum Dots (red) were imaged in mouse skin using either a flat (left) or optimised (right) mirror shape.

We first measured the level of Src activity in control and dasatinib treated PDAC cells, in order to establish the range of fluorescence lifetimes associated with high and low Src activity. This enabled us to classify individual cells as being Src active or inactive. Using this approach we found that following drug washout, cells in culture rebounded to a high level of Src activity, which peaked at 3 hours and returned to baseline by 6 hours. A similar phenomenon was observed when the same cells were grown subcutaneously in nude mice and subjected to 3 days of dasatinib treatment, however the rebound peak occurred at 16 hours and return to baseline was complete by 24 hours. To investigate spatial aspects of Src activation during invasion, PDAC cells expressing the Src FRET probe were plated onto organotypic cultures derived from acid extracted collagen I. After 6 days, cells had invaded over 120 µm into the matrix. Interestingly, we observed a gradient of Src activation within the invading cells: the percentage of Src active cells increased with increasing invasion depth. When the same cells were grown subcutaneously, we observed there were significantly fewer Src active cells in central tumour regions compared to the tumour margins. Dasatinib treatment did not alter the fraction of Src active cells in the tumour core but significantly reduced the fraction of those at the tumour margin, i.e. the region where local tissue invasion occurs. Finally, we examined response to dasatinib treatment as a function of proximity to vasculature (Fig. 2). We found that over a range of ~100 µm the fraction of Src active cells increased with increasing distance from the nearest blood vessel, with about 90% of cells classified as Src active over 100 µm. Dasatinib treatment significantly reduced the fraction of Src active cells at all of the distances measured, although the effects were strongest for cells nearer to blood vessels.

Development of adaptive optics for deep tissue multiphoton imaging

Adaptive optics have the potential to increase signal intensity and spatial resolution by sharpening the focus of a laser beam used for multiphoton excitation deep within tissue. This approach can be use to correct for aberrations in focus introduced by both the optics of the imaging system and refractive index variations of the sample. In collaboration with Amanda Wright and Keith Mathieson (Institute of Photonics, University of Strathclyde), Caroline Müllenbroich spent six months working with Ewan McGhee on a fellowship funded by INSPIRE to develop an adaptive optics module for use on one of our LaVision TRIM microscopes. The module they developed was based on a deformable mirror placed in the OPO illumination pathway and imaged via a 4f system into the back-focal plane of the microscope objective. The mirror shape was determined using a random search algorithm that was optimised on the basis of signal intensity from the sample. Significantly, we found that mirror shapes optimised using one sample were transferrable to other samples, meaning that the lengthy optimisation procedure does not need to be performed prior to each imaging session. Also, the signal derived from collagen second harmonic generation (SHG) can be used in place of two-photon excited fluorescence as a signal for mirror optimisation. This is advantageous because unlike fluorescence, the second harmonic signal does not photo-bleach over time. As a result, signal improvements of between two and four times were achieved in a variety of samples, including organotypic cultures, zebrafish embryos, and freshly excised mouse skin and intestinal tissue (Fig. 3).

Publications listed on page 76

TRANSLATIONAL CANCER THERAPEUTICS

www.beatson.gla.ac.uk/jeff_evans

Group Leader
Jeff Evans

Associate Scientist
Jennifer Morton

Research Scientist
Amy Au[1]

Scientific Officer
Saadia Karim

[1] University of Glasgow

Work in our group is aimed at developing novel laboratory models that will allow us to understand the biological function of key tumour suppressor genes and oncogenes *in vivo*. We aim to identify and characterise the signalling pathways deregulated at different stages of pancreatic cancer and those that are potential therapeutic targets. Using these models, we will also determine how potential anti-cancer agents might best be evaluated in subsequent clinical trials.

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

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

Evaluation of putative anti-invasive therapies in pancreatic cancer models

One aim of our work is to determine how potentially anti-invasive agents might best be used in the clinical management of epithelial cancers. In our initial studies, we defined the mode of action of anti-cancer drugs that are currently in clinical evaluation and tested the hypothesis that these agents may have anti-migratory and hence anti-invasive and/or anti-metastatic properties. Initially, we used clinically relevant, pharmacologically active anti-cancer agents as experimental tools.

We demonstrated that Src kinase may be a relevant target for therapeutic intervention following resection of PDAC and that Src activity is upregulated during progression to invasive PDAC in the *Pdx1-Cre*, *Z/EGFP*, *LSL-Kras*$^{G12D/+}$, *LSL-Trp53*$^{R172H/+}$ model. We also showed that treatment with dasatinib, an inhibitor of Src, *in vitro* resulted in a dose-dependent inhibition of Src activity, and of migration and invasion of PDAC cells. Furthermore, we demonstrated that *in vivo* treatment with dasatinib from 10 weeks of age significantly reduced the number of mice with metastases compared with those treated with vehicle control. However, there was no improvement in survival when compared with vehicle control due to the morbidity of the primary tumour burden. Consequently, we are also exploring whether inhibition of metastases by dasatinib can be combined with local disease control of the primary tumour by gemcitabine, radiation therapy or both to improve survival in the mice with locally advanced, unresectable disease but without evidence of visible metastatic disease. Treatment with dasatinib in combination with twice-weekly injection of gemcitabine resulted in a further inhibition of metastatic spread compared with dasatinib treatment alone (11% exhibited metastasis compared with 33%). Most excitingly, however, we also observed that average survival was significantly increased by over 60 days. These observations have contributed to the development of a global, commercially sponsored clinical trial that aims to improve progression-free and overall survival in patients with locally advanced disease by inhibiting the development of metastases.

Currently, we are pursuing these observations further to determine if the administration of dasatinib, as monotherapy or in combination with gemcitabine, after potentially curative resection of the localised primary tumour can inhibit the development of metastases and therefore reduce or delay disease recurrence and improve relapse-free and overall survival. However, the *Pdx1-Cre*, *Z/EGFP*, *LSL-Kras*$^{G12D/+}$, *LSL-Trp53*$^{R172H/+}$ model develops multiple tumours throughout the pancreas making it difficult to accurately stage disease and achieve therapeutically effective tumour removal. We are now using a number of different approaches to address this problem, including generating an 'inducible' model (*Pdx1-Cre-ERT, KrasG12D, p53R172H or Elastase-CreER, KrasG12D, p53R172H*) in which we can alter the timing and efficiency of induction of genetic modifications thus targeting a smaller number of cells in the adult pancreas rather than generating tumour promoting genetic modifications in the pancreas at an embryonic stage as in the *Pdx1-Cre*, *Z/EGFP*, *LSL-Kras*$^{G12D/+}$, *LSL-Trp53*$^{R172H/+}$ model.

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

Biological function of key tumour suppressor genes and oncogenes in PDAC

We have also developed a number of novel models with a range of genetic backgrounds in collaboration with Owen Sansom's group, including those with targeted deletion of *Pten*, *Apc*, *Myc* or *Arf*. These models will help us understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours. They will also allow us to identify and characterise the signalling pathways that are deregulated at the early stages of pancreatic cancer, during the development and progression of the invasive and metastatic phenotype, and in advanced disease.

For example, we previously demonstrated that *Lkb1* haplo-insufficiency cooperates with *Kras*^{G12D} to cause PDAC. Mechanistically, we showed that LKB1 deficient KRAS^{G12D}-induced tumours exhibited reduced levels of the tumour suppressors p53 and p21, and we proposed that this reduction in p53 and p21 allows KRAS^{G12D} bearing cells to overcome a senescent barrier to tumour formation. Moreover, haplo-insufficiency for *p21* also synergised with *Kras*^{G12D} to drive PDAC. We have extended these observations to show that activation of the PI3K/Akt/mTOR pathway, which occurs in approximately 20% of patients, is associated with poor prognosis in human PDAC patients. Loss or deficiency of *Pten* (and consequent activation of Akt) abrogates Ras-induced senescence and leads to acceleration of PDAC progression in laboratory models.

Currently, we are using putative inhibitors of a number of pathways as clinically relevant tools to explore the activity of these agents in laboratory models with the relevant genetic background. Based on these observations, we are developing the concept of an adaptive design 'umbrella' clinical trial in which patients are recruited into multiple treatment arms based on their molecular profile. Critical to these approaches will be identifying potential biomarkers in murine models and confirming the potential clinical relevance of these in human tissue microarrays, and developing robust assays for patient selection to enrich the clinical trial population and to demonstrate a biological, as well as a clinical, anti-tumour effect.

Publications listed on page 77

CELL MIGRATION AND CHEMOTAXIS

www.beatson.gla.ac.uk/robert_insall

Group Leader

Robert Insall

Research Scientists

Xiaoli Du[2]
Jason King[3]
Jan Ohotski[4]
Olivia Susanto

Scientific Officer

Peter Thomason

Graduate Students

Clelia Amato[4]
José Batista[1]
Andrew Davidson
Yvette Koh[4]

[1] FCT
[2] until December
[3] until August
[4] from October

When tumours metastasise, the regulation of cell movement goes wrong - cancer cells invade other tissues, and spread through the blood and lymph systems to form secondary tumours. This spreading behaviour is one of the most feared features of cancer and a principal driver of death in patients. Despite this, we do not understand what makes cells move or what steers their direction. Our group aims to improve understanding using a wide range of models and techniques.

Cell movement is a central part of biology involved in embryo development, wound healing and immune responses. We are interested in two related processes. The first is chemotaxis, in which external signals orient and attract cells, and which is increasingly seen as a fundamental cause of metastasis. The second is the regulation of the Arp2/3 complex, an assembly of proteins that promotes movement by driving the formation of actin microfilaments.

Most mammalian cells use pseudopods made of polymerised actin to power migration. Our research focuses on the proteins and pathways that control these pseudopods. We use three approaches. For genetic studies we use *Dictyostelium*, taking advantage of its ease of manipulation, and prominent cell movement and chemotaxis. To apply our knowledge to cancer, we use melanoma cell lines as well as tumours from mouse models and, when possible, from patients. We also develop computational models of single cells in collaboration with the Mathematics Department, University of Strathclyde, and of populations of moving cells with the Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow. In the long term, we will work on anything that will help us to understand the conserved and fundamental mechanisms that drive cell movement.

Regulators of the Arp2/3 complex

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

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

Our experiments are currently focused on identifying what regulates each component of the complex. SCAR and the other complex members are phosphorylated at multiple sites but the biological significance of these is not understood. We have shown that control of SCAR phosphorylation is important – nearly all the cellular SCAR is heavily phosphorylated but a rare dephosphorylated form seems to be particularly significant. It is very active in extending pseudopods and very unstable, explaining its rarity. We are now seeking the phosphatases. Unexpectedly, we have also shown 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.

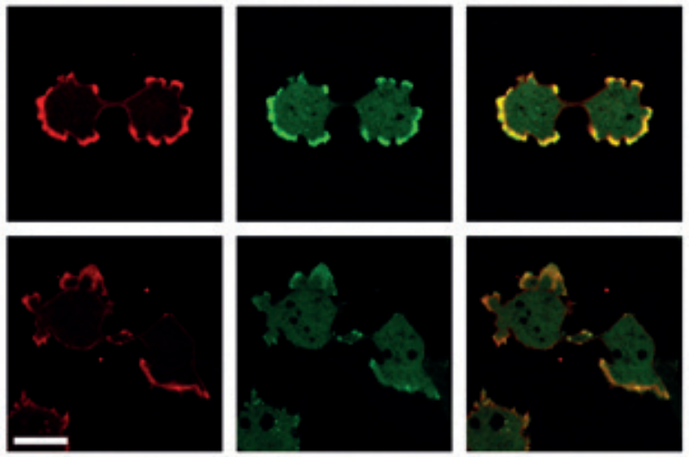

Figure 1
Cytokinesis in *Dictyostelium* cells lacking WASP. Red panels show F-actin visualised with RFP-lifeact and green panels show Arp2/3 complex. Normal cells (top) are well-organised and polarised away from the site of cell division. WASP mutants (bottom) are disorganised, with a pronounced midbody.

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

Mechanisms underlying chemotaxis

Chemotaxis is emerging as a major driver of tumour metastasis. We have shown that in *Dictyostelium* cells it works by a different mechanism than that which is usually described. Pseudopods are constantly generated in random directions, then the ones that point in the best directions are selected and maintained. We are now performing similar studies with cancer cells. We will use high-resolution, three-dimensional microscopy determine whether human tumour cells use the same mechanism as *Dictyostelium* for chemotaxis and where chemotactic signals originate. Parameters such as cell speed are widely used but are broadly irrelevant in our new model. Instead, we measure the rate at which pseudopods are made and change shape, the instantaneous velocity at which pseudopods move, and the regulation of pseudopod retraction. We also want to identify which proteins are used to regulate tumour cell chemotaxis and whether they are the same for all metastatic cells or if not, how broad the range is. It is now clear that there are at least two dissimilar mechanisms that drive tumour cell movement. We want to know whether there really are exactly two mechanisms or something more variable. Recent experiments suggest a particularly attractive model - that melanoma cells generate chemotactic gradients themselves, before migrating up them. Thus cells drive their own dispersal through chemotaxis. We have also used chemotaxis chambers to show that melanoma cells are exquisitely chemotactically sensitive. We are now working on the molecular details of the attractant in serum and the chemotactic receptors that detect it.

We are collaborating with the Mathematics Department, University of Strathclyde to make computational models representing moving cells. Our models already faithfully mimic some aspects of the movement of *Dictyostelium* cells. We are now using the model to test our predictions about the underlying mechanisms of chemotaxis, and the proteins that are involved. We are showing that chemotaxis is mostly likely mediated by several dissimilar mechanisms acting in parallel, including regulated pseudopod growth, pseudopod retraction and the control of adhesion. Our theory of self-generated gradients implies that cells behave in a similar way to herds of animals in the wild. We are therefore also collaborating with mathematical ecologists at the University of Glasgow to determine whether this comparison can yield useful predictions about cancer cell behaviour.

Publications listed on page 79

MIGRATION, INVASION AND METASTASIS

www.beatson.gla.ac.uk/laura_machesky

Group Leader
Laura Machesky

Research Scientists
Amelie Juin
Ben Tyrrell

Scientific Officer
Heather Spence

Clinical Research Fellows
Hayley Morris[1]
Richard Stevenson

Graduate Students
Loic Fort
Emma Woodham

Visitor
Emmy Borgmastars

[1] MRC

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

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

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

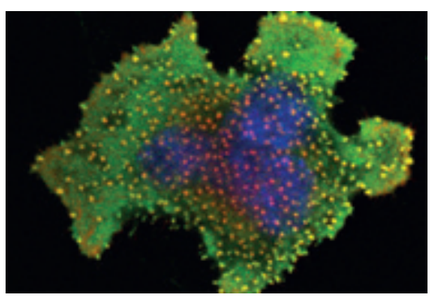

Figure 1
Mouse megakaryocyte labelled with phalloidin (actin filaments, red), ARPC2 antibody (Arp2/3 complex, green) and DAPI (DNA, blue). The prominent dots are podosomes. Photo by Hannah Schachtner.

Together with former postdoc Xinzi (Amber) Yu and scientific officer Heather Spence and in collaboration with Lynn McGarry and Emma Shanks (RNAi Screening), we completed a targeted screen for genes regulating invasion. We continue to validate and study several new targets involved in three-dimensional migration and invasion. The most promising will be taken forward into *in vivo* screens for invasion and metastasis. Amber has moved on to a postdoctoral position with Iain McNeish (University of Glasgow).

This year, we (student Hannah Schachtner and postdoc Simon Calaminus) published a study showing that the platelet precursor cells, megakaryocytes assemble actin-based podosomes that resemble invadopodia of cancer cells (Fig. 1 and Schachtner *et al.*, Blood 2013; 121: 2542). Megakaryocyte podosomes contain actin, Arp2/3 complex and WASP and they are used for adhesion, spreading and motility. Podosomes form an interconnected network in cells that can sense and respond to changes in the extracellular matrix environment. We also found that megakaryocyte podosomes could degrade matrix and protrude through

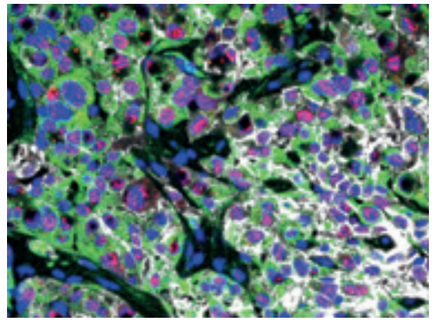

Figure 2
Expression of fascin in a well-differentiated mouse PDAC. Photo shows immunofluorescence staining of a murine tumour expressing *Pdx-1Cre KRasG12D* and $p53^{R172H}$. Fascin is green, E-cadherin is white, Slug is red and DNA is blue. Photo by Ang Li.

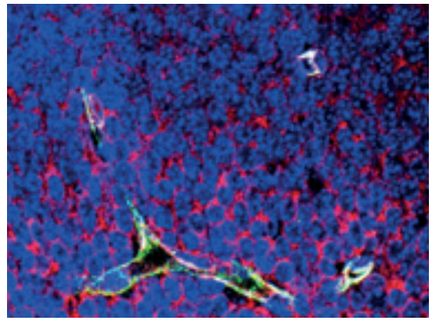

Figure 3
Hindbrain immunostain showing PECAM for blood vessels (green), fascin (red) and DNA (DAPI, blue) and co-localisation in white of fascin with endothelial cells. From Ma *et al.*, 2013. Photo by Ang Li.

native basement membranes. We speculate that megakaryocytes may use podosomes for extending proplatelet arms through the walls of sinusoids or blood vessels to release platelets into the bloodstream. In our review (Schachtner *et al.*, Cytoskeleton 2013; 70: 572), we discuss podosomes as mechanosensors and mediators of adhesion and matrix remodelling and the relationship between podosomes and cancer cell invadopodia. Hannah has taken up a postdoc position with Tanja Maritzen in Berlin, while Simon has moved on to a lectureship in Hull.

We also continued our studies of the role of WASH in actin networks of *Dictyostelium* cells (together with Robert Insall, Park *et al.*, Dev Cell 2013; 24: 169) and in breast cancer cells. Postdoc Tobias Zech found a potential connection between WASH and ERBB2 signalling, which we are studying further *in vitro* and *in vivo* with the help of a pilot grant from Breast Cancer Campaign. Tobias has recently taken up a lectureship at the University of Liverpool.

Role of actin regulatory proteins in colorectal and pancreatic cancer

Clinical research fellow Richard Stevenson identified a role of the actin bundling protein fascin in tumour formation in colorectal cancer models as well as a potential role in colitis. We started new projects with MRC funded clinical research fellow Hayley Morris on the role of N-WASP in colorectal cancer (in collaboration with Owen Sansom) and with postdoc Amelie Juin on the role of N-WASP in pancreatic ductal adenocarcinoma (with Jen Morton and Owen Sansom). We are interested to know whether the role of N-WASP in invadopodia translates into differences in tumour formation, progression and spread in these models.

We are also studying the role of fascin-1 in pancreatic ductal adenocarcinoma progression, a project initiated by former student Ang Li (now at Rockefeller University in New York) and now continued by Amelie Juin, Hayley Morris and students Emma Woodham and Loic Fort, and in collaboration with Jen Morton and Owen Sansom (Fig. 2). We found that fascin is a target of the epithelial to mesenchymal transition in pancreatic cancer and has a key role in early tumour formation as well as metastatic dissemination. Fascin mediates metastasis to the peritoneal cavity via the formation of actin-rich filopodia that enable cells to intercalate and migrate through mesothelial cell layers and seed a new tumour. Fascin also contributes to liver metastasis. This work complements our ongoing efforts to develop fascin inhibitor compounds together with Martin Drysdale (Drug Discovery).

Role of actin regulatory proteins in melanoblast migration and melanoma

We also started new projects with Emma Woodham and postdoc Ben Tyrrell on the role of Rho family GTPases in melanocyte migration. We previously showed that loss of *Rac1* causes major defects in melanoblast migration and proliferation during development (Li *et al.*, Dev Cell 2011; 21: 722) and now we are studying the roles of RhoA and Cdc42 in melanoblasts, together with Cord Brakebusch (University of Copenhagen, Denmark). With postdoc Yafeng Ma, we published this year that loss of fascin causes defects in melanoblast migration and proliferation that are subtler than loss of *Rac1* (Ma *et al.*, Development 2013; 140: 2203). Fascin is transiently expressed in murine melanoblasts during their migration and is lost later when they differentiate into melanocytes. Fascin is expressed in some melanomas but is not tightly correlated with stage or progression as it is in many epithelial cancers.

Fascin is also present in tumour stroma and is expressed by vascular endothelial cells, smooth muscle cells and pericytes. Despite the abundance of fascin in blood vessel walls, we found that transplanted B16 melanomas showed only slight differences in vascularisation when the host was a fascin knockout. Tumour growth was not affected by these small differences, making fascin dispensable for tumour angiogenesis. Likewise, developmental angiogenesis does not seem to be greatly affected by loss of fascin (Fig. 3 and Ma *et al.*, Biol Open 2013; 2: 1187).

Publications listed on page 81

INTEGRIN CELL BIOLOGY

www.beatson.gla.ac.uk/jim_norman

Group Leader

Jim Norman

Research Scientists
Joanna Birch
Jesica Diaz-Vera[1]
Emmanuel Dornier
Elena Rainero[2]
Peter van den Berghe

Associate Clinical Scientist
Iain MacPherson

Scientific Officer
Louise Mitchell

Graduate Students
Cassie Clark
Christine Gundry
Nikki Heath
Elena Ruengeler

[1] Fundacion Canaria Doctor Manuel Morales

[2] West of Scotland Women's Bowling Association

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

Use of phosphoproteomics to characterise RhoGTPase signalling downstream of mutant p53/RCP-dependent trafficking

We knew from our previous work that Rab-coupling protein (RCP) dependent recycling influences the way that α5β1 integrin signals to Rho subfamily GTPases. As RhoA signalling is likely important to dictating how RCP controls invasion, we used a phosphoproteomic approach to look at signalling events downstream of RCP trafficking. From this, we have identified a novel PKB/Akt substrate, RacGAP1, which is phosphorylated as a consequence of RCP-dependent α5β1 trafficking. Phosphorylation of RacGAP1 promotes its recruitment to IQGAP1 at the tips of invasive pseudopods. Live cell imaging experiments using FRET reporter probes for Rho subfamily GTPases showed that phospho-RacGAP1 locally suppresses the activity of Rac and promotes the activity of RhoA in this subcellular region (Fig. 1). This Rac to RhoA switch promotes extension of pseudopodial processes and invasive migration into the extracellular matrix, in a RhoA-dependent manner.

An endocytic pathway transporting ligand-occupied integrins from fibrillar adhesions to late endosomes supports lysosomal recruitment and activation of mTOR

Recycling of internalised integrin is known to occur at the front and back of migrating cells to coordinate membrane protrusion and retraction respectively but less is known about the spatio-temporal organisation of integrin endocytosis and the processes that this controls. We have developed a novel photoactivation-in-TIRF approach to show that ligand-bound integrins are internalised at fibrillar adhesions (but not at focal adhesions) located under the nucleus and are trafficked directly to nearby late endosomes/lysosomes without passage through early endosomes. This clathrin-independent internalisation route requires tensins-1, -2 and -3 and the class II Arf subfamily GTPase, Arf4. Suppression of tensins or Arf4 disrupts flow of ligand-bound integrins to late endosomes/lysosomes and their degradation within these compartments. Integrin internalisation from fibrillar adhesions is required for proper lysosome positioning, and recruitment and activation of mTOR at this cellular sub-compartment. These findings identify integrin endocytosis as a mechanism to support mTOR signalling and point to the extracellular matrix as a possible source of nutrients for invading cancer cells (Fig. 2).

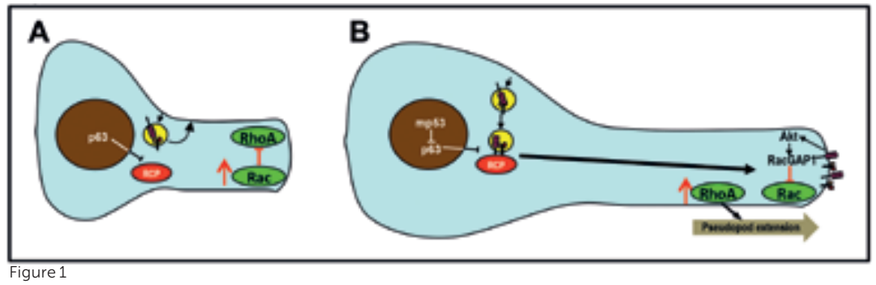

Figure 1

Figure 1
Influence of RCP-driven α5β1 recycling on localisation of RacGAP1 and downstream signalling to RhoGTPases. (A) In p53 null cells, p63 suppresses association of RCP with α5β1, so the integrin is not trafficked to the cell front and Rac signalling predominates at the leading edge. (B) Gain-of-function mutant p53 inhibits p63 to promote association of RCP with α5β1. Production of phosphatidic acid by DGKα within the tips of protruding pseudopods recruits RCP/α5β1/EGFR1 vesicles, and localises downstream signalling via Akt to this subcellular region. Here Akt phosphorylates RacGAP1, allowing inactivation of Rac and activation of RhoA to promote pseudopod extension and invasion.

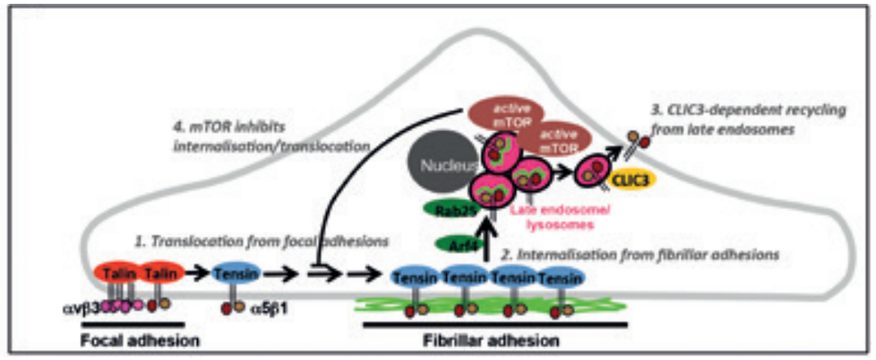

Figure 2

Figure 2
Schematic diagram depicting the relationship between trafficking of α5β1 and mTOR activation at late endosomes/lysosomes.

Use of SILAC mass spectrometry to identify new components of the invasive 'recyclome'

We have used gene expression arrays to identify *RAB17* as a gene whose expression must be suppressed in order for MAP kinase signalling pathways to drive invasive migration. To identify which of the receptor cargoes of RAB17 are responsible for its ability to oppose tumour cell invasion, we have developed a novel SILAC (stable isotope labelling in cell culture) approach. By using this quantitative mass spectrometry approach in combination with membrane purification methods, we are able to compare the distribution of proteins between the plasma membrane and recycling endosomes under control conditions and when RAB17 levels are suppressed. By careful analysis of peptides from proteins that are differentially trafficked, we have identified VAMP8 as a novel effector of RAB17 and have gone on to show that RAB17/VAMP8 influences invasion by controlling trafficking of neuropilin 2 (NRP2). Thus, we have developed a new mass spectrometry approach that is capable of identifying novel effectors and cargoes of Rab GTPases that function during cancer invasion. We anticipate that further characterisation of the invasive 'recyclome' will identify more new receptor cargoes that are key to invasion, and that these may represent new targets at which to aim anti-cancer drugs.

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MOLECULAR CELL BIOLOGY

www.beatson.gla.ac.uk/michael_olson

Group Leader
Michael Olson

Research Scientists
Mads Gabrielsen[1]
Felipe Lourenco
Katerina Mardilovich[1]
Nicola Rath
Mathieu Unbekandt[2]
Serge Zander

Scientific Officer
June Munro

Clinical Research Fellow
Jennifer Brown[3]

Graduate Students
Jenifer Cameron[4]
Linda Julian
Dominika Rudzka

Visiting Students
Laura Bello Figueroa
Dominika Kowalczyk
Haneela Qadir
Maike Schuldt
Stephanie Schumacher

[1] CRUK Discovery Committee
[2] MRC DPFS
[3] CRUK Glasgow Centre
[4] until October

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

Crystal structure of human cofilin 1

The actin cytoskeleton is the chassis that gives a cell its shape and structure, and supplies the power for numerous dynamic processes including motility, endocytosis, intracellular transport and division. To perform these activities, the cytoskeleton undergoes constant remodelling and reorganisation. One of the major actin remodelling families is the cofilin family of proteins, made up of cofilin 1, cofilin 2 and actin depolymerising factor (ADF), that sever aged ADP-associated actin filaments to reduce filament length and provide new potential nucleation sites. Despite the significant interest in cofilin as a central node in actin cytoskeleton dynamics, to date the only forms of cofilin for which crystal structures have been solved are from the yeast, Chromalveolata and plant kingdoms; none have previously been reported for an animal cofilin protein. Two distinct regions in animal cofilin are significantly larger than in the forms previously crystallised, suggesting that they would be uniquely organised. Therefore, we sought to determine the structure of human cofilin 1 by X-ray crystallography to elucidate how it could interact with and regulate dynamic actin cytoskeletal structures. Although wild-type human cofilin 1 proved to be recalcitrant, a C147A point mutant yielded crystals that diffracted to 2.8 Å resolution. These studies revealed how the actin binding helix undergoes a conformational change that increases the number of potential hydrogen bonds available for substrate binding.

Regulation of cofilin 1 activity by cysteine oxidation

Cell migration is essential for many processes including embryonic development, wound healing and inflammation. It is also involved in the local invasion and metastasis of cancer cells. In order for cells to migrate the actin-myosin cytoskeleton must be constantly rearranged, which is mediated by a number of proteins that act downstream of the Rho GTPases. These proteins are tightly regulated both spatially and temporally to ensure that cell migration occurs at the appropriate time and in the correct direction. One of the proteins that has a central role is the actin binding protein, cofilin 1. Cofilin 1 has been demonstrated to increase the rate of actin monomer (G-actin) dissociation from the pointed end of filamentous actin (F-actin) and to sever F-actin. Additionally, at high concentrations cofilin can promote the nucleation of actin filaments and actin rod formation. A further post-translational modification that can alter cofilin function is cysteine oxidation, which predominantly results in the establishment of disulphide bonds.

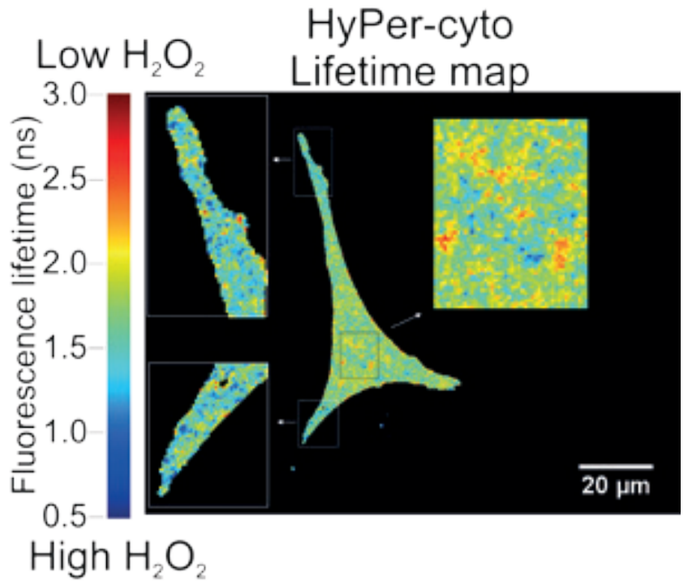

Figure 1
Hydrogen peroxide is generated in protrusions during cell movement. Fluorescence lifetime imaging was used to detect changes in the optical properties of the hydrogen peroxide biosensor probe HyPer-cyto. Subcellular changes in fluorescence lifetime are indicated by the heat map, with magnitudes of these changes indicated in nanoseconds (ns) on the left. Boxed regions show higher magnification of hydrogen peroxide being produced in cell protrusions, relative to lower hydrogen peroxide in the cell body.

We found that H_2O_2 levels are elevated in migrating relative to stationary cells with the highest levels being observed at cell protrusions. In addition, protein oxidation is increased in migrating compared to stationary cells and we identified cofilin as one of these oxidised proteins. Moreover, the oxidation of cofilin inhibits its ability to decrease F-actin levels and this is dependent on cysteine residues 139 and 147. The oxidation of these cysteine residues is also required for cell adhesion and directional migration. Taken together these results provide a direct link between the increased production of reactive oxygen species in cell protrusions and dynamic changes in the actin cytoskeleton that take place in this region to enable cell migration.

LIM kinases in prostate cancer

Prostate cancer affects a large proportion of the male population and is primarily driven by androgen receptor (AR) activity. First-line treatment typically consists of reducing AR signalling by hormone depletion but resistance inevitably develops over time. One way to overcome this issue is to block AR function via alternative means, preferably by inhibiting protein targets that are more active in tumours than in normal tissue. By staining prostate cancer tumour sections, elevated LIM kinase 1 (LIMK1) expression and increased phosphorylation of its substrate cofilin were found to be associated with poor outcome and reduced survival in patients with non-metastatic prostate cancer. Pharmacological inhibition of LIMK activity reduced proliferation and increased apoptosis in androgen-dependent prostate cancer cells more effectively than in androgen-independent prostate cancer cells. LIMK inhibition blocked ligand-induced AR nuclear translocation, and reduced AR protein stability and transcriptional activity, consistent with its effects on proliferation and survival acting via inhibition of AR activity. Furthermore, inhibition of LIMK activity increased αTubulin acetylation and decreased AR interactions with αTubulin, indicating that the function of LIMK in regulating microtubule dynamics contributes to AR function. Finally, treatment of androgen-independent prostate cancer cell lines reduced cell motility, indicating that LIMK inhibitors could be beneficial for the treatment of prostate cancer both by reducing nuclear AR translocation, leading to reduced proliferation and survival, and by inhibiting prostate cancer cell dissemination.

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COLORECTAL CANCER AND WNT SIGNALLING

www.beatson.gla.ac.uk/owen_sansom

Group Leader

Owen Sansom FRSE

Research Scientists
Patrizia Cammareri[1]
Fatih Ceteci[1]
Julia Cordero[2,11]
William Faller[3]
Dritan Liko[4,12]
Bryan Miller
Claudio Murgia
Kevin Myant[5]
Alessandro Scopelitti[6,11]
Myriam Solar Abboud
Ee Hong Tan
David Vincent[5]

Scientific Officers
Tam Jamieson
Rachel Ridgway
Andrew Campbell[3,13]

Clinical Research Fellows
Douglas Morran[7,14]
Colin Steele[8]

Graduate Students
Sahra Derkits
David Huels[1]
David Gay[3,15]
Pedro Salgueiro[9]

MRes Student
Josh Leach[10,16]

[1] EU PRIMES
[2] Royal Society
[3] ERC
[4] EMBO
[5] AICR
[6] NC3Rs (joint with Marcos Vidal)
[7] MRC
[8] Wellcome Trust
[9] joint with A*STAR
[10] CRUK Glasgow Centre
[11] until October
[12] until November
[13] from November
[14] until August
[15] from October
[16] until February

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

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

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

mTORC1 is required for the proliferation of APC deficient cells following *Apc* loss, although this requirement is lost following additional *Kras* mutations

Another pathway that is activated following *Apc* loss, in a MYC-dependent manner, is the mTORC1 signalling pathway. mTORC1 is a kinase that is required to coordinate proliferation and cell growth, and has long been viewed as an attractive pathway for cancer therapy. The major reason for this is that there are a number of inhibitors of this pathway that work well *in vivo* and are either licensed for use in patients or clinical trials. We have shown that APC deficient cells are absolutely dependent on mTORC1 to proliferate. Genetic inhibition or use of rapamycin, a chemical inhibitor, completely removes the proliferative capacity of APC deficient cells. This is associated with a slowing of translational elongation and hence, given the massive burst of transcriptionally activity of APC deficient cells, cells enter growth arrest (Fig.2). Although this growth arrest is reversible, as rapamycin is a well-tolerated drug it is possible that this would be beneficial for those patients that are predisposed to colorectal cancer.

In colorectal cancer, *KRAS* is often co-mutated with *APC* (in approximately 40% of cases). These mutations are activating and tumours carrying *KRAS* mutations have a poorer prognosis and are less likely to respond to therapy. Therefore, my laboratory has been investigating the cooperation of *Apc* and *Kras* mutations in models of colorectal cancer. The additional mutation of *Kras* exacerbates many of the phenotypes of *Apc* loss, increasing proliferation rates and accelerating tumourigenesis. Importantly, APC deficient cells that also carry a *Kras* mutation are intrinsically resistant to mTORC1 inhibition. This may help explain why later stage colorectal cancer trials using mTORC1 inhibitors have not worked.

Mechanistically, *Kras* mutation causes the activation of mTORC1 targets in an mTORC1-independent manner. We are now examining the pathways of resistance in these cells and hope to come up with rationale combination therapies that will overcome this resistance mechanism and specifically target cells carrying both of these mutations. Importantly, loss of *RAC1* or *mTORC2*, a less well studied member of the mTORC family, both appear to be required for the proliferation of cells carrying a *KRAS* mutation in a number of different cancers.

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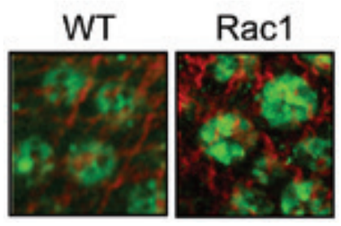

Figure 1
In vivo multiphoton imaging of the intestinal stem cell marker Lgr5 shows Rac deficiency suppresses the increased levels following *Apc* loss. In collaboration with Kurt Anderson, we have quantified the levels of the stem marker Lgr5 through the use of a reporter GFP in murine intestinal whole-mounts. As can be seen here, there is a marked increase in the amount of Lgr5-GFP following *Apc* loss *in vivo* (APC), however following combined loss of *Apc* and *Rac* (APC RAC) this is restored to wild-type levels. Importantly, loss of *Rac* alone (RAC) has equivalent levels of GFP to wild-types. Red here is second harmonics imaging of collagen.

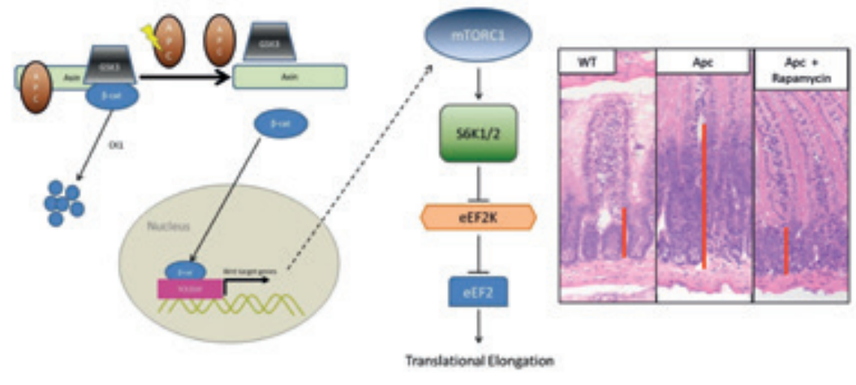

Figure 2
Inhibition of mTORC stops the proliferation of APC deficient cells. A) Schematic of mechanism of how loss of *Apc* causes the activation of mTORC signalling. B) H&E stained sections of intestines, wild-type (WT) or APC deficient (APC). As can be seen by the red bar, wild-type intestinal crypts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

DROSOPHILA APPROACHES TO CANCER

www.beatson.gla.ac.uk/marcos_vidal

Group Leader
Marcos Vidal

Research Scientists
Federica Parisi
Alessandro Scopelitti

Scientific Officer
Yachuan Yu

Graduate Students
Christin Bauer
Joseph Hodgson
Juan Macagno
Rhoda Stefanatos

Intern
Paula Climent-Canto

We utilise the fruit fly *Drosophila* to understand fundamental aspects of cancer biology. We have previously reported an inflammatory reaction by the innate immune system when tumours arise, comparable to the one observed in cancer patients. Importantly, powerful fly genetic tools allowed us to define for the first time the contexts dictating 'good' versus 'bad' inflammation. Recently, we have dissected systemic crosstalk between tumours and peripheral energy stores such as adipose tissue. Remarkably, our data demonstrate that adipocytes can sense tumours at a distance, and react by activating pro-inflammatory pathways that act as tissue non-autonomous tumour suppressor factors. These results could shed light on the complex systemic relationships between tumours, chronic inflammation and peripheral tissues in human cancer patients. We also characterised a novel role of LGR receptors - known as stem cell markers in numerous tissues - as tumour suppressors directing stem cell quiescence.

Systemic crosstalk between *Drosophila* adipocytes and epithelial tumours

High tumour burden is associated with increased levels of circulating inflammatory cytokines that influence the pathophysiology of the tumour and its environment. The cellular and molecular events mediating the organismal response to a growing tumour are poorly understood. We revealed a bidirectional crosstalk in *Drosophila* between epithelial tumours and the fat body - a peripheral immune tissue akin the mammalian liver and white adipose tissue. Epithelial tumours trigger a systemic immune response through local activation of Eiger/TNF signalling that leads to the recruitment and activation of hemocytes (fly macrophages) and to Toll pathway upregulation in distant adipocytes. Reciprocally, Toll elicits a tissue non-autonomous programme in adipocytes that drives tumour cell death. Hemocytes play a critical role in this system by producing the ligands Spätzle and Eiger, which are required for Toll activation in the fat body and for tumour cell death. Indeed, we demonstrated that Eiger protein produced by macrophages is internalised by tumour cells but not by normal ones (Fig. 1). Altogether, our results provide a novel paradigm for a long-range tumour suppression function of adipocytes in *Drosophila* that may represent an evolutionary conserved mechanism in the organismal response to solid tumours.

Mechanism for LGRs as tumour suppressor genes

The mechanisms regulating stem cell niche plasticity remain poorly understood. Mammalian leucine-rich repeat-containing G protein-coupled receptors (LGRs) have recently been characterised as somatic stem cell markers in multiple epithelial tissues. However, their biological roles in adult tissues are largely unexplored. We have identified a novel function of LGRs as inhibitors of intestinal stem cell (ISC) proliferation in *Drosophila*. Our results uncover a new paracrine signalling mechanism composed of neuroendocrine cells and mesenchyme/visceral muscle (VM) that constrains ISC proliferation. Midgut neuroendocrine cells secrete Bursicon, which acts as a paracrine factor on the VM through DLGR2, the ortholog of mammalian LGRs. Bursicon/DLGR2 restricts ISC proliferation and upregulation of cAMP in the visceral muscle. In the absence of this pathway, the intestine hyperproliferates resulting in excess cells and epithelial multi-layering (Fig. 2). Our results identify a novel paradigm in the regulation of ISC quiescence through modifications of stem cell niche signalling, which uncovers previously unknown roles of neuroendocrine cells and the conserved ligand/receptor Bursicon/DLGR2. Indeed, while the current knowledge indicates that LGRs drive stem cell proliferation via activation of Wnt signalling, clinical data indicates they are silenced or mutated in numerous tumours, indicating they can act as tumour suppressor genes. Our results provide a mechanistic framework to understand these recent clinical observations.

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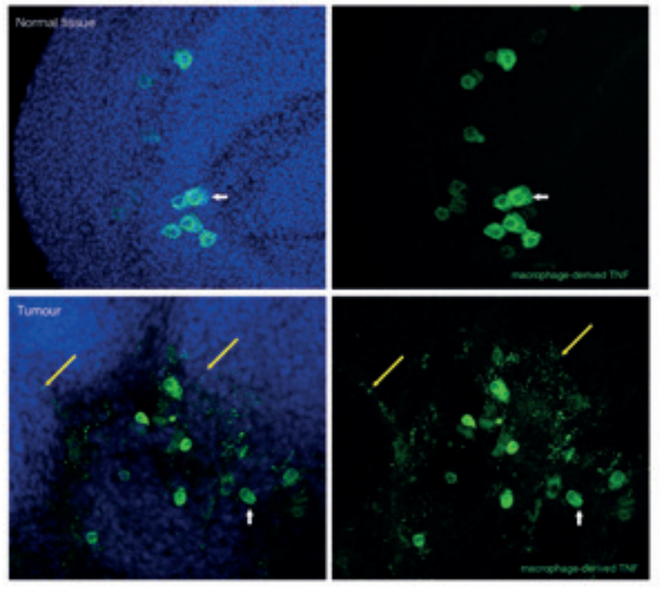

Figure 1
Confocal images from *Drosophila* imaginal disc tissues in a transgenic background expressing a Venus-tagged form of Eiger/TNF specifically in the macrophages (white arrows). Note the vesicles of macrophage-derived TNF incorporated into epithelial tumour cells (yellow arrows), a process absent in normal tissue.

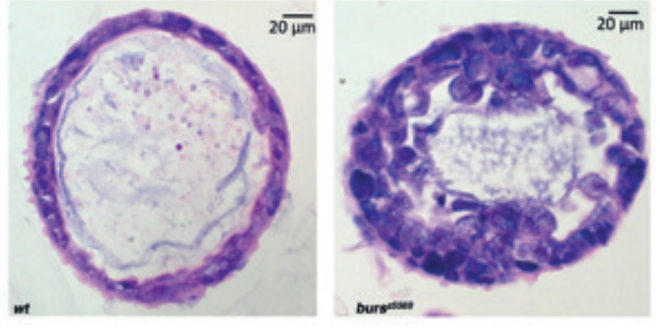

Figure 2
Haematoxylin and eosin staining of paraffin embedded sections from wild-type or *burs* mutant *Drosophila* intestines. Note the hypercellularity and epithelial multi-layering in *burs* mutant midguts.

VASCULAR PROTEOMICS

www.beatson.gla.ac.uk/sara_zanivan

Group Leader
Sara Zanivan

Research Scientists
Juan Ramon Hernandez
Francesca Patella

Scientific Officer
Lisa Neilson

Graduate Students
Fernanda Kugeratski
Steven Reid

Visitor
Maartje van den Biggelaar

Tumour angiogenesis, the process of vessel growth from pre-existing ones, is a well-recognised hallmark of cancer. Indeed, tumour vessels provide the oxygen and nutrients necessary to sustain the uncontrolled proliferation of tumour cells and provide a way for them to escape the primary tumour and form distant metastases. Additionally, in a clinical context, tumour vessels can impact on the success of conventional therapies. Indeed, tumour vessels are generally leaky and function poorly, which can result in reduced efficiency of chemotherapeutic drug delivery and limited efficacy of radiotherapy. For this reason, much effort is devoted to developing therapies that interfere with the angiogenic process in cancer for use in conjunction with conventional chemo- and radiotherapy.

Our group is working with state-of-the-art mass spectrometry in combination with SILAC (stable isotope labelling with amino acids in cell culture) to perform quantitative proteomic and post-translational modification - such as phosphorylation - analyses. We combine the use of this technology with a variety of cellular and molecular approaches with the aim of better understanding the complexity of the signalling that drives (tumour) vessel formation, with a particular focus on how the environment surrounding newly forming vessels impacts on this process.

Endothelial cell signalling in angiogenesis

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

When endothelial cells are plated on an appropriate matrix, such as matrigel, they assemble into tubular structures complete with a lumen, and thereby morphologically recapitulate the *in vivo* process. By using SILAC-based quantitative mass spectrometry we have profiled the proteomic changes associated with this process and identified the proteins CLEC14A and MMRN2 as ECM components required for angiogenesis *in vitro*, and blood vessel markers modulated during tumour progression in genetic mouse models of multistep carcinogenesis (Zanivan *et al.*, Mol Cell Proteomics 2013; 12: 3599). Additionally, this proteomic study highlighted that the morphogenetic process is associated with major metabolic changes in endothelial cells, and that this alteration is functional. We are currently investigating this aspect further using a unique combination of proteomics, mathematical modelling (in collaboration with Eytan Ruppin, University of Tel Aviv) and metabolomics (in collaboration with Eyal Gottlieb).

Our future work will be devoted to better characterisation of the complex signalling network during vessel formation with a particular focus on lipid metabolism. We aim to integrate these data with those previously generated to draw a more comprehensive picture of the molecular mechanisms regulating the formation of new vessels, and to identify and further characterise new key proteins as possible targets to interfere with during tumour vascularisation in the context of anti-cancer therapies.

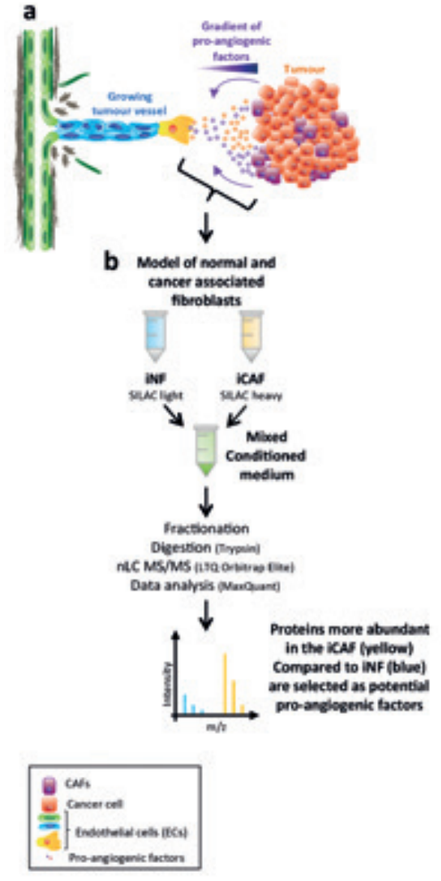

Figure 1
Quantitative proteomics in angiogenesis. (a) Schematic overview of the formation of a new tumour blood vessel from a pre-existing one induced by the secretion of pro-angiogenic factors from tumour cells and cancer-associate fibroblasts (CAFs). The arrows indicate the direction of the secreted factors. (b) Schematic overview of a mass spectrometry shotgun proteomic approach used to identify pro-angiogenic factors secreted by CAFs. Immortalised normal (iNFs) and cancer-associated (iCAFs) human fibroblasts are used in combination with SILAC for quantitative proteomic analysis. In a typical workflow, SILAC proteins secreted into the conditioned medium by iNFs and iCAFs are mixed together, fractionated, digested and peptides are analysed by nano-liquid chromatography (nLC)-high resolution tandem mass spectrometry (MS/MS). MS data are analysed with the MaxQuant computational platform to identify proteins secreted in high levels by iCAFs.

The extracellular environment drives tumour progression and angiogenesis

The environment surrounding cells strongly affects their behaviour. Importantly, the extracellular environment is diverse in physiological and pathological conditions. In cancer, the tumour stroma, composed of ECM and non-cancer cells such as endothelial cells, fibroblasts and immune cells, has a different composition and structure compared to normal tissue. This contributes to tumour progression to malignancy. Accordingly, our *in vivo* quantitative proteomic study of mouse skin carcinogenesis revealed that the ECM composition differs between benign and malignant tumours, and that specific cell adhesion proteins are highly expressed in tumour cells associated with malignancy (Zanivan *et al.*, Cell Reports 2013; 3: 552). In the context of angiogenesis, tumour stroma cells such as cancer-associated fibroblasts (CAFs), can affect endothelial cell functions by altering the ECM's composition and mechanical properties, and by secreting pro-angiogenic factors (Fig. 1a). Therefore, there is a clear link between CAFs and the angiogenic process in cancer, and we aim to investigate this further.

We have applied SILAC-based quantitative proteomics to characterise in depth the phosphoproteome and proteome of immortalised human fibroblasts of different origins, normal (iNF) and cancer-associated (iCAF), to provide a detailed picture of proteins specifically expressed or with an altered level of expression in the tumour environment. Since we observed that iCAFs were more pro-angiogenic than iNFs in *in vitro* models of angiogenesis, we have additionally analysed the ECM and conditioned medium produced by these cells by mass spectrometry with the aim of identifying previously uncharacterised pro-angiogenic factors (Fig. 1b). We are currently investigating the functions of some of the identified proteins in the context of angiogenesis using *in vitro*, *ex vivo* and *in vivo* models.

In addition, we are exploring the signalling networks activated in endothelial cells by the ECM's mechanical properties and composition, which are typically altered in the tumour environment. This will hopefully reveal signalling and identify specific proteins involved in tumour angiogenesis. Candidate proteins will be further characterised as potential suitable targets to interfere with the process of angiogenesis in cancer.

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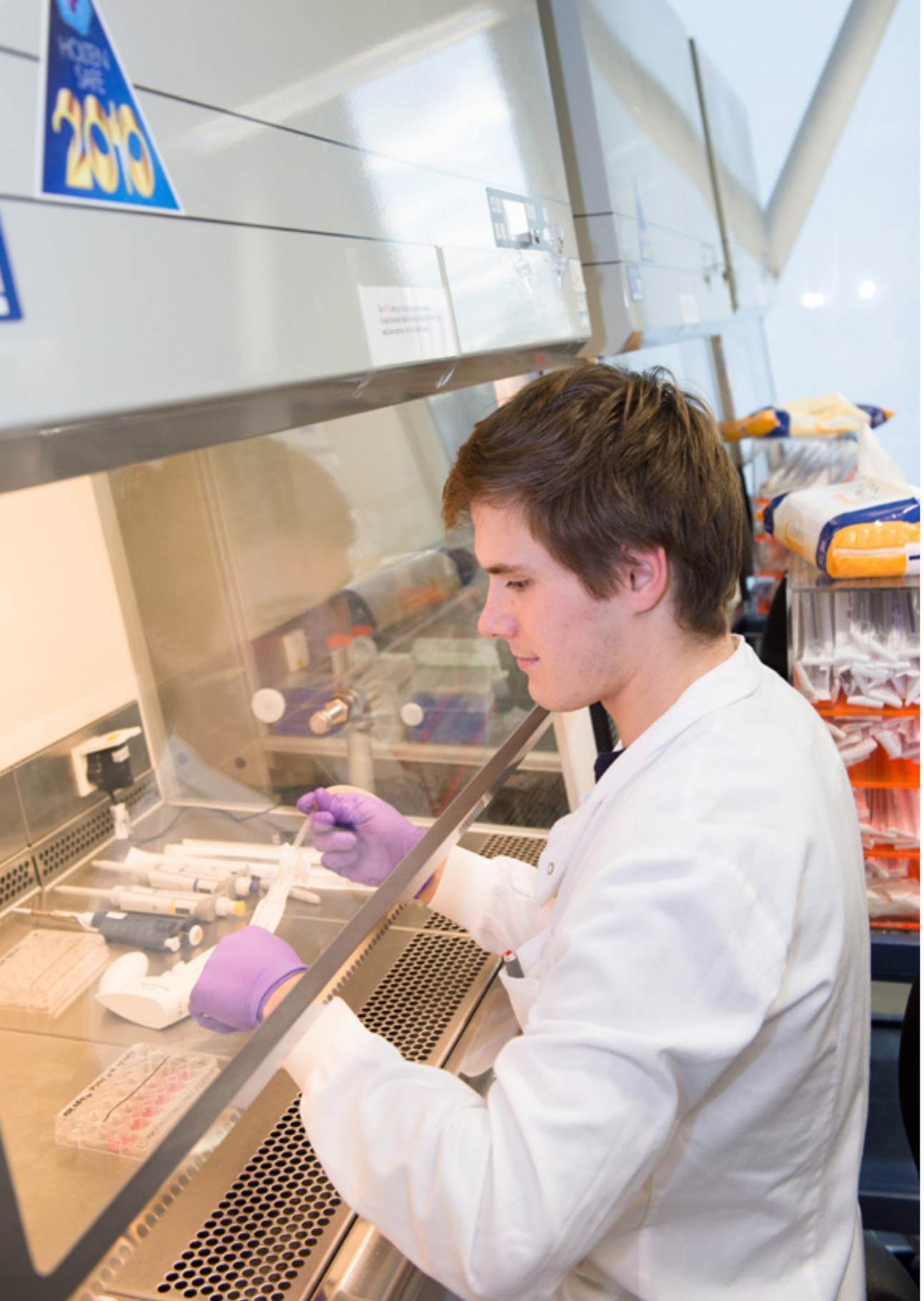

DRUG DISCOVERY

CANCER RESEARCH UK BEATSON INSTITUTE

Martin Drysdale - Drug Discovery Programme

DRUG DISCOVERY PROGRAMME

www.beatson.gla.ac.uk/ddp

Group Leader

Martin Drysdale

Chemistry
Catherine Barber
Simone Belshaw
Justin Bower
Keneth Davies
Emma Dick
Stuart Francis
Kate Gill
Duncan McArthur
Charles Parry
Angelo Pugliese
Mairi Sime

Biology
Diane Crighton
Dan Croft
Laura McDonald
Patricia McConnell
Mokdad Mezna

Structural Biology
Kenneth Cameron
Gillian Goodwin
Chris Gray
Jen Konczal
Andrew Pannifer
Alex Schuettelkopf

The vast majority of deaths from cancer result directly from metastatic spread of the disease, however there are currently no therapeutics specifically designed to combat this process. During the past year we have made significant advances in two of our projects targeting novel approaches to inhibiting invasion and metastasis; these are the kinase MRCK (myotonic dystrophy kinase-related Cdc42-binding kinase), a key effector in the Rho signalling pathway, and the actin-bundling protein fascin. We have also continued to utilise our fragment-based hit identification expertise to target other protein-protein interactions that, although challenging biological targets, have a high degree of validation as cancer targets. In particular, we are targeting the RAS oncogene, which is one of the best validated cancer targets and found to be mutated in 30% of all human cancers.

Myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK)

Mounting evidence suggests MRCK as an attractive target for anti-metastatic therapy. MRCK is a key effector in the Rho GTPase signalling pathway that acts to promote cell movement by initiating actomyosin contractility and reorganisation of the actin cytoskeleton. An increasing number of tumour types show mis-regulated expression of a number of components of this pathway. Elevated Rho GTPase expression, loss of negative regulators and/or upregulation of downstream effectors such as MRCK, results in enhanced signalling through the Rho GTPase pathway (Unbekandt and Olson, J Mol Med 2014; 92: 217).

As part of a collaboration with Mike Olson's group, we are developing small molecule inhibitors that target the α and β isoforms of MRCK. These inhibitors could be used either alone or in combination for the management of metastatic disease. Our lead series has delivered BDP-00007215, which has high potency for MRCKα and β and excellent selectivity over other key kinases such as Rho kinases (ROCKs). This translates into exquisite selectivity in our selective cell-based assays where we specifically target MRCKβ (IC_{50} = 0.05mM) or ROCK1 (IC_{50} > 30mM) (Fig. 1).

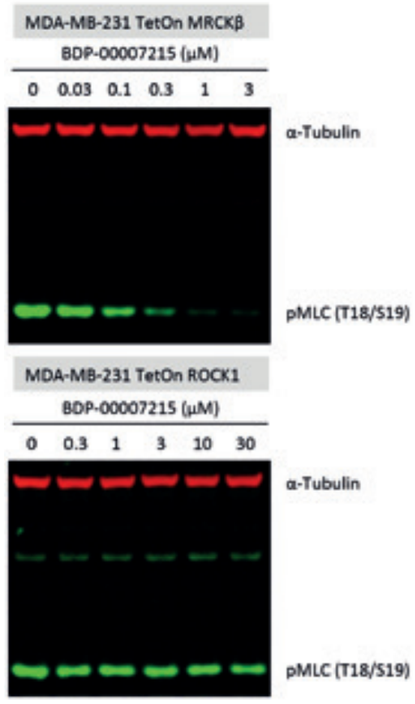

Figure 1
MDA-MB-231 D3H2LN cells stably expressing tetracycline inducible MRCKβ (top panel) or ROCK1 (bottom panel) were treated with BDP-00007215 as indicated in the presence of doxycycline. Levels of phosphorylated MLC (pMLC; lower band) and α-Tubulin (upper band) were measured by western blotting and quantified by LI-COR infrared scanning.

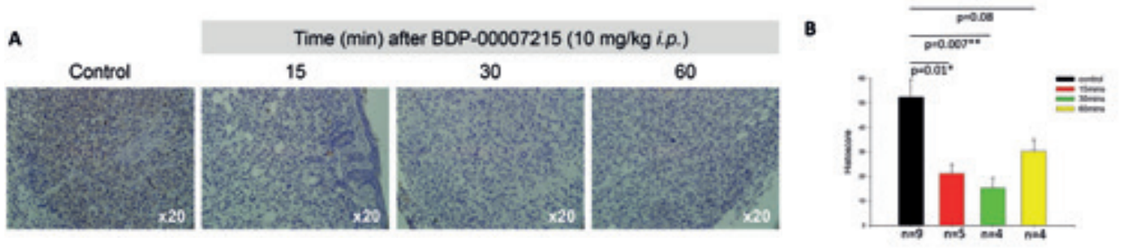

Figure 2
Mice bearing subcutaneous MDA-MB-231 D3H2LN tumours were treated with BDP-00007215 (10mg/kg, *i.p.*) and tumour sections stained for expression of phospho-Ser990 using a phospho-specific antibody developed in the Olson lab. (A) Immunohistochemistry carried out at 15, 30 and 60 minutes post dose. (B) Semi-quantitation of staining using the weighted Histoscore method.

Using BDP-00007215 we have carried out pharmacokinetic/phamacodynamic (PK/PD) *in vivo* studies in mice bearing MDA-MB-231 D3H2LN subcutaneous breast tumours. At 10mg/kg *i.p.* administration BDP-00007215 reduces the staining of active MRCKα using the phospho-specific antibody developed in collaboration with the Olson lab (Fig. 2). Additional analogues are being profiled in *in vivo* pharmacodynamic (PD) studies ahead of selection of the best compound for *in vivo* efficacy studies.

Fascin

The actin-binding protein fascin crosslinks filamentous actin into tightly packed bundles that drive the formation of cell surface protrusions involved in cell migration and degradative invasion into extracellular matrix. Elevated fascin expression is strongly correlated with invasiveness and poor clinical outcome in a variety of tumour types, therefore making fascin a compelling drug discovery target. We have now solved multiple crystal structures of fragments bound to fascin and these have been shown to bind to four distinct sites within the four β-trefoil domains that make up the fascin structure (Fig. 3).

Site-directed mutagenesis data derived in-house and also from published literature has directed us to two of these sites as being most likely to be functionally relevant and these have been prioritised for further study. Using structure-guided drug design we have developed hits for these two sites. Improvements in affinity of 10-1000 fold have been achieved and we now have >60 crystal structures of ligands bound to our two main sites of interest.

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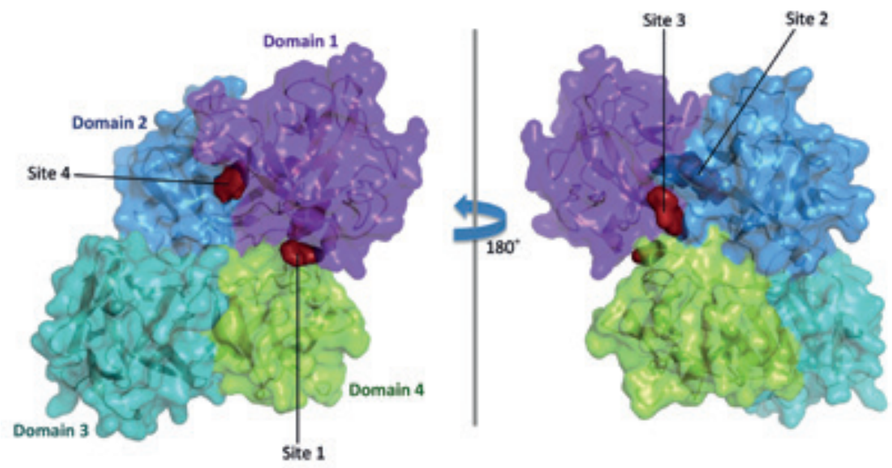
Figure 3
Fragments identified binding to four distinct sites on fascin



ADVANCED TECHNOLOGIES

CANCER RESEARCH UK BEATSON INSTITUTE

Kurt Anderson - Beatson Advanced Imaging Resource (BAIR)
Gabriela Kalna - Bioinformatics and Computational Biology
Gillian Mackay - Metabolomics
Nick Morrice - Proteomics and Mass Spectrometry
Emma Shanks - RNAi Screening
Karen Blyth - Transgenic Models of Cancer
Douglas Strathdee - Transgenic Technology

BEATSON ADVANCED IMAGING RESOURCE (BAIR)

www.beatson.gla.ac.uk/advanced_technologies

Head
Kurt Anderson

Staff Scientist
Gaurav Malviya

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

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

Concomitant with the development of new genetic tools, there have been tremendous advances in fluorescence imaging technology to visualise molecular dynamics in living cells, tissues, and organisms. These powerful techniques are increasingly sought by researchers, who require assistance in both the evaluation and application of imaging technology in order to address fundamental questions in cancer biology.

Our mission is to support basic imaging and the development of advanced applications. Basic imaging support primarily consists of training users in simple acquisition and analysis techniques. Development of advanced applications requires close work with our users to understand their scientific questions and help them develop appropriate imaging strategies. The following advanced techniques have been identified as important: medium throughput long term time lapse imaging; high resolution live cell imaging; confocal microscopy, especially for the use of photo-activation, -bleaching, and –switching; total internal reflection fluorescence (TIRF) microscopy; intravital microscopy; and fluorescence lifetime imaging for the determination of fluorescence resonance energy transfer (FLIM-FRET). More recently PET, SPECT and CT imaging were identified as techniques having great potential to support the extensive use of preclinical cancer models at the Beatson. Collectively we provide our users with a powerful technology toolbox for cellular and molecular level investigations of disease and response to therapy *in vitro* and *in vivo*.

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

Changes this year

This year we celebrated our first published use of PET with one of Kevin Ryan's papers: (Rosenfeldt *et al.*, Nature 2013; 504: 296). In order to meet the rising demand for preclinical use of PET, SPECT and CT, Gaurav Malviya was recruited to a Staff Scientist post. Gaurav brings expertise in radiolabelling and characterisation of antibodies for SPECT and PET imaging to the Beatson, and will lead on tracer development projects. In May we began the installation of a new multiphoton TRIM microscope from LaVision Biotec. This system will have increased sensitivity through the use of hybrid detectors for both intensity and lifetime imaging. It will also have a 'cloud scanner' that increases signal and minimises photo-damage by scanning the sample with four closely spaced laser foci instead of one. Continuing our successful use of adaptive optics on the OPO beam path of our old TRIM microscope, the new system will have a developmental adaptive optics unit built into the scanhead. In partnership with M Squared Lasers, we were awarded a Technology Strategy Board project in July entitled 'Optimised Lasers for Multicolour Multiphoton imaging' that will explore the use of semiconductor disk lasers as alternative excitation sources for two-colour multiphoton imaging. Finally, much time and effort this year went into 'shaking down' the Albira and the Andor Spinning Disk systems. The Andor system has suffered from software problems and some alignment instability but is now performing well and has been enthusiastically embraced for live imaging of actin dynamics in *Dictyostelium*.

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

www.beatson.gla.ac.uk/advanced_technologies

Head
Gabriela Kalna

Informaticians
Ann Hedley
Matthew Neilson

The unit provides support for a range of research projects that require computational approaches, advanced statistical analyses or mathematical modelling. Despite the continuing demand for data analysis from high-throughput technologies, we strive to ensure that even the smallest task receives our full attention in terms of experimental design, the application of appropriate statistical tests and the clear presentation of results for use in publications.

Our team focuses on exploratory data analysis, with the ultimate goal of providing insights that enhance our understanding of cancer biology. We offer routine processing of data, differential expression, supervised and unsupervised machine learning, and graph and network theory-based analyses. Many of our data analysis tasks are performed using specialised computer software such as Partek Genomics Suite, MATLAB (most notably the Bioinformatics and Statistics Toolboxes) and the open source Bioconductor package for R. We also make use of analytical routines that have been developed in-house in collaboration with our colleagues in Mathematics, Statistics, Computer Science and Biology. In addition to Ingenuity Pathway Analysis, GeneGo Meta Core and CLC Genomics Workbench, which are available to all researchers at the Beatson, we use the Oncomine Research Premium Edition database to satisfy the increasing demands of researchers who wish to exploit publicly available datasets.

In 2013 we focused on improving and expanding our service, particularly in the fields of next generation sequencing and mathematical modelling. To develop our skills in working with sequencing data, we attended the EMBO Practical Training Course on Analysis of High-Throughput Sequencing Data. Our team also expanded to three in January with the arrival of Matthew Neilson, who has since employed his expertise in mathematical modelling to embark on several modelling and simulation-based projects.

Obstacle detection has been incorporated into our existing computational model for pseudopod-centred cell migration and chemotaxis, such that simulated cells are capable of interacting with their environment. This exciting development provides us with the computational capability to simulate a wide range of processes, such as phagocytosis and contact inhibition. We will also employ this model to investigate the effect of membrane rigidity on a cell's ability to migrate through constrictions of varying widths.

Finally, we have been developing a particle-based spatial simulator that aims to provide some quantitative insight into the role of E-cadherin interactions in cell-cell adhesions. E-cadherin molecules are capable of forming three distinct interaction types, which allow them to act as a 'glue' that binds cells together. This challenging project has matured in recent months and we are currently performing parameter studies to determine regions of the model's parameter space that produce agreement between simulated and experimentally observed behaviour. Once the model has been calibrated, we intend to use it to test various researcher-defined hypotheses that specifically relate to E-cadherin.

We are pleased that there is a constant interest in our services, and we will continue our efforts to deal with all incoming projects effectively and within a reasonable timeframe. Our aim is to ensure that we provide Beatson researchers with the best possible service and we welcome any opportunities to offer advice on topics such as experimental design, statistical methodologies and data presentation.

Publications listed on page 80

METABOLOMICS

www.beatson.gla.ac.uk/advanced_technologies

Head

Gillian Mackay

Scientific Officer
Niels van den Broek

The Metabolomics facility forms part of the Beatson's new Cancer Metabolism Research Unit (headed by Eyal Gottlieb) and, in addition, is involved in collaborations with several other research groups at the Institute. Our aim for the future is to be able to measure all the low molecular weight metabolites present in the human body. The Human Metabolome Database currently contains over 40,000 metabolites.

We concentrate on targeted metabolomics, analysing for specific known metabolites, to determine the relative quantification between experimental and control groups as well as examining metabolic flux. We can also perform untargeted metabolomics, producing a detailed metabolic profile that can be used to look for novel metabolic changes.

For metabolomics projects, we provide research groups with advice on study design, sample preparation, data analysis and quantification of metabolites. The scope of our current analyses includes amino acids, organic acids, sugars, sugar phosphates and nucleotides, although other small polar molecules can be detected in the same screens.

We have two members of staff in the core facility, and two other research scientists, from Eyal Gottlieb and Karen Vousden's labs, who use the mass spectrometers for their own work and occasionally for other specific projects in their groups.

We have two Thermo Scientific Exactive LC-MS systems for metabolite analysis. The Exactive mass spectrometer has a polarity-switching feature, allowing many metabolites to be detected in the same run. Our focus on glycolysis and the tricarboxylic acid cycle has resulted in robust LC-MS methods for metabolites of these pathways. We have expertise in Thermo's data analysis package LCquan for targeted metabolomics, and for untargeted data analysis we use Thermo's Sieve software.

Working together with the Cancer Metabolism Research Unit, we have determined metabolic flux in cell culture experiments. Using labelled carbon or nitrogen tracers, such as 13C glucose, we can detect the different isotopes of many intracellular metabolites over time. We have accurately quantified specific metabolite concentrations and calculated intracellular isotopomer levels (nmol/µg protein) at different time points. Quantification of the concentration of metabolites in cell culture medium over time has enabled us to calculate the rate of uptake or secretion of metabolites by cells from the medium. This extracellular metabolite exchange rate is a key parameter for metabolic flux modelling.

In August, we purchased an Agilent GC-MS/MS system, and have developed a promising method for fatty acid analysis. We are also currently developing other GC-MS methods for a range of metabolites. With our Exactive LC-MS, we are investigating UPLC analysis to dramatically reduce analysis time for some of our standard methods, as well as developing LC-MS methods for fatty acid analysis. A Q-Exactive system has been purchased and will be installed in January 2014, to enable LC-MS/MS analysis for the identification of unknown metabolites, and provide structural information on compounds.

Publications listed on page 82

PROTEOMICS AND MASS SPECTROMETRY

www.beatson.gla.ac.uk/advanced_technologies

Head

Nick Morrice

Research Scientist
Emma Carrick

Scientific Officers
Sergio Lilla
David Sumpton

The facility has five mass spectrometers used for both proteomics and metabolomics: two Orbitrap Velos mass spectrometers (one of which is used by Sara Zanivan's group) coupled to Easy-LC II nano HPLCs; two Exactive mass spectrometer systems for metabolomics (operated by Gillian Mackay, see page 50); and an AB-Sciex 5600 triple TOF system.

The Orbitrap Velos systems are primarily used for protein and peptide identification, post-translational modification analysis and quantitative proteomics using SILAC. The 5600 Triple TOF is used for quantitative proteomics using SILAC, iTRAQ or label-free analyses. We have also introduced data independent LC-MS using SWATH technology and parallel reaction monitoring (pRM) using the Orbitrap Velos system, where peptide ions of interest are constantly selected for MS/MS and then quantified using Skyline software from the MacCoss group in Seattle. This approach is at least two orders of magnitude more sensitive than LC-MS using conventional data dependent acquisition (DDA) and has been used successfully by our group to quantify proteins and phosphopeptides that were 'invisible' in the DDA analysis.

The experienced staff members provide advanced knowledge and expertise for initiation of proteomics projects and state-of-the-art proteomics analyses to the Institute's scientific community. It is advisable to contact one of the staff members prior to initiating a project so that the best experimental design can be drawn up. Although most of the activity of the service is related to the analyses of samples for protein identification and characterisation by MS, the analysis of post-translational modifications, especially protein phosphorylation, is a specialty. Along with the investment in MS instrumentation, we are expanding the informatics capability of the facility. There is now a dedicated MaxQuant server for the analysis of SILAC data, a new Mascot server, Proteome Discoverer 1.4, Progenesis 4 and Skyline for the analysis of label free/SWATH or pRM data. Data can be disseminated to researchers using Scaffold Q + S, which has a free, user friendly viewer. Training is always available from the facility staff on how to use any of these software packages.This year, in collaboration with Robert Insall's group, we have set up lipidomic analyses for LPAs, LPCs, PAs, DAGs and, to a more limited extent, other phospholipids on our 5600 Triple TOF system. The primary assay has been to develop a very quick analysis of LPAs in media in which melanoma cells have been grown. These cells are now known to show chemotaxis towards LPA gradients and measuring the individual LPA levels has become a high priority. An example of this type of analysis is shown in Figure 1.

The facility focuses on four major areas:

1. Quantification of protein complexes, proteomes and secretomes using label and label-free methods.
2. Post-translational modification analysis of individual proteins, in particular phosphorylation, acetylation, methylation and ubiquitination. Recent research has involved the chemical modification of proteins by naturally occurring metabolites such as succinylation of cysteine/lysine and kyneurinin modification of cysteine/histidne/lysine.
3. Lipidomic profiling of known, naturally occurring phospholipids such as LPA, LPC, PA, PS, PI, PC and cardiolipin.
4. Method development for proteomic and small molecule analysis.

Publications listed on page 82

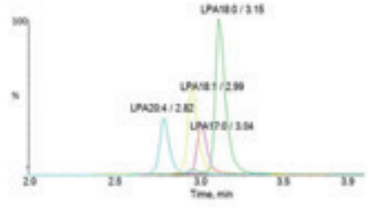


Figure 1
LC-MS analysis of lysophosphatidic acids.

RNAi SCREENING

www.beatson.gla.ac.uk/advanced_technologies

Head

Emma Shanks

Scientific Officer
Lynn McGarry

Informatics Manager
Daniel James

Graduate Student
Matthew Davidson

The RNAi Screening facility couples high throughput RNA interference (RNAi) screening with high content imaging (HCI) to translate fundamental cancer research towards new therapies. We can sequentially knockdown every gene in the genome and quantify its effect on a biological system. This approach is a powerful tool for elucidating new interactions and identifying novel drug targets and/or drug interacting partners to improve existing cancer therapeutic approaches.

Over the past two years, the facility has collaborated with nine groups and completed 17 screens, including 11 in 2013. Notable projects in the past year include identification of genes that are synthetic lethal with i) *Kras*^{G12D}/*p53*$^{-/-}$ in a pancreatic cancer cell line (Owen Sansom), ii) MYC expression in U2OS cells (Daniel Murphy) and iii) miR21 in colorectal cancer (Nicola Valeri). We also conducted an investigation into drugs that are synthetically lethal with radiation exposure in glioblastoma.

Viability based on nuclear count is our most requested output, yet HCI facilitates quantification of a suite of phenotypic parameters pertaining to nuclei and/or cells. For example, nuclear size can be indicative of cellular senescence or the scattering of cells may be suggestive of a metastatic phenotype. We now routinely offer quantification of additional phenotypic parameters to maximise the return on the investment of conducting a screen. Moreover, with the implementation of the Columbus Image Data Storage and Analysis System (Perkin Elmer), we have the additional flexibility to conduct machine learning, where we can manually teach software what phenotype(s) are of interest and then apply the resulting algorithms to an entire screening dataset. This is a very powerful way to conduct phenotypic analysis. We have also been working with Owen Sansom's group to develop our three-dimensional quantification capabilities. Currently, we are able to quantify cancer cell spheroids using our metabolic, fluorometric screening assay and are developing an image-based assay to complement this.

Over the last year, we have supported the acquisition of a number of bespoke siRNA collections designed to target specific processes, in particular a metabolism focused one of 1,800 genes. We also offer access to three libraries of clinically tested/approved drugs for a wide range of indications unrelated to cancer, which are used to support a drug re-purposing approach. As the principal targets of these drugs are established, we often overlay these data with siRNA screening datasets to isolate the most pertinent targets for validation. To date, we have conducted drug re-purposing screens in pancreatic cancer, glioblastoma and breast cancer models, the latter being funded by Breast Cancer Campaign.

Finally, we welcomed graduate student Matthew Davidson whose project will utilise siRNA screening and drug re-purposing to identify and validate innovative therapeutics for the treatment of oral/oropharyngeal cancers. The facility also relocated into the Wolfson Wohl Cancer Research Centre to provide some much needed additional space.

Future developments

To meet increasing demand, we have purchased an additional Operetta HCI system, which has confocality. This will increase our ability to conduct more challenging screens and improve our capacity to support validation experiments through more detailed imaging acquisition and analysis. Further to this, we aim to develop our aptitude in conducting screens within a three-dimensional environment.

TRANSGENIC MODELS OF CANCER

www.beatson.gla.ac.uk/advanced_technologies

Head

Karen Blyth

Research Scientists
Kirsteen Campbell[1]
Laura McDonald

Scientific Officers
Dimitris Athineos
Susan Mason

Clinical Research Fellow
Anne McKillop[2]

Graduate Students
Nicola Ferrari
Alessandra Riggio

Intern
Sinead Griffin[3]

[1] Royal Society

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

[3] University of Glasgow

The Transgenic Models Laboratory uses *in vivo* models to accurately recapitulate human cancer in a physiologically relevant way to better understand how cancers develop and metastasise. With sophisticated preclinical models we can assess the genetic causes of cancer allowing us to develop biologically relevant systems for testing novel therapies. The lab has a particular interest in the *RUNX* gene family and is investigating the role of *RUNX1* and *RUNX2* in breast and prostate cancer.

Preclinical genetic models have significantly contributed to our understanding of how cancers develop and metastasise, and the role of the Transgenic Models lab is to exploit these cancer models to address important biological questions in a physiologically relevant *in vivo* setting. In collaboration with other Beatson research groups, we are using genetic and transplantation models to assess the signalling pathways and genetic mutations that are associated with cancers such as pancreatic, colorectal, breast and prostate cancer, and melanoma. These cancer models, which faithfully recapitulate the genetic regulation, sporadic development, tumour pathology and organ-specific metastatic spread of specific human epithelial cancers, are particularly valuable for monitoring responses to therapeutic agents in our efforts to develop improved and novel targeted therapies to the common cancers.

RUNX1 and RUNX2 in breast and prostate cancer

RUNX1 and *RUNX2*, which are essential for normal mammalian development, are genes that are associated with cancer. *RUNX1* is one of the most frequently altered genes in leukaemia, where loss of function caused by chromosomal translocation and mutation predisposes to adult myeloid and childhood lymphoid leukaemia. Recent evidence suggests these genes may also have a role in breast cancer. In collaboration with researchers at the University of Glasgow, we have been assessing archival human breast cancer tissues for expression of RUNX1 and RUNX2. We find that overexpression of RUNX1 in a specific subtype of human breast cancer, the so-called triple negative (ER/PR/HER2-negative) breast cancers, correlates with poorer survival (Fig. 1) and may be an independent prognostic marker for this patient group. This is particularly important, as there is currently no good biomarker or targeted therapy for this subtype of breast cancer. We also find RUNX2 to be overexpressed in some triple negative breast cancers and indeed have evidence that *Runx2* may be specifically involved in the squamous metaplastic form of the disease. Interestingly RUNX2 is also expressed in the stromal component of breast cancers where it correlates with worse survival. Current projects in the lab are looking at the role of *Runx2* as a regulator of mammary stem and/or progenitor cells, the role of *Runx1* and *Runx2* in the maintenance of mammary epithelium homeostasis and the dysregulation of these genes in epithelial cancers, in particular in breast and prostate cancer.

Publications listed on page 76

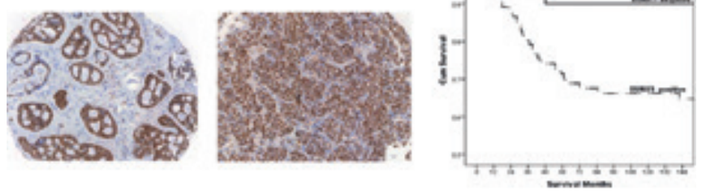


Figure 1
RUNX1 may be a new prognostic marker in triple negative breast cancer. Immunohistochemistry images demonstrating high levels of RUNX1 in human breast cancer tissues. High RUNX1 expression is associated with poorer overall survival in patients with triple negative breast cancer as shown by the survival graph.

TRANSGENIC TECHNOLOGY

www.beatson.gla.ac.uk/advanced_technologies

Head
Douglas Strathdee

Scientific Officers
Laurence Cadalbert
Farah Naz Ghaffar

Graduate Student
Nicola Laprano[1]

[1] from October

The Transgenic Technology Laboratory uses genetic manipulation technology to help understand gene function in cancer. This also allows the generation of new and more accurate models of human cancers. By the use of gene targeting in stem cells and direct genome editing we can precisely introduce defined alterations of specific genes into the germline, allowing us to create increasingly sophisticated cancer models.

Using stem cells to model cancer

Embryonic stem cells have a number of properties that make them an extremely useful tool for the analysis of gene function. One of the most important of these is their high rate of DNA recombination, termed homologous recombination. We can exploit this property to engineer very defined alterations of target genes to allow us to interrogate the function of these genes in cells. These can be, for example, knockout alleles where the function of a specific gene is compromised. This allows direct assessment of the role of the target gene in specific cellular processes. Also, we can use the same technology to introduce point mutations that mimic the exact changes uncovered in the analysis of human cancers. Taken together these techniques allow us to carefully interrogate the role of genes in cell and tissue homeostasis and the development of cancer. We collaborate on a wide variety of different projects and use a number of strategies, such as point mutations or conditional knockouts, to produce finely controlled alterations in gene activity. Once the desired genetic alteration has been introduced into stem cells, the cells can be phenotyped to assess the consequence of the desired mutation on the biology and behaviour of the cells. One advantage of using stem cells is that they can be differentiated in culture to produce a wide variety of cell types. So, the consequence of the mutation can be analysed not only in stem cells themselves but also in a variety of specialised cell types derived from them.

Employing state-of-the-art genetic manipulation techniques

In addition to maximising the potential of existing technologies, we are interested in implementing new technologies with the aim of refining and improving current cancer models. In addition to standard knockout and transgenic approaches, we are testing new approaches to allow us to extend the range of projects we can consider. To this end we are currently testing shRNA methods, designed to allow us to control the level of gene expression in tumours directly. Methods that allow us to regulate the level of a protein in this manner should enable, for example, the validation of candidate genes as potential targets for drug development. In collaboration with the Sanger MGP (Mouse Genetics Project) and the IMPC (International Mouse Phenotyping Consortium), we are currently establishing a screen to analyse the role of a variety of genes in intestinal homeostasis (Fig. 1). Candidate genes are identified and the function of these in maintaining cells populations in the intestine is assessed. The aim of this work is to identify candidate genes that may play a role in the maintenance of cell number in the intestinal epithelium and are therefore good candidate genes that may play an important role in tumour formation in the intestine.

Publications listed on page 86

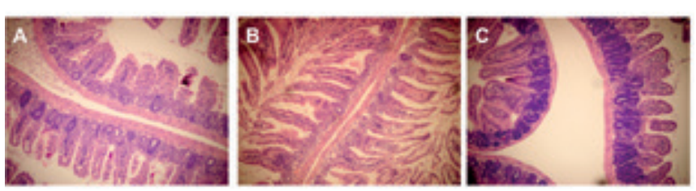


Figure 1
A screen for gene involved in regenerating intestinal epithelium. The number of cells that are responsible for populating the intestinal epithelium are assessed in controls (A). Phenotypes can be observed in samples lacking the function of particular genes where the number of cells in the crypts is reduced (B) or where the number of cells is greater than the controls (C). Genes identified as playing a role in intestinal regeneration represent good candidate genes for a potential role in tumourigenesis.

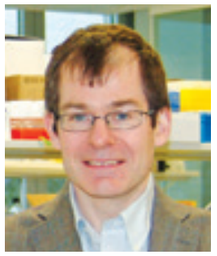
BEATSON ASSOCIATES

UNIVERSITY OF GLASGOW

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

EPIGENETICS OF CANCER AND AGEING

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



Group Leader

Peter D. Adams

Beatson Associate

Research Scientists
Dina Dikovskaya
Andrejs Ivanov
Tony McBryan
David Nelson
Jeff Pawlikowski
Nikolay Pchelintsev
Taranjit Singh Rai
John van Tuyn

Clinical Research Fellows
Annie Latif
Douglas MacKenzie

Scientific Officer
Claire Brock

Graduate Students
Rachael Hewitt
Indrani Manoharan
Desiree Piscitello[1]

[1] joint with David Gillespie

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

Cell senescence is an irreversible proliferation arrest instigated by a variety of molecular triggers including acquisition of activated oncogenes, and shortened telomeres caused by excess rounds of cell division. In addition, senescent cells secrete a cocktail of inflammatory cytokines, chemokines and matrix proteases (the 'inflammatory secretome' or senescence-associated secretory phenotype, SASP) that is capable of influencing behaviour of neighbouring cells, including immune cells. Compelling evidence now indicates that cell senescence is a potent tumour suppression mechanism, notably in cells harbouring activated oncogenes. Senescence-associated proliferation arrest and the SASP act in concert to achieve tumour suppression: proliferation arrest directly curtails tumour growth and the SASP calls on innate immune cells to eliminate the offending damaged cells. Because of senescence, most primary human cells have a finite proliferative lifespan, and evidence has been presented that senescence contributes to tissue ageing *in vivo*, in part by limiting the proper self-renewal of stem cells and tissues. In sum, cell senescence has both beneficial and detrimental effects, and bypass of senescence can lead to tumour formation through uncontrolled proliferation of damaged cells (Fig. 1).

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

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

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

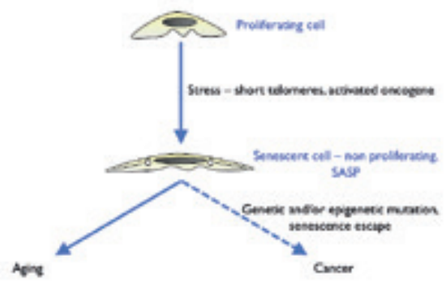


Figure 1
Senescence as a tumour suppressor mechanism. Acquisition of an activated oncogene or inactivation of a tumour suppressor initially causes a proliferative burst. Ultimately, senescence kicks in to arrest proliferation of the cells harbouring the oncogenic event. Proliferation arrest is reinforced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

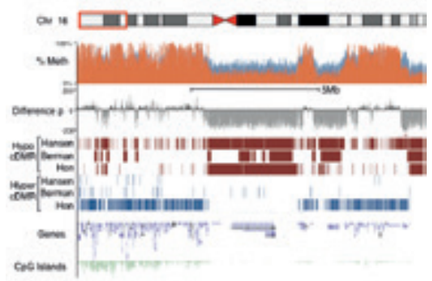


Figure 2
DNA methylation gains and losses in senescent cells overlap with hyper- and hypomethylation in cancer. Paradoxically, this suggests that the epigenome of senescent cells might be primed for progression to cancer. In turn, this suggests that senescence might be an imperfect tumour suppressor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

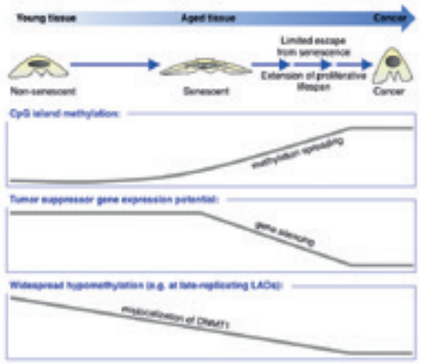


Figure 3
The altered epigenome of senescent cells might promote an age-associated increase in the incidence of human cancers. Our analyses and previous studies and ideas lead to the following model: Senescent cells accumulate with age in human tissues. These senescent cells harbour low-level methylation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

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

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

Senescent cells appear 'epigenetically primed' to form cancer cells

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

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ONCOGENE-INDUCED VULNERABILITIES

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

Group Leader
Daniel J.Murphy
Beatson Associate

Research Scientist
Meera Raja

Scientific Officer
Alan McVie

Graduate Students
Tiziana Monteverde
Nathiya Muthalagu
Sarah Neidler
Jennifer Port

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

MYC in cancer

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

MYC-induced metabolic vulnerability

Rather than attempting to target MYC directly, a number of groups including our own have turned to 'synthetic lethal' screening approaches in order to identify induced vulnerabilities, i.e. proteins and pathways to which cells overexpressing MYC – but, crucially, not normal cells – are addicted for survival. Our siRNA screen of the human kinome surprisingly failed to return predicted cooperating oncogenes such as *RAS* or *PI3K*. Instead, we identified a number of metabolic regulators, including the AMP-activated protein kinase, AMPK and the closely related ARK5 (aka NUAK1), as required for viability specifically when MYC is overexpressed. Why these kinases? MYC-driven biosynthetic processes cause cells to consume ATP at a much higher rate than normal and, despite elaborate reorganisation of carbon metabolism to balance ATP production with biosynthetic precursor provision, this engenders a state of energetic stress that leads to AMPK activation. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with production. For reasons that are presently unclear, ARK5 is required for this homeostatic feedback mechanism. Depletion of either AMPK or ARK5 thus leads to ATP collapse and consequently loss of viability, selectively in cells overexpressing MYC, strongly suggesting that targeting these kinases may be therapeutically effective against MYC overexpressing cancers. In 2013, the lab developed and acquired a number of key tools that will now enable us to understand the molecular basis of the role of ARK5 in bio-energetic homeostasis as well as to genetically model the therapeutic impact of ARK5 suppression on MYC-dependent tumours *in vivo*.

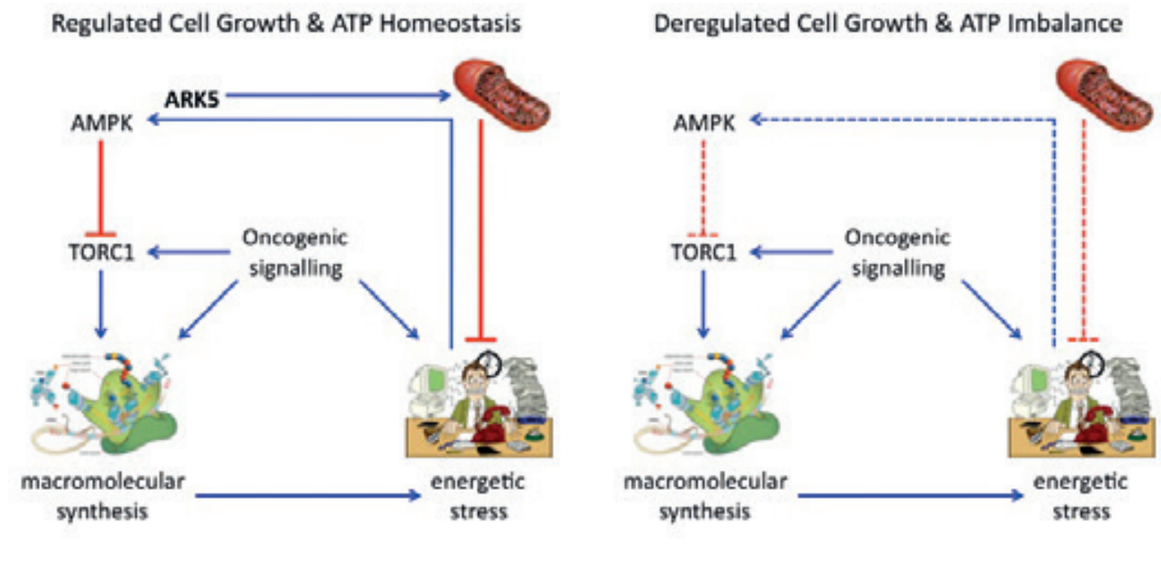

Figure 1
Induced dependencies need not reflect direct molecular interactions. Oncogene-induced cell growth, typically requiring signal transduction via the MTOR (mechanistic target of rapamycin) pathway, drives rampant ATP consumption that must be compensated for through increased cellular intake of fuel (e.g. glucose, amino and fatty acids) combined with AMPK-mediated attenuation of macromolecular synthesis. Upon suppression of ARK5, this feedback mechanism is impaired, leading to ATP depletion and bio-energetic catastrophe. In principle, any intervention that similarly impairs bio-energetic homeostasis may selectively kill tumour cells.

MYC-induced lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for some 18% of all cancer related deaths, and incidence of lung cancer is on the rise especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have developed a model for early tumour progression using tractable combinations of conditional alleles, including *Kras* and *MYC*. We are using laser-capture microdissection combined with gene expression analysis and functional screening to identify proteins with enzymatic activities that are required for progression from benign to malignant disease. The dual goal of this endeavour is to identify both new candidate therapeutic targets and surrogates for early detection.

This year was focused on integrating into our new environment and establishing the tools and collaborations necessary to drive our research programme forward. The lab attracted competitive funding from the British Lung Foundation, European Union and CRUK Centre Development Fund, as well as entering a funded collaboration with Merck Sharp & Dohme, together enabling us to welcome four new lab members, including two graduate students. We have generated a number of exciting preliminary results to build upon and look forward to a highly productive and successful 2014.

MITOCHONDRIA AND CELL DEATH

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

Group Leader
Stephen Tait
Royal Society University Research Fellow
Beatson Associate

Research Scientists
Martina Haller
Gabriel Ichim[1]
Maria Karvela

Graduate Student
Evangelos Giampazolias[2]

Visitor
Jonathan Lopez

[1] EMBO
[2] CRUK Glasgow Centre

Cell death is a potent tumour suppressor mechanism that must be evaded in order for cancer to develop. Because anti-cancer therapies often act by killing cells, cell death sensitivity also governs therapeutic efficacy. Paradoxically, besides powering our cells, mitochondria are essential for apoptosis, the major form of programmed cell death. We aim to understand how mitochondria regulate cell death and define how this process is deregulated in cancer. Our goal is to clinically translate these findings in order to improve existing anti-cancer therapies and develop new means to selectively kill cancer cells.

Mitochondria, cell death and cancer

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

Regulating the mitochondrial gateway to death

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

Cell survival following mitochondrial permeabilisation

Following MOMP, cells often die irrespective of caspase activation, suggesting that MOMP represents a 'point-of-no-return'. However, we have found that cells can sometimes survive and proliferate following MOMP. This may be important in cancer development and regulating therapeutic sensitivity as cancer cells often display defective caspase activity. We determined that survival following mitochondrial permeabilisation was dependent, at least in part, upon glycolysis and autophagy. More recently, using novel live cell imaging techniques, we have discovered that MOMP can be incomplete such that some mitochondria remain intact. Intact mitochondria are required for cell survival following MOMP probably by serving as a source of healthy mitochondria that allows mitochondrial repopulation. We found that the ability of certain mitochondria to evade MOMP is due to increased anti-apoptotic Bcl-2 protein expression on their outer membrane. Following from this, treatment with BH3-mimetic compounds effectively induced complete MOMP and prevented clonogenic survival, a result that may have therapeutic relevance. Current work is aimed at addressing the importance of post-MOMP cell survival in tumourigenesis and treatment response using a variety of approaches including developing a means to report cell survival following MOMP *in vivo*. Secondly, we are investigating the mechanisms that enable cell survival, focusing upon the protective role of autophagy in this process.

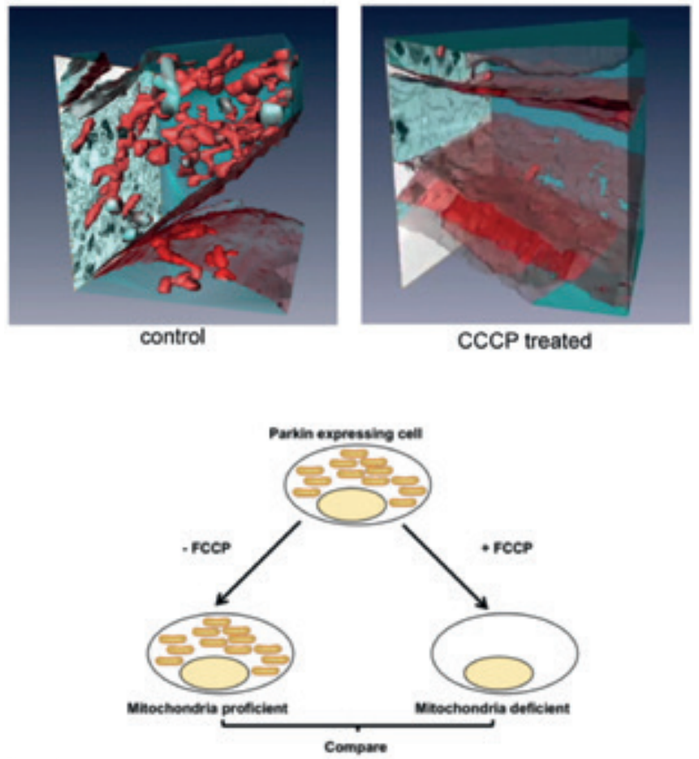

Figure 1

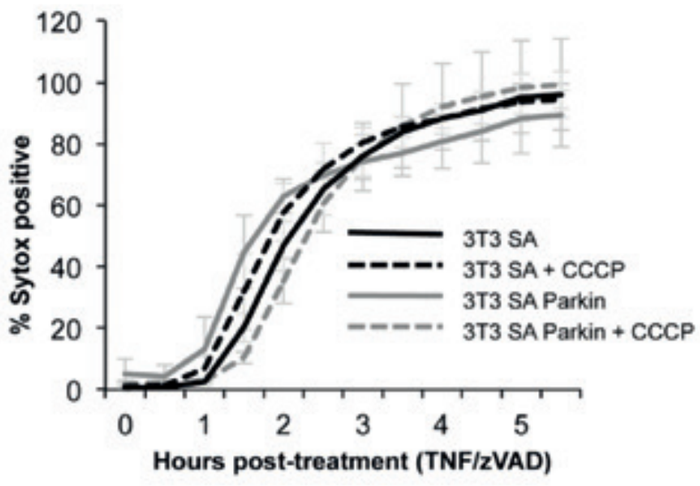

Figure 2

Figure 1
Defining mitochondrial functions through Parkin-mediated mitophagy. Top: Three-dimensional EM showing mitochondrial depletion in Parkin-expressing cells specifically following CCCP treatment (mitochondria are in red, cytosol is in blue). Bottom: Using this approach, we can address the importance of mitochondria in cell death and other processes by comparing mitochondria proficient and deficient cells.

Figure 2
RIPK3-dependent necroptosis does not require mitochondria. 3T3-SA cells expressing vector or Parkin were treated with or without CCCP then treated with TNF/zVAD to induce necroptosis. Cell death was measured by Sytox Green uptake in an Incucyte imager. No difference in the kinetics or extent of necroptosis was observed between mitochondria in replete versus depleted (Parkin + CCCP) cells.

Mitochondria and non-apoptotic cell death

Various other forms of programmed cell death exist besides apoptosis, including caspase independent cell death, necroptosis and mitotic cell death. These alternate forms of cell death function as either back-up pathways in the event of failed apoptosis or are distinct cell death programmes in themselves. Although these cell death modalities are likely important in cancer (particularly in response to therapy) little is known about their underlying molecular mechanisms. Mitochondria have been implicated as major effectors in necroptosis, a form of regulated cell death that requires activation of the kinase RIPK3. We have directly tested a role for mitochondria in necroptosis by generating mitochondria-free cells through Parkin-mediated mitophagy (Fig. 1). Using this method, we find that the kinetics and extent of necroptosis are not affected by mitochondrial depletion thereby ruling out a major role for mitochondria executing necroptosis (Fig. 2). Our currents studies focus upon investigating if mitochondrial dysfunction can serve to initiate necroptosis in addition to defining the role for necroptosis in tumour suppression and therapy-induced cell death.

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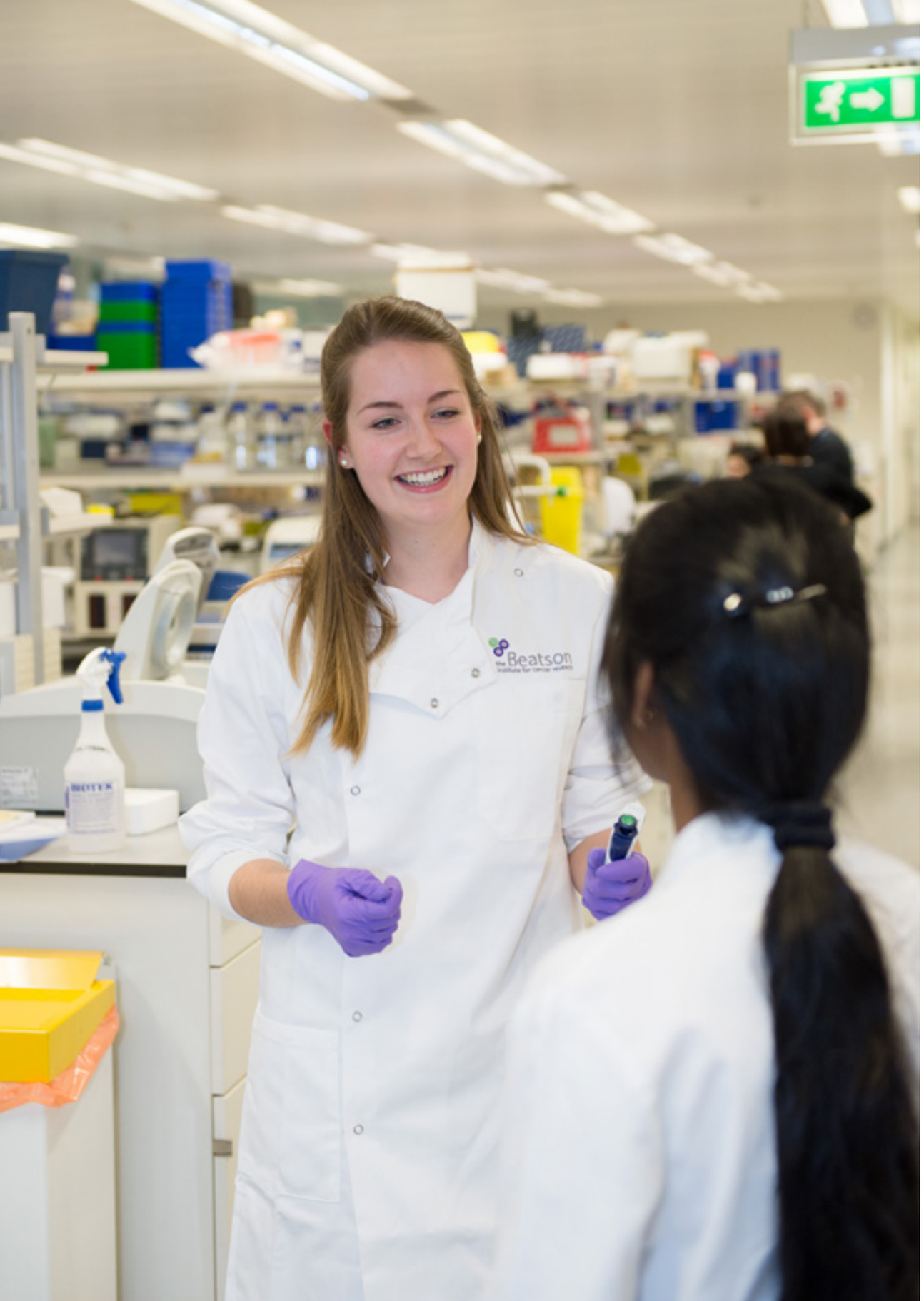


RESEARCH GROUPS

UNIVERSITY OF GLASGOW

Jeff Evans - Institute of Cancer Sciences

INSTITUTE OF CANCER SCIENCES

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

Director
Jeff Evans

The Institute of Cancer Sciences (ICS) at the University of Glasgow spans fundamental cancer biology, translational and clinical cancer research with a major focus on cancer genomics and disease-specific research. The ICS also spans three campuses, at the Garscube Estate (adjacent to the CRUK Beatson Institute), Gartnavel (including the Paul O'Gorman Leukaemia Research Centre [POG-LRC] and the Beatson West of Scotland Cancer Centre) and the New South Glasgow Hospital. Its primary goal is to deliver world-class research that can be translated to patient benefit and to provide a leading-edge environment for research and training.

This year, the ICS was a major partner in the successful renewal of the CRUK Glasgow Centre, and a major contributor to the University's Research Excellence Framework (REF) submission including a number of impact case studies. Notable achievements in 2013 included the completion and occupation of the Wolfson Wohl Cancer Research Centre (WWCRC) building. We also continued our ambitious recruitment of international research leaders. **Andrew Biankin** took up his post as the Regius Chair of Surgery and Director of the WWCRC and **Sean Grimmond** joined us as Chair of Medical Genomics. We also welcomed **David Chang** (Senior Clinical Lecturer in Surgery), **Liz Musgrove** (Senior Research Fellow in Cancer Genomics), **Prabs Rajan** (CRUK Clinician Scientist, Urological Surgery), **Katie Wakeham** (Clinical Lecturer in Clinical Oncology), while **Julia Cordero** and **Vignir Helgason** were both appointed as University Leadership Fellows. We congratulated **Mhairi Copland** and **Paul Shiels** on promotion to Professorships, while **Kamil Kranc** was appointed to a prestigious position at the University of Edinburgh and both **Nicola Valeri** and **Chiara Braconi** were appointed to the Institute of Cancer Research, London.

Fundamental cancer biology, translational and clinical cancer research

Major interests of our cancer biologists include cellular senescence, ageing, and cancer stem cells. The malignant properties of cancer cells have their origins in DNA mutations and other forms of genetic damage but also in epigenetic changes. These are heritable through cell division but are not directly coded in the DNA. Instead, they are coded within chromatin, through DNA methylation, non-canonical histone variants, histone modifications, histone binding proteins and higher order patterns of chromatin folding. A number of research groups (**Peter Adams** [see page 56], **David Vetrie**, **Adam West**, **Katherine West**) are interested in basic mechanisms of chromatin regulation, epigenetic inheritance and how epigenetic dysfunction contributes to ageing, cancer and response to cancer therapies. For example, a study in the Vetrie and West groups, published in *Blood*, described the chromosomal interactions that regulate the expression of the *TAL1* gene. *TAL1* is frequently mis-regulated in T-cell acute lymphoblastic leukaemia (T-ALL). This study found that chromosomal looping facilitates both normal and aberrant TAL1 expression and may predispose to genomic rearrangements that drive T-ALL.

Stem cells maintain tissue homeostasis by adjusting their function to micro-environmental or 'niche' signals. Deregulation of such signals results in uncontrolled stem cell proliferation, which is often associated with cancer. **Julia Cordero**'s laboratory combines studies on the *Drosophila* midgut and the mammalian intestine to understand how cell autonomous and niche-derived signals integrate to regulate stem cell proliferation in response to damage as well as tumourigenesis of adult self-renewing tissues.

Cell death is a key tumour suppressor mechanism that must be inhibited in order for cancer to develop, and sensitivity to cell death also governs therapeutic efficacy. **Stephen Tait**'s group (see page 60) is focused on understanding how mitochondria control apoptotic cell death and addressing how this is deregulated in cancer. Similarly, cell immortality is one of the hallmarks of cancer development and is directly controlled by telomerase. Research in **Nicol Keith**'s laboratory has demonstrated the validity of this target through diverse telomerase targeting approaches. The lab has discovered key targets that regulate telomere homeostasis and is translating these findings on telomerase, cell senescence and telomere dysfunction into biomarker clinical studies in patients. **Paul Shiels**' group has also developed and validated biomarkers of ageing for use in basic research and translational applications. Specifically, they demonstrated that the CDKN2 locus could be used to gauge the impact of non cell-autonomous senescence in disease states. For example, they demonstrated that cellular biological age, proliferation and immune cell infiltrates were independent prognostic factors in a cohort of patients with colorectal cancer.

Pancreatic cancer biology and genomics is a major research focus for a number of research groups, and with potential clinical application (**Andrew Biankin**, **Sean Grimmond**, **David Chang**, **Liz Musgrove**). Pancreatic cancer is one of the most lethal and molecularly diverse malignancies. Re-purposing of therapeutics that target specific molecular mechanisms in different disease types offers potential for rapid improvements in outcome. Anti-HER2 therapies have a proven track record in the treatment of breast cancer, and the groups have now established diagnostic criteria for HER2 amplification in pancreatic cancer. Although the incidence of HER2 amplification in pancreatic cancer is low (2%), it represents an attractive therapeutic target because treatment options are so limited in this disease.

A collaborative study with Cold Spring Harbor Laboratories and the Memorial Sloan-Kettering Cancer Centre has shown that inhibition of signalling through PDGFRβ can block metastasis promoted by mutations in the *TP53* gene, which are common in pancreatic cancer. High PDGFRβ expression correlates with poor disease-free survival in pancreatic, colon and ovarian cancer patients, implicating PDGFRβ as a prognostic marker and possible target for attenuating metastasis in p53 mutant tumours.

The groups also contributed to a major international catalogue of somatic mutations in cancer genomes. The results reveal the diversity of mutational processes underlying the development of cancer, with potential implications for understanding of cancer aetiology and prevention. Importantly, they also identify mutational patterns that may provide a basis for a mutational phenotype-guided therapeutic strategy, in which next generation sequencing is used to screen for mutations that characterise cancers that are vulnerable to specific existing targeted therapies.

Other emerging research interests include oncogene-induced vulnerabilities (**Daniel Murphy**, see page 58), while **Katie Wakeham** is developing a research programme investigating the prognostic significance of human papillomavirus in vulvar intraepithelial neoplasia, vulvar cancer and anal cancer, and the prevalence of HPV type-specific genotypes in oropharyngeal cancers diagnosed in the West of Scotland.

Ovarian cancer research is led by **Iain McNeish**. One major research focus is the biology of acquired platinum resistance in high grade serous ovarian cancer. The BriTROC project, funded by Ovarian Cancer Action and Cancer Research UK, and run in collaboration with James Brenton at the CRUK Cambridge Institute, is the largest-ever translational study of relapsed high-grade serous disease. Tumour biopsies are being acquired at relapse from 300 women in 9 UK centres, to allow comparative

genomic analysis of relapsed disease with disease in the same patient at the time of diagnosis. The other main research area focuses on novel biological therapies, especially oncolytic virus therapy. Work in 2013 demonstrated that oncolytic vaccinia induces death through pathways of programmed necrosis and an ongoing collaboration with Stephen Tait will extend this work to adenovirus and HSV. In addition, the group demonstrated that taxane resistance increases activity of oncolytic adenoviruses through two separate mechanisms - increased expression of the primary adenovirus receptor CAR and dysregulated cell cycle control. These results have shaped the design of the phase 1 OCTAVE trial (intraperitoneal administration of oncolytic adenovirus ColoAd1 in relapsed ovarian cancer), which will open in May 2014. Collaboration with Gerry Graham (University of Glasgow) will investigate how innate immune responses to oncolytic adenovirus and HSV modulate overall anti-cancer efficacy. The relationship between p53 mutation, inflammation and survival in high-grade serous ovarian cancer is being pursued by **Patricia Roxburgh** (Clinical Lecturer in Medical Oncology) in collaboration with Karen Vousden and Iain McNeish.

Breast cancer is the research focus for **Iain MacPherson**, **Torsten Stein** and **Joanne Edwards**. Iain Macpherson studies the role endo/exocytic trafficking in breast cancer in collaboration with Jim Norman's group, ultimately with the aim of developing novel classes of therapeutic agents. A specific focus is on the diacylglycerol kinase/Rab-coupling protein axis that the Norman lab has previously identified as a key regulator of integrin and receptor tyrosine kinase trafficking from recycling endosomes, and to test the hypothesis that this a major determinant of the aggressiveness of luminal B and HER2-positive breast cancers. **Torsten Stein**'s interest is in mammary gland morphogenesis and how these processes may be hijacked or deregulated in breast cancer. He has demonstrated that annexin A8 expression is associated with a subgroup of breast cancers with poor prognosis, including hereditary breast cancers that carry a dysfunctional BRCA1 gene. One of the major causes of breast cancer related mortality is the development of endocrine resistance. **Joanne Edwards** has identified IKKα as a target in endocrine resistant breast cancer and is now working in collaboration with University of Strathclyde to assess IKKα inhibitors and identify markers to predict response to these in patients with breast cancer. The group has extended its studies of endocrine resistance to prostate cancer, having demonstrated that androgen receptor (AR) phosphorylation at serine 81 in response to androgens may predict response to Abiraterone, and AR phosphorylation at serine 213 by Akt may predict response to PI3K/Akt inhibitors. The Prostate Cancer Biology group is led by **Hing Leung** (see page 16), with **Prabs Rajan** and **Imran Ahmad** as junior investigators in clinical academic urology.

Leukaemias are a major disease-specific research interest in the Institute of Cancer Sciences, led by **Tessa Holyoake** (Director, POG-LRC). These diseases of the haemopoietic system arise from normal stem and progenitor cells that have acquired corrupted cell fate decisions. These aberrant leukaemic stem cells (LSCs) initiate and drive the process of leukaemogenesis. The research programmes within the POG-LRC collectively focus on understanding the fundamental mechanisms governing normal haemopoietic stem cell and LSC functions, identifying novel therapeutic targets against LSCs and exploiting these targets in clinical trials.

The Chronic Myeloid Leukaemia (CML) group (**Tessa Holyoake**) focuses on the identification and characterisation of normal versus LSCs in CML aiming to elucidate survival factors or pathways that selectively maintain leukaemic stem and progenitor cells, and to develop novel approaches to target LSC maintenance. A systems biology approach to compare normal versus LSCs in terms of gene, miRNA and protein expression and activity, and to understand drug resistance in the LSC population, is being adopted.

Recently opened Wolfson Wohl Cancer Research Centre building

The Autophagy group (**Vignir Helgason**) is focused on the inhibition of autophagy, a survival process that can protect CML LSCs following tyrosine kinase inhibition (TKI) drug treatment. The group has identified alternative signalling pathways that provide survival signals following oncoprotein inhibition, providing a rationale for testing the effect of specific inhibitors against these pathways on the survival of TKI-resistant cells and primary cells from TKI-resistant patients.

The Paediatric and Adult Acute Leukaemia group (**Karen Keeshan**) is primarily focused on mouse models and acute leukaemias. The group's interest is in identifying and characterising novel factors that play a role in paediatric and adult leukaemia, extending into the study of molecular events that have a role in stem and progenitor cell function and lineage fate in normal haematopoiesis and in both adult and paediatric AML.

The Molecular Lymphopoiesis group (**Alison Michie**) initially focused research efforts on investigating the molecular events that regulate lymphocyte lineage commitment and development. While investigating the role of PKCα in early lymphocyte development, the group established that subversion of PKCα signalling acts as an oncogenic trigger in B lymphocytes, resulting in the development of chronic lymphocytic leukaemia.

The Stem Cell group (**Helen Wheadon**) focuses on developmental pathways involved in normal and aberrant haemopoiesis, especially signals acting along conserved embryonic morphogenic pathways. Increasing evidence suggests that these embryonic morphogenic pathways are important for controlling self-renewal behaviour of haemopoietic and cancer stem cells (CSCs). The main focus is to determine the role these pathways play in CSC self-renewal and leukaemia disease progression.

The Leukaemia Stem Cell Self-Renewal group (**Mhairi Copland**) studies the Notch and Hedgehog signalling pathways in myeloid leukaemias, and quiescence as a mechanism of drug resistance in LSCs. Additional research

interests include the interactions between LSCs and their bone marrow niche, in particular via the CXCL12/CXCR4 axis, and validation of induced pluripotent stem cells as an acceptable alternative model for preclinical drug screening in haematological malignancies.

The Experimental Radiation Oncology group (**Rob Mairs**) is developing targeted strategies to improve the biological specificity and effectiveness of radiation treatment by the selective delivery of radionuclides to tumour cells, by enhanced uptake of radiolabelled targeting agents and by the selective sensitisation of tumour cells to radiation. Their observations of synergy between targeted therapy using [131I]meta-iodobenzylguanidineand topotecan without marrow toxicity provided the rationale for current combination approaches applied clinically in Europe and the USA.

The key objectives of the Translational Radiation Biology group (**Anthony Chalmers**) are to evaluate the clinical potential of novel inhibitors of the DNA damage response when delivered in combination with radiotherapy and/or chemotherapy. The effects of DNA repair inhibitors in combination with radiotherapy and chemotherapy have been evaluated using a panel of intracranial models of glioblastoma that recapitulate the key features of the disease in patients. The mechanisms underlying the *in vivo* effects are being investigated in two- and three-dimensional in vitro models of glioblastoma that incorporate glioma stem cells, hypoxia and other features of the tumour micro-environment. The effects of novel anti-invasive drugs on glioblastoma models *in vitro* and *in vivo* are being pursued in collaboration with Mike Olson and Martin Drysdale, supported by the Brain Tumour Charity. The clinical potential of these approaches are being evaluated in a number of early phase clinical studies of PARP inhibition in combination with chemotherapy in recurrent glioblastoma and with radiotherapy in newly diagnosed disease.

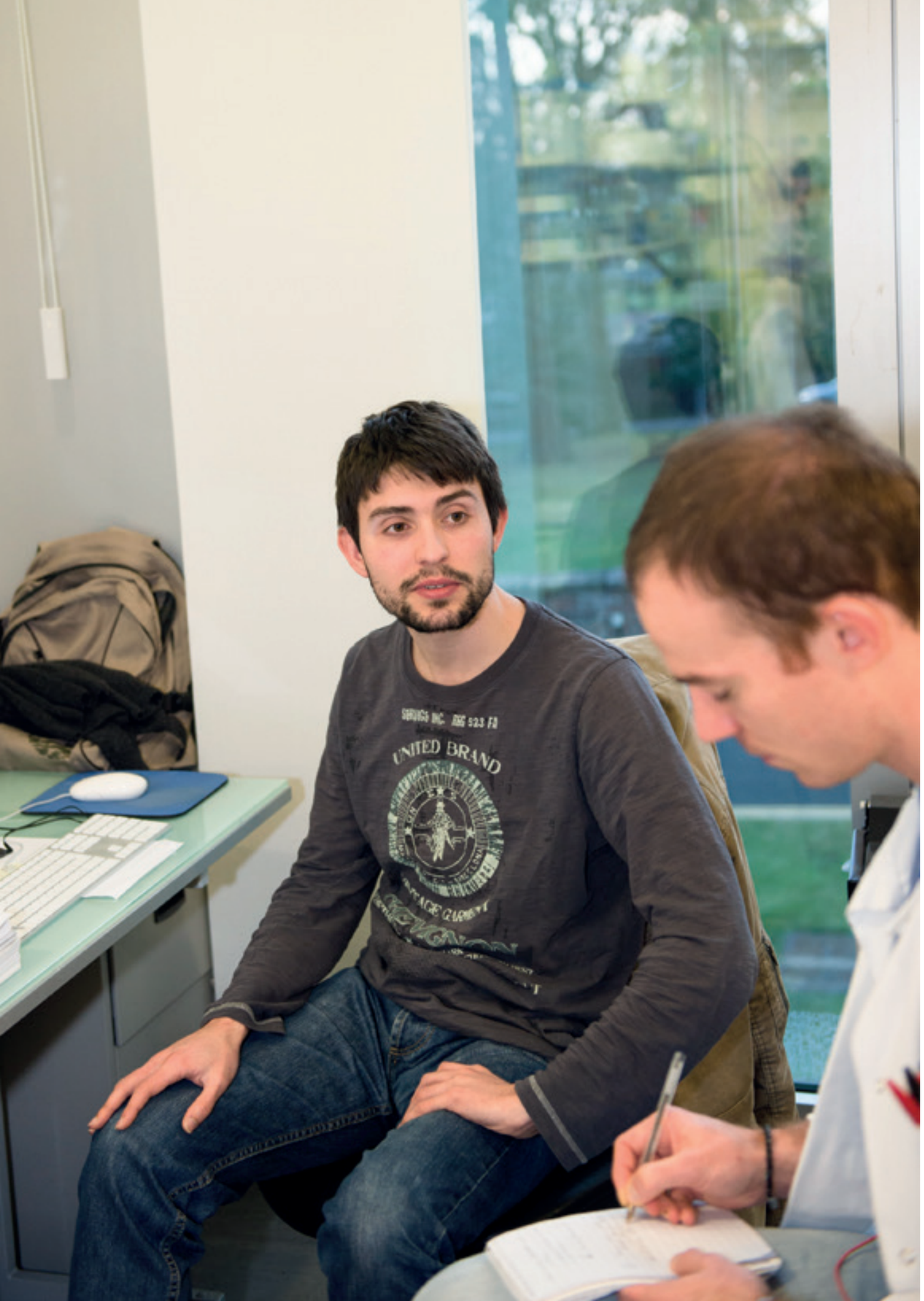
Translational research often requires high quality human tissue samples, which are collected with full patient consent and accompanying clinical, pathological and follow-up information. In Glasgow, such tissue samples are collected, stored and distributed to researchers by the Glasgow Bio-Repository, led by **James Going**. This resource is funded by the NHS, Chief Scientist Office and CRUK (via the Glasgow ECMC), and its importance to research has been underscored by the successful contribution to phase 1 of CRUK's Stratified Medicine Programme, led by **Karin Oien**. The concept of molecular profiling to optimise patient management is currently being explored in the national stratified medicine trial in patients with advanced lung cancer and in the CUP-ONE study, which evaluates a number of molecular classifiers in patients with carcinoma of unknown primary to refine treatment regimens based on the molecular classification of the tumour and its likely primary origin (Karin Oien).

The Glasgow Experimental Cancer Medicine Centre (ECMC), led by **Jeff Evans**, and the CRUK Glasgow Clinical Trials Unit are the conduits to translate our findings through to clinical evaluation, while **Stefano Schipani** leads the Clinical Radiotherapy Research group in functional imaging, stereotactic sblative radiotherapy and drug-radiotherapy combination studies.

The objectives of the Glasgow ECMC are to take ideas from laboratory programmes into clinical studies, with a specific emphasis of early phase clinical trials (including industry alliances) and in biological markers including pharmacokinetic, predictive and pharmacodynamic bioassays. Highlights of the early phase trials programme include the completion of the phase 1b component of an ECMC- Astra Zeneca Alliance study (FGFR inhibitor) and a successful funding application for an Alliance study to support a chemo-radiation study in locally advanced pancreatic cancer. The laboratory component of the ECMC is the Analytical Services Unit (ASU) in which sample acquisition, handling, processing and reporting are all performed to standards that allow us to make important decisions on the drug development pathway based on the results of these bioassays. These include PK studies in support of hydroxychloroquine (CML) and docetaxel (prostate cancer), and PD assays of Mdm2 (prostate cancer) and PARP inhibition (pancreatic cancer) and investigation of the role of markers associated with cellular senescence in blood samples from patients with gastro-oesophageal cancer during chemotherapy treatment. Exploratory studies of novel biomarkers include collaborations with Gerry Graham (University of Glasgow, melanoma), Chiara Braconi (HCC), and Rhian Touyz (University of Glasgow) into mechanisms of hypertension induced by VEGF inhibition.

The CRUK Clinical Trials Unit (**Rob Jones**) develops, co-ordinates and delivers national and international multicentre studies, and has UKCRC (UK Clinical Research Collaboration) registration. Several new trials have been designed and funded from a variety of sources over the past year that will ensure our central place in the organisation of large-scale trials in the UK and internationally, and our collaborations with industry via NCRN/CRUK and the ECMC Combination Alliances continue to flourish. These trials play on our core strengths in gynaecological cancer, GI cancer, urological malignancies and radiotherapy studies, and we are also developing a portfolio of studies in lung cancer. Many of these studies reflect our growing expertise in collaborating with international collaborative groups, including the IRCI (International Rare Cancer Initiative). Specific highlights are the completion of accrual to the SCOT study, which recruited 6,087 patients from 237 investigator sites across 7 countries, and completion of accrual to KPS (Ketamine Pain Study), which is the largest randomised phase 3 trial of pain intervention in the UK (n=214). In support of the clinical trials strategy, Rob Jones, Iain McNeish, Iain Macpherson, Anthony Chalmers, Andrew Biankin, Karin Oien, Mhairi Copland and Jeff Evans are all members of NCRN Clinical Studies Groups or sub-groups, and we have consolidated our longstanding collaborative links with our research active cancer researchers in NHS Greater Glasgow & Clyde, particularly through the NHS Research Scotland research awards from the Chief Scientist Office, Scotland.

Public engagement remains an important theme to our research programmes and Chloe Cowan, CRUK Senior Research and Information Nurse, leads on public engagement and education. In addition to our research programmes, education remains an important core activity and Karin Oien, Katherine West and Torsten Stein have led the development of new postgraduate taught courses that will commence in 2014.



RESEARCH SUPPORT AND MANAGEMENT

CANCER RESEARCH UK BEATSON INSTITUTE

RESEARCH FACILITIES

Head of Research Facilities
Sue Fowler

Research Facilities supports research groups at the Beatson Institute and University of Glasgow on the Beatson site. This year we said farewell to Robert McFarlane who retired as Laboratory Manager after 42 years at the Beatson. We welcomed Laura Bence who joined in May and took over as the new Laboratory Manager. We congratulate Richard Selkirk our Health and Safety Officer who has attained chartered membership of the Institute of Occupational Health and Safety (IOSH). There has been continued investment in new equipment for Information Services and Histology. Information Services have invested in new equipment to increase the available network storage and Histology has purchased a fully automated Leica slide scanner for the capture and storage of high quality digital images. Building Facilities have been active with a number of projects to adapt laboratory space to accommodate specialist equipment.

Building Facilities

Alistair Wilson, Alex Kernahan, Michael Daly, Don MacBean[1]

[1] until January

Building Facilities manage the outsourced services provision for catering, cleaning and janitorial services. We provide maintenance support for the Beatson Institute buildings and manage alterations and refurbishments. This year the workshop repair and modification service was closed due to space constraints, as the building where it was located is due for demolition.

Use of the online helpdesk facility continues to be an effective means of logging reactive calls for maintenance and repair. Minor project work continues at a fairly high level. A number of building services have been altered or extended to accommodate new equipment. This year PAT testing has been completed for all portable appliances in the Institute.

Central Services

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

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

Histology Service

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

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

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

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

The Institute has a Leica LMD6500 laser microdissection system that allows subpopulations of tissue cells to be procured from histological prepared slides under microscopic visualisation. We are able to cut sections from both cryostat and paraffin blocks onto specialised slides, which can be stained appropriately allowing cellular material to be identified and separated to permit subsequent downstream analysis to be performed. Consultation regarding the downstream analysis is imperative prior to work beginning as this allows the correct protocols and procedures to be used to maximise the results obtained from the specific analysis required. Both DNA and RNA material can be retrieved from the tissue sections for downstream analysis.

A fully automated large capacity Leica SCN400F slide scanner has been installed into Histology capable of capturing bright-field or fluorescent images. This allows high quality digital images to be scanned and stored and if required quantitative interpretation can be performed.

Information Services

Peter McHardy, Iain White

Information Services provide a wide range of support services, including server support, hardware cover, an on-site helpdesk providing both repair and software support as well as help in hardware selection and user training. There are over 350 users with nearly 400 PCs on site comprising a mixture of Windows computers, Apple Macs and Linux machines, with central authentication, central file store and print sharing.

The servers provide in excess of 500 TB of online storage with nightly backups and tapes stored off-site, to provide support for microscopy, DNA sequencing and mass spectrometry data. Data backup facilities are now available for laptop users when they are off-site, aimed at reducing data loss.

All PCs are built with a common desktop environment, around Windows or Mac OS X and Microsoft Office and are actively managed and upgraded to ensure the best possible working environment. Mac OS X Mountain Lion has been rolled out across the site and we have completed the upgrade of relevant Windows computers to Windows 7. All e-mail services run on Microsoft Exchange which allows local client-based access and web access to email as well as delivering email, diaries and address books to mobile devices including iPhones, iPads and other smart phones.

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

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

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

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

New Laboratory Manager
Laura Bence

Laboratory Management

Laura Bence[1], Robert McFarlane[2], Richard Selkirk, Michael McTaggart, Joe McFadden, George Monteith

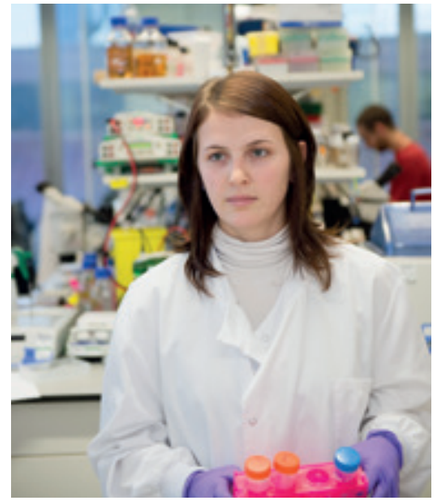
[1] from May, [2] until August

Laboratory Management is responsible for providing advice and information to scientists on health and safety, particularly on how to carry out risk assessments and on appropriate control measures. Safety plays an important part of everyday life in the laboratory and in running the building services. We administer the monitoring and training elements on a day-to-day basis, identifying training needs and ensuring adequate provision is made to fulfil the Institute's legal obligations to staff. This year we have trained a number of new first aiders and fire marshalls. To enhance safety we have introduced an improved training programme for users of our cryostore and radiation suites.

A major function of Laboratory Management is the overseeing of shared equipment servicing, replacement and the purchase of new equipment to facilitate the needs of researchers. We have effective procurement processes and liaise with Cancer Research UK purchasing to take advantage of any centralised agreements. A further essential role is the monitoring of all outgoing orders to ensure compliance with Institute safety procedures, particularly those relating to COSHH.

Service contracts for core equipment are procured centrally and maintenance or repairs are coordinated to ensure these are dealt with as efficiently as possible. This year we have installed a new freezer alarm system that allows us to monitor and interrogate freezer alarms remotely. This enables us to attend to alarm situations more efficiently and to forestall any potential issues before a freezer fails.

The stores facility stocks a wide range of consumables with rapid re-stocking to ensure high use materials are always available. Items can be withdrawn on a self-service basis with automatic cost centre allocation via swipe card. A porter service is run to deliver external orders to the researchers, while stores processes any outgoing samples or materials for courier collection. We continue to review the services provided by stores to try to improve what is on offer to the scientific staff. This has required negotiating preferential pricing with our suppliers at a local level. As a result of these negotiations and better turnaround times from suppliers, we have been able to reduce the overall value of stock held without compromising supply lines to the laboratories.



Molecular Technology and Reagent Services

Billy Clark, Deborah Gardner, Andrew Keith

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

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

Human cell line authentication using the Promega Geneprint 10 Kit is available as an internal service. The samples are run on the Applied Biosystems 3130xl Sequencer (Gene Fragment Analysis) and analysed using Genemapper v4.0 software (Applied Biosystems). Regular cell line authentication is important both to confirm integrity of data and is increasingly requested by journals as a requirement prior to publication

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

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

RESEARCH PUBLICATIONS

Kurt Anderson (page 24)
Tumour Cell Migration

Primary Research Papers
Müllenbroich CM, McGhee EJ, Wright AJ, Anderson KI, Mathieson K.
Adaptive nonlinear microscopy for whole tissue imaging. ***Proc. SPIE 8588***, Multiphoton Microscopy in the Biomedical Sciences XIII, 85881X (February 22, 2013); doi:10.1117/12.2006375

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ROS production and NF-kappaB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. ***Cell Stem Cell*** 2013; 12: 761-73

Nobis M, McGhee EJ, Morton JP, Schwarz JP, Karim SA, Quinn J, Edward M, Campbell AD, McGarry LC, Evans TR, Brunton VG, Frame MC, Carragher NO, Wang Y, Sansom OJ, Timpson P, Anderson KI.
Intravital FLIM-FRET imaging reveals dasatinib-induced spatial control of src in pancreatic cancer. ***Cancer Res*** 2013; 73: 4674-86

Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, Mackay G, Mrowinska A, Au A, Rai TS, Zheng L, Ridgway R, Adams PD, Anderson KI, Gottlieb E, Sansom OJ, Ryan KM.
p53 status determines the role of autophagy in pancreatic tumour development. ***Nature*** 2013; 504: 296-300

Trinidad AG, Muller PA, Cuellar J, Klejnot M, Nobis M, Valpuesta JM, Vousden KH.
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Nobis M, Carragher NO, McGhee EJ, Morton JP, Sansom OJ, Anderson KI, Timpson P.
Advanced intravital subcellular imaging reveals vital three-dimensional signalling events driving cancer cell behaviour and drug responses in live tissue. ***FEBS J*** 2013; 280: 5177-97

Karen Blyth (page 53)
Transgenic Models of Cancer

Primary Research Papers
Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, Athineos D, Kang TW, Lasitschka F, Andrulis M, Pascual G, Morris KJ, Khan S, Jin H, Dharmalingam G, Snijders AP, Carroll T, Capper D, Pritchard C, Inman GJ, Longerich T, Sansom OJ, Benitah SA, Zender L, Gil J.
A complex secretory program orchestrated by the inflammasome controls paracrine senescence. ***Nat Cell Biol*** 2013; 15: 978-90

Cheung EC, Athineos D, Lee P, Ridgway RA, Lambie W, Nixon C, Strathdee D, Blyth K, Sansom OJ, Vousden KH.
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Hock AK, Lee P, Maddocks OD, Mason SM, Blyth K, Vousden KH.
iRFP is a sensitive marker for cell number and tumor growth in high-throughput systems. ***Cell Cycle*** 2013; 13: 220-6

Myant KB, Cammareri P, McGhee EJ, Ridgway RA, Huels DJ, Cordero JB, Schwitalla S, Kalna G, Ogg EL, Athineos D, Timpson P, Vidal M, Murray GI, Greten FR, Anderson KI, Sansom OJ.
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Zheng L, Mackenzie ED, Karim SA, Hedley A, Blyth K, Kalna G, Watson DG, Szlosarek P, Frezza C, Gottlieb E.
Reversed argininosuccinate lyase activity in fumarate hydratase-deficient cancer cells. ***Cancer Metab*** 2013; 1: 12

Martin Drysdale (page 44)
Drug Discovery Programme

Other publications
Drysdale MJ.
Fragment-based Drug Discovery: the Shape of Things to Come. ***Aust J Chem*** 2013; 66: 1544-9

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Fragment-based hit identification: thinking in 3D. ***Drug Discov Today*** 2013; 18: 1221-7

Jeff Evans (page 26)
Translational Cancer Therapeutics

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Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, Athineos D, Kang TW, Lasitschka F, Andrulis M, Pascual G, Morris KJ, Khan S, Jin H, Dharmalingam G, Snijders AP, Carroll T, Capper D, Pritchard C, Inman GJ, Longerich T, Sansom OJ, Benitah SA, Zender L, Gil J.
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Activation of the IL-6R/Jak/stat pathway is associated with a poor outcome in resected pancreatic ductal adenocarcinoma. ***J Gastrointest Surg*** 2013; 17: 887-98

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Eyal Gottlieb (page 12)

Apoptosis and Tumour Metabolism

Primary Research Papers

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*corresponding authors

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Danny Huang (page 14)

Ubiquitin Signalling

Primary Research Papers

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Robert Insall (page 28)

Cell Migration and Chemotaxis

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Predicting cell shapes in melanomas. ***Pigment Cell Melanoma Res*** 2014; 27: 5-6

Gabriela Kalna (page 49)
Bioinformatics

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Gillian Mackay (page 50)

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Nick Morrice (page 51)

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Owen Sansom (page 36)

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Douglas Strathdee (page 54)
Transgenic Technology

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Marcos Vidal (page 38)
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Karen Vousden (page 20)
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Sara Zanivan (page 40)
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Chk1 is essential for the development of murine epidermal melanocytes. ***Pigment Cell Melanoma Res*** 2013; 26: 580-5

Talapatra SK, Anthony NG, Mackay SP, Kozielski F.
Mitotic kinesin Eg5 overcomes inhibition to the phase I/II clinical candidate SB743921 by an allosteric resistance mechanism. ***J Med Chem*** 2013; 56: 6317-29

Ulaganathan V, Talapatra SK, Rath O, Pannifer A, Hackney DD, Kozielski F.
Structural insights into a unique inhibitor binding pocket in kinesin spindle protein. ***J Am Chem Soc*** 2013; 135: 2263-72

Peter D. Adams (page 56)

Epigenetics of Cancer and Ageing

Primary Research Papers

Cruickshanks HA, McBryan T, Nelson DM, Vanderkraats ND, Shah PP, van Tuyn J, Singh Rai T, Brock C, Donahue G, Dunican DS, Drotar ME, Meehan RR, Edwards JR, Berger SL, Adams PD.
Senescent cells harbour features of the cancer epigenome. ***Nat Cell Biol*** 2013; 15: 1495-506

Howard L, Mackenzie RM, Pchelintsev NA, McBryan T, McClure JD, McBride MW, Kane NM, Adams PD, Milligan G, Baker AH.
Profiling of transcriptional and epigenetic changes during directed endothelial differentiation of human embryonic stem cells identifies FOXA2 as a marker of early mesoderm commitment. ***Stem Cell Res Ther*** 2013; 4: 36

Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, Shah PP, Hewitt G, Korolchuk VI, Passos JF, Wu H, Berger SL, Adams PD.
Lysosome-mediated processing of chromatin in senescence. ***J Cell Biol*** 2013; 202: 129-43

Pawlikowski JS, McBryan T, van Tuyn J, Drotar ME, Hewitt RN, Maier AB, King A, Blyth K, Wu H, Adams PD.
Wnt signaling potentiates nevogenesis. ***Proc Natl Acad Sci USA*** 2013; 110: 16009-14

Pchelintsev NA, McBryan T, Rai TS, van Tuyn J, Ray-Gallet D, Almouzni G, Adams PD.
Placing the HIRA histone chaperone complex in the chromatin landscape. ***Cell Rep*** 2013; 3: 1012-9

Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, Mackay G, Mrowinska A, Au A, Rai TS, Zheng L, Ridgway R, Adams PD, Anderson KI, Gottlieb E, Sansom OJ, Ryan KM.
p53 status determines the role of autophagy in pancreatic tumour development. ***Nature*** 2013; 504: 296-300

Sawicka M, Pawlikowski J, Wilson S, Ferdinando D, Wu H, Adams PD, Gunn DA, Parish W.
The specificity and patterns of staining in human cells and tissues of p16INK4a antibodies demonstrate variant antigen binding. ***PLoS One*** 2013; 8: e53313

Shah PP, Donahue G, Otte GL, Capell BC, Nelson DM, Cao K, Aggarwala V, Cruickshanks HA, Rai TS, McBryan T, Gregory BD, Adams PD*, Berger SL*.
Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape. ***Genes Dev*** 2013; 27: 1787-99
*corresponding authors

Other publications

Lund K, Adams PD, Copland M.
EZH2 in normal and malignant hematopoiesis. ***Leukemia*** 2014; 28: 44-9 Epub 2013/10/08

Pawlikowski JS, Adams PD, Nelson DM.
Senescence at a glance. ***J Cell Sci*** 2013; 126: 4061-7

Stephen Tait (page 60)

Mitochondria and Cell Death

Primary Research Papers

Tait SW, Oberst A, Quarato G, Milasta S, Haller M, Wang R, Karvela M, Ichim G, Yatim N, Albert ML, Kidd G, Wakefield R, Frase S, Krautwald S, Linkermann A, Green DR.
Widespread Mitochondrial Depletion via Mitophagy Does Not Compromise Necroptosis. ***Cell Rep*** 2013; 5: 878-85

Other Publications

Tait, S.
DNA: leukemia's secret weapon of bone mass destruction. ***Oncogene*** 2013; 32: 5199-200

Tait SW, Green DR.
Mitochondrial regulation of cell death. ***Cold Spring Harb Perspect Biol*** 2013; 5: a008706

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 Barbara Chaneton from Eyal Gottlieb's group. Barbara's work aims to characterise the metabolic adaptations made by cancer cells with the goal of then targeting these as anti-cancer treatments.

Theses

Cameron, Jenifer (2013) Investigating the role of cofilin oxidation in cancer cell migration and invasion [PhD thesis, University of Glasgow, Beatson Institute]

Derkits, Sahra (2013) Investigating the role of PTEN and LKB1 in traditional and serrated mouse models of colorectal cancer [PhD thesis, University of Glasgow, Beatson Institute]

Dou, Hao (2013) Mechanism of ubiquitin transfer by RING E3 ligases [PhD thesis, University of Glasgow, Beatson Institute]

Dowding, Sarah (2013) The regulation of RNA polymerase III-mediated transcription by p53, AP-1 and JNKs [PhD thesis, University of Glasgow, Beatson Institute]

Ferrari, Nicola (2013) Investigating Runx transcription factors in mammary gland development and breast cancer [PhD thesis, University of Glasgow, Beatson Institute]

Galbraith, Laura (2013) The role of cardiolipin in mitophagy [PhD thesis, University of Glasgow, Beatson Institute]

Klejnot, Marta (2013) Structural and Biochemical insights into members of the kinesin and ubiquitin ligase families [PhD thesis, University of Glasgow, Beatson Institute]

Moreaux, Guenièvre (2013) Investigating downstream effectors of KRas signalling in vivo: Dusp6 and Fra1 [PhD thesis, University of Glasgow, Beatson Institute]

Macagno, Juan (2013) A study of focal adhesion kinase in cancer using Drosophila melanogaster [PhD thesis, University of Glasgow, Beatson Institute]

Muinonen-Martin, Andrew (2013) Melanoma cells induce LPA gradients that drive chemotactic dispersal and invasion [PhD thesis, University of Glasgow, Beatson Institute]

Rosenfeldt, Mathias (2013) p53 and the role of autophagy in pancreatic cancer development [PhD thesis, University of Glasgow, Beatson Institute]

Roxburgh, Patricia (2013) Manipulating the p53 pathway for cancer treatment [PhD thesis, University of Glasgow, Beatson Institute]

Schachtner, Hannah (2013) Investigation of the functional and structural role of podosomes in megakaryocytes [PhD thesis, University of Glasgow, Beatson Institute]

Smith, Joanne (2013) Role of checkpoint kinase 1 in malignant melanoma [PhD thesis, University of Glasgow, Beatson Institute]

Stefanatos, Rhoda (2013) Modelling tumourigenesis and the stress response in Drosophila melanogaster [PhD thesis, University of Glasgow, Beatson Institute]

Stevenson, Richard (2013) Investigating the role of fascin in murine models of inflammatory bowel disease and intestinal tumourigenesis [PhD thesis, University of Glasgow, Beatson Institute]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Targeting the Tumour Stroma

7 - 10 July 2013

Bute Hall, University of Glasgow

Scientific Committee: Jim Norman, Jeff Evans, Michael Olson, Owen Sansom, Marcos Vidal, Sara Zanivan

This year's conference, which started a little earlier than previously, offered participants a packed and exciting programme. On the first evening, the Colin Thomson Memorial Keynote Lecture, sponsored by the Association for International Cancer Research, was given by Robert Kerbel (Toronto) who described his studies to develop better models for predicting the outcome of clinical trials. Throughout the four days, there was a range of other excellent speakers including Alexander Anderson, Neta Erez, Elisa Espinet, Yi Feng, Robert Insall, Jennifer Munson, Alexandra Naba, Juan Rodriguez-Manzaneque, Michael Samuel, Herbert Schiller and Viviana Vallacchi who gave selected short talks and Owen Sansom whose talk was sponsored by the journal Disease Models & Mechanisms. Neta Erez (Tel Aviv) was awarded the Portland Press sponsored prize for her talk describing work to characterise the role of cancer-associated fibroblasts in breast cancer progression. Following the poster session, Jean Albrengues (Nice), working on LIF cytokine and invasion, and Thomas Cox (Copenhagen), using proteome analysis to study bone metastasis, were jointly awarded the AMSBIO-Trevigen sponsored prize. The meeting was generously co-sponsored by Cancer Research UK and the Association for International Cancer Research.

The 2014 meeting will focus on the oncogenic signals that initiate and regulate metabolic rewiring as well as the adaptability of the metabolic network to stress. New therapeutic opportunities will also be discussed (see www.beatson.gla.ac.uk/conf for details).

Beatson Retreat

An Institute retreat was held in our lecture theatre on 27th September, giving everyone a chance to hear about the latest, exciting work being done by researchers here as well as some of that planned for the recently opened Translational Cancer Research Centre (TCRC) next door. Laura Machesky and Kevin Ryan described ongoing projects in their groups, while Martin Drysdale gave an update on the progress of the Drug Discovery Programme, which had its first quinquennial review this year. There were talks by Beatson Associates Daniel Murphy and Stephen Tait, who are relatively recent arrivals, and by Iain McNeish and David Chang from the TCRC who outlined their plans, which focus on ovarian and pancreatic cancer respectively.

Sir Paul Nurse FRS on a visit to the Institute (image courtesy of Andreas Hock)

Visit by Royal Society President, Sir Paul Nurse FRS

Paul Nurse and members of the Royal Society visited the Institute on 1st November. Graduate students Emma Woodham, Linda Julian and Barbara Chaneton along with clinical research fellow Colin Steele gave talks about their work, while graduate student Rhoda Stefanatos chaired a lively question and answer session with Paul and around a hundred students and postdocs. Paul was very generous with his time not only listening to the talks and quizzing the speakers but also then answering questions on a wide range of topics. It was a thought provoking and inspiring event for all who attend, especially the junior researchers.

Open Evening

Our opening evening, which was held on 13th March this year, attracted a large number of attendees, particularly local school pupils. The talks, given by Andrew Biankin (Director, TCRC), Owen Sansom (Deputy Director, Beatson Institute), Jen Morton (Associate Scientist) and Max Nobis (PhD Student), focused on the topical theme of personalised medicine and were followed a series of lab tours and demos by our hardworking volunteers.

For the second year in a row, we also offered 12 high school students the opportunity to visit us for a week in July to find out more about our work and get some hands-on experience in the labs. The feedback from this event was extremely positive and many thanks must go to Colin Nixon, Emmanuel Dornier, Louise Mitchell, Amy Au, Saadia Karim, Kirsteen Campbell, Emma Woodham, Olivia Susanto, Catherine Barber and Ken Davies who all did a considerable amount of work to make this a success.

Poster for 2014 conference

SEMINARS AT THE BEATSON INSTITUTE

The following seminars were held at the CR-UK Beatson Institute during 2013.

January

Wolfgang Wick, Department of Neuro-oncology, University of Heidelberg, Germany

Elton Zeqiraj, Samuel Lunenfeld Research Institute, Toronto, Canada

Paul Clarke, Division of Cancer Research, University of Dundee

Markus Ralser, Department of Biochemistry, University of Cambridge

Steve Kaiser, St Jude Children's Research Hospital, Memphis, USA

Ruth Muschel, CR-UK/MRC Gray Institute for Radiation Oncology & Biology, University of Oxford

February

Rob Cairns, Princess Margaret Hospital, Toronto, Canada

Ronen Zaidel-Bar, Mechanobiology Institute and NUS Division of Bioengineering, Singapore

Shehab Ismail, Max Planck Institute of Molecular Physiology, Dortmund, Germany

Elisabetta Dejana, Department of Biomolecular Sciences and Biotechnologies, University of Milan, Italy

Krishnaraj Rajalingam, Institute of Biochemistry II, Goethe University School of Medicine, Frankfurt, Germany

Juri Rappsilber, Wellcome Trust Centre for Cell Biology, University of Edinburgh

March

Hannah Brown, Department of Oncology, University of Sheffield

David Sabatini, Whitehead Institute, Cambridge, USA

Jean–Philippe Theurillat, Dana-Farber Cancer Institute, Boston, USA

Christian Pilarsky, Medical Faculty "Carl Gustav Carus", Technical University Dresden, Germany

Tatjana Crnogorac-Jurcevic, Centre for Molecular Oncology, Queen Mary University of London

Julia Newton-Bishop, Section of Epidemiology and Biostatistics, University of Leeds

April

Sakari Vanharanta, Sloan-Kettering Institute, New York, USA

Neil Rodrigues, Boston University, USA

Ghassan Mouneimne, Harvard Medical School, Boston, USA

Melda Tozluoglu, CR-UK London Research Institute

Jurre Kamphorst, Lewis-Sigler Institute for Integrative Genomics, Princeton University, USA

Martin McMahon, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, USA

David Adams, The Wellcome Trust Sanger Institute, London

Marion MacFarlane, MRC Toxicology Unit, Leicester

Kairbaan Hodivala-Dilke, Barts and The London School of Medicine and Dentistry

David Tuveson, CSHL Cancer Centre, Cold Spring Harbor Laboratory, New York, USA

May

Martin Sos, Cellular & Molecular Pharmacology, University of California, San Francisco School of Medicine, USA

June

Pascal Meier, The Breakthrough Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London

Andrew South, Division of Cancer Research, University of Dundee

Nigel Waterhouse, Queensland Institute for Medical Research, Brisbane, Australia

Fabien Llambi, St. Jude Children's Research Hospital, Memphis, USA

July

Oliver Hofmann, Bioinformatics Core, Harvard School of Public Health, Boston, USA

Chiara Gorrini, The Campbell Family Institute for Breast Cancer Research, Toronto, Canada

Christine Watson, Department of Pathology, University of Cambridge

August

Richard Gibbs, Human Genome Sequencing Center, Baylor College of Medicine, Houston, USA

Scott Lyons, CR-UK Cambridge Institute

Timour Baslan, Cold Spring Harbor Laboratory, New York, USA

September

Alexei Vazquez, Department of Radiation Oncology and Center for Systems Biology, Robert Wood Johnson Medical School, New Brunswick, USA

Clemens Schmitt, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Thomas Cox, Biotech Research and Innovation Centre, University of Copenhagen, Denmark

Shankar Balasubramanian, Department of Chemistry, University of Cambridge

October

Joao Pedro de Magalhaes, Institute of Integrative Biology, University of Liverpool

Jose Lizcano, Autonomous University of Barcelona, Spain

Jason Locasale, Division of Nutritional Sciences, Cornell University, New York, USA

David Bryant, Department of Anatomy University of California, San Francisco, USA

Jon Lane, School of Biochemistry, University of Bristol

Bin-bing Zhou, Shanghai Jiaotong University School of Medicine, China Elke Markert, Department of Medicine and Center for Systems Biology, Rutgers Cancer Institute of New Jersey, USA

John Stingl, Cancer Research UK, University of Cambridge

Richard Youle, National Institutes of Health, Bethesda, USA

November

Pierre Leopold, Institute of Biology Valrose, Université Nice Sophia Antipolis, France

Robert Goldman, Department of Cell and Molecular Biology, Northwestern University, Chicago, USA

Helen Mott, University of Cambridge

Jane Endicott, Northern Institute for Cancer Research, Newcastle University

December

Patrizia Agostinis, KU Leuven, Belgium

Xin Lu, Ludwig Institute, Oxford

STUDENTSHIPS AND POSTDOCTORAL FELLOWSHIPS

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

PhD Studentships

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

Our students are registered at the University of Glasgow and are allocated a supervisor and an advisor who are jointly responsible for supporting and monitoring their performance and progress. The supervisor is responsible for developing the student's abilities, providing all practical support required for the project and dealing with any administrative matters required in relation to the University or funding body. The advisor gives additional guidance by providing independent advice on any matters concerning the studentship and by attempting to resolve any problems that may arise.

Postdoctoral Research Scientists and Fellows

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

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

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

ADMINISTRATION

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

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

Finance & Human Resources

Peter Winckles, Caroline Preacher
Jacqui Clare, Karen Connor, Nicki Kolliatsas, Elaine Marshall, Frank McGeoghegan, Gary Niven, Lynn Wilson, Patricia Wylie

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

Secretarial

Laraine Kernahan *(PA to Professor Vousden), Rebecca Gebbie, Barbara Laing, Catriona Lambert, Sarah Price*

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

Scientific Administration

Jackie Beesley, *Catherine Winchester*

The Scientific Administration team supports scientists at the Beatson Institute by taking minutes at a range of scientific and administrative meetings, editing publications such as the Scientific Report, maintaining an up-to-date website, administering the graduate student training programme, and providing reports and answering queries for both internal and external audiences, including Cancer Research UK. The team also assists researchers in identifying and applying for external grant funding and has a role in providing oversight , training and advice in all aspects of research integrity.

Cancer Research Technology

Maria Lopalco

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

THANKS FOR SUPPORTING US

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

Cancer Research UK Beatson Institute

Kurt Anderson
Carestream, Technology Strategy Board

Karen Blyth
Royal Society

Martin Drysdale
Medical Research Council (with MO)

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

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

Robert Insall
Wellcome Trust

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

Laura Machesky
Association for International Cancer Research, Breast Cancer Campaign, Medical Research Council

Nick Morrice
Association for International Cancer Research

Jim Norman
Breast Cancer Campaign, CSIC (Spanish National Research Council)

Michael Olson
Breast Cancer Campaign, Medical Research Council (with MD)

Kevin Ryan
Association for International Cancer Research, EMBO

Owen Sansom
Association for International Cancer Research, Bioven, EMBO, European Community, Institute of Cancer Research, Medical Research Council, Medimmune, NC3Rs (with MV), Royal Society, Wellcome Trust

Emma Shanks
Breast Cancer Campaign

Marcos Vidal
NC3Rs (with OS)

Karen Vousden
Association for International Cancer Research, European Community, Netherlands Organisation for Scientific Research, NHS Greater Glasgow & Clyde Health Board Endowment Fund, Rubicon, West of Scotland Women's Bowling Association

Sara Zanivan
Breast Cancer Campaign

Beatson Associates

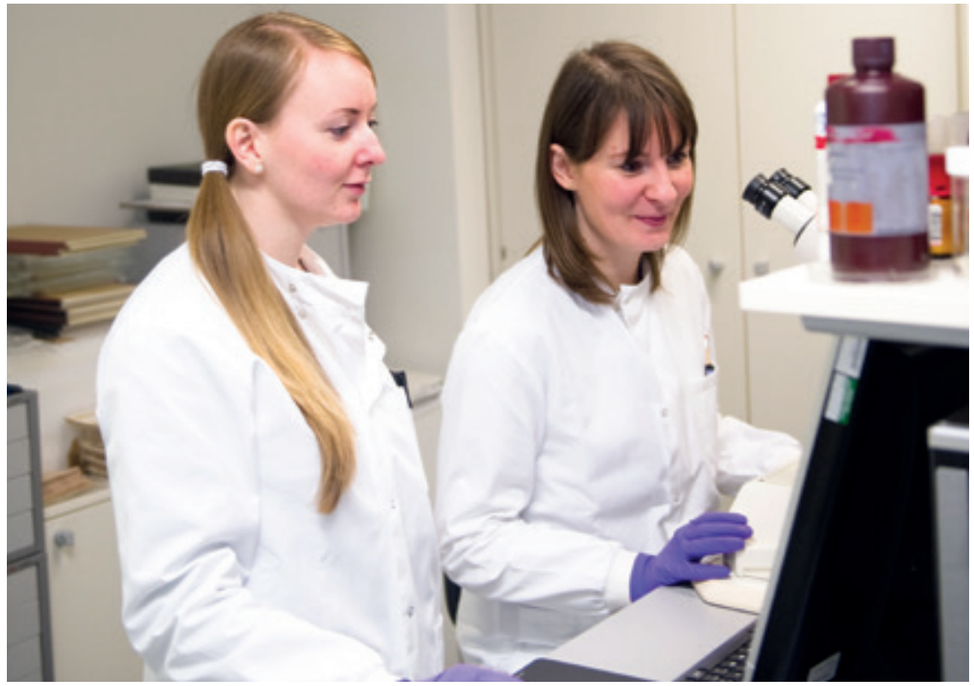
Peter Adams
BBSRC, Medical Research Council, NIH with National Institute on Aging & National Cancer Institute (USA)

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

Stephen Tait
ARC, BBSRC, EMBO, EU Marie Curie Actions, Royal Society

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

- Aggreko UK Ltd
- AMV Ltd
- Anachem Ltd
- Legacy of the late Mary Paterson Dunning Anwyl-Jones
- Staff of Aviva Insurance
- Bearsden Primary School
- Beattie
- Blair & Bryden Solicitors
- Margaret G Brown
- T Brown
- Cambridge Bioscience Ltd
- David N Campbell
- Elizabeth M Campbell, in memory of Mr Alasdair Campbell
- Cathcart Trinity Church, Art & Craft Club
- Central District Grand Chapter, Order of the Eastern Star
- Clyde Travel
- Crookston Castle Primary School
- Holly Docherty, her friend Owen and her sister Carly
- Don Whitley Scientific Ltd
- Dowling
- Dumbarton & District Probus Club
- Edrington
- Noel Elliott
- Enserve Technical Services LLP
- Fermentas (part of Thermo Fisher Scientific)
- Glenfield Bowling Club
- May Gow
- J Graham
- Avril Haddow
- In memory of the late Alma Harper
- Hendry, in memory of John and Jean Grant
- RA Hockley, in memory of Dr Stuart Gerrard Hoggar
- Hodder & Stoughton Ltd
- Mary Hopkin, in memory of her husband Bill
- E Irving, in memory of her husband
- The James Inglis Trust
- Barbara Jordan
- Kelvinside Hillhead Parish Church, in memory of Dr Stuart Gerrard Hogger
- Emily Sharpe, Demlza Hart, Khyra Cannon and Parmis Taghizadeh of Killermont Primary, Bearsden
- Lanarkshire Women's Bowling Association
- Rita Lindsay
- Legacy of Mr Magnus John Mackay
- Legacy of Miss Grace F MacLean
- E McCrum
- H McCulloch
- Christina McDougall, in memory of her husband John
- W McGuineness, in memory of Mrs Isabel Adam



- Ian McHale and members of 1 Section at MOD Police, RNAD Coulport
- Fiona and Sarah McNeill, M&S Simply Food Store, Bearsden
- Fiona McNeill and family
- B Millar
- Mosshead Primary School
- Mrs Agnes Hill, in memory of Aline Legros
- Murray, Gillies & Wilson
- Employees of Nationwide Building Society
- Christine Neale
- North View Housing Association
- Novus Bio
- Anne O'Hare, Hillpark Bowling Club and Hillpark Ladies Bowling Section
- Dr & Mrs J D Olav Kerr's Charitable Trust
- Worthy Matron, Worthy Patron, Office Bearers and Members of the Order of the Eastern Star, Lily of the Valley
- Order of the Eastern Star, Priory Chapter No 21
- Pacitti Jones
- Maurice Paterson
- PEQLAB Ltd
- Sarah Percy and Irene Kennedy
- Promega UK Ltd
- Gordon Ronney, in memory of Ian O'Friel from Dr Wilma Gemmell Trust
- Rotary Club of Clydebank
- Rutherglen Vogue Celtic Supporters Club
- GCH Shepherd
- P Sommerville
- Source Bioscience UK Ltd
- St Andrew of Glasgow Royal Arch Chapter No.69
- Members, friends and family of St Rollox Bowling Club
- In memory of Mr Bob Sharp
- In memory of the late Aline Legros
- In memory of the late Duncan MacLullich
- StarLab (UK) Ltd
- Edward Stephen, in memory of his wife Mrs Mary Johanna Stephen
- Stratech Scientific Ltd
- Strathblane Bowling Club
- Children of Struther Hill Activity Group
- Mr & Mrs Sweeney
- Synantix
- John Teevan, in memory of his late mother
- The Local Charity Shop
- The West Coast Motors
- Thornhill Gardening Society
- UK Labs Direct Ltd
- J Walker
- H Wardell, in memory of her husband Mr Anthony Richard Wardell
- Mr & Mrs Webb, in memory of Mr Philip Alfred James
- West of Scotland Women's Bowling Association D & M Wiseman

PATRONS AND BOARD OF GOVERNORS

Patrons

His Grace the Duke of Hamilton
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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.

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Dr Iain Foulkes
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Mr Peter Winckles
Cancer Research UK Beatson Institute

CONTACT DETAILS

The Beatson Institute for Cancer Research
Director: Professor Karen H Vousden
Tel: +44(0) 141 330 3953
Fax: +44(0) 141 942 6521

Address
Cancer Research UK Beatson Institute
Garscube Estate, Switchback Road
Bearsden, Glasgow G61 1BD

e-mail: beatson@gla.ac.uk
web site: www.beatson.gla.ac.uk

Registered address
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Inland Revenue Charity Ref. SC006106
Registered as a company limited by guarantee in Scotland No. 84170
Registered address: Cancer Research UK Beatson Institute,
Garscube Estate,
Switchback Road, Bearsden,
Glasgow G61 1BD

Tel +44(0) 141 330 3953
www.beatson.gla.ac.uk

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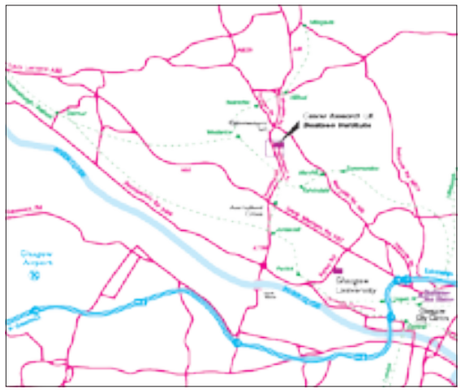

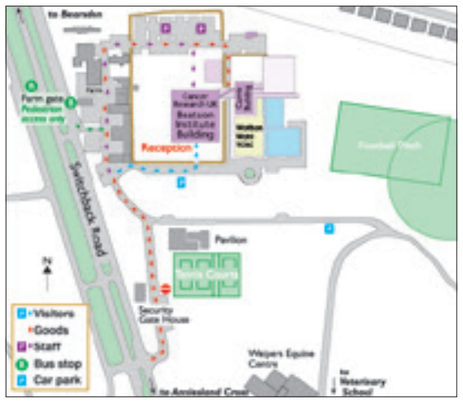

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Cancer Research UK
Beatson Institute
Garscube Estate
Switchback Road
Bearsden
Glasgow
G61 1BD
Telephone +44(0) 141 330 3953

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