



2009
Annual Scientific Meeting

Wednesday 24 June 2009

Pavilions of Harrogate

PROGRAMME

PROGRAMME
2009 YCR ANNUAL SCIENTIFIC MEETING
Pavilions of Harrogate
WEDNESDAY 24 JUNE 2009

- 8.45 a.m. Registration and Coffee
- 9.30 a.m. Welcome
Professor R Cartwright
(Chairman, Scientific Advisory Committee)
- 9.35 a.m. Open Papers - Scientific Session 1
- 10.35 a.m. Coffee, Poster Session and Scientific Exhibition
- 11.05 a.m. Open Papers – Scientific Session 2
- 12.05 p.m. Buffet Lunch and Scientific Exhibition
- 1.00 p.m. Poster viewing
- 2.00 p.m. Open Papers – Scientific Session 3
- 3.15 p.m. Tea, Poster Session & Scientific Exhibition
- 3.45 p.m. Guest Lecture, Professor Alan R Clarke
(Cardiff School of Biosciences, Cardiff University)
“Building better models of colon cancer in the mouse”
- 4.45 p.m. Announcement of Poster Award
- 4.50 p.m. Closing address
Mrs Elaine King
(Chief Executive)
- 5.00 p.m. Wine and Canapes

Scientific presentations to be 15 minutes duration and the Guest Lecture is
1 hour including questions.

The Open Papers and Guest Lecture will be held in the
Aire Room.

The Poster presentations, Refreshments and Scientific Exhibition
will all be held in the Wharfe Room.

Please stand by your Posters from 1pm to 2pm and remove them from the stands at the end of
the afternoon Poster Session.

Organising Committee:	This meeting was sponsored by:	
Professor R Cartwright	Applied Biosystems	Illumina Ltd
Professor G E Blair	Autogen Bioclear & Geneservice	New Brunswick UK Ltd
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Professor P Andrews	Fisher Scientific UK	Starlab UK Ltd
Dr D Gilham	Geneflow Ltd	ThermoFisher Scientific
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OPEN PAPERS – SCIENTIFIC SESSION 1

Chair: Professor L Turnbull

- 9.35 a.m. **DISCOVERY OF A NOVEL CHEMOKINE RECEPTOR ANTAGONIST USING MOLECULAR MODELLING AND VIRTUAL HIGH THROUGHPUT SCREENING**
K. Afarinkia, C.W.G. Fishwick*, M.V. Vinader and L.H. Patterson
The Institute of Cancer Therapeutics, University of Bradford, West Yorkshire BD7 1DP.
*School of Chemistry, University of Leeds, Leeds, LS2 9JT
- 9.50 a.m. **IN SILICO MODELLING OF DOXORUBICIN PENETRATION THROUGH MULTICELL LAYERS**
C.J. Evans^a, P.F. Jones^c, P.M. Loadman^b, B.D. Sleeman^d, C.J. Twelves^c, S.W. Smye^e and R.M. Phillips^b
^aDivision of Medical Physics, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, LS2 9JT
^bInstitute of Cancer Therapeutics, University of Bradford, Bradford, BD7 1DP
^cSection of Oncology and Clinical Research, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, LS9 7TF
^dDepartment of Applied Mathematics, University of Leeds, Leeds, LS2 9JT
^eDepartment of Medical Physics and Engineering, Leeds Teaching Hospitals, St James's University Hospital, Leeds LS9 7TF.
- 10.05 a.m. **LONGITUDINAL AND INTRATUMORAL HETEROGENEITY OF KRAS AND BRAF MUTATION STATUS IN PATIENTS WITH ADVANCED COLORECTAL CANCER (aCRC)**
S. D. Richman, P. Chambers, S. Grant, C. Daly, M. Seymour and P. Quirke
All authors Leeds Institute for Molecular Medicine, University of Leeds, Leeds LS9 7TF
- 10.20 a.m. **MEASURING WATER T₂ AND WATER:FAT SIGNAL RATIOS WITH MR SPECTROSCOPY (TEA-PRESS) AND CHEMICAL SHIFT IMAGING (IDEAL): PRELIMINARY RESULTS IN BREAST CANCER NEOADJUVANT CHEMOTHERAPY PATIENTS**
D. J. Manton¹, G. P. Liney², P. Gibbs¹, M. Lowry¹, M. D. Pickles¹ and L. W. Turnbull¹
¹YCR Centre for MR Investigations, The University of Hull
²Medical Physics Department, Hull and East Yorkshire Hospitals NHS Trust
- 10.35 a.m. **COFFEE, POSTER SESSION AND SCIENTIFIC EXHIBITION**

OPEN PAPERS – SCIENTIFIC SESSION 2

Chair: Professor P Andrews

- 11.05 a.m. **SCREENING OF GASTROINTESTINAL CANCERS FOR ADENOVIRUS RECEPTORS: A BASIS FOR TARGETED CANCER GENE THERAPY**
N. Fox¹, S. Priestman¹, C. Verbeke² and G. E. Blair¹
¹Institute of Molecular & Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT
²Department of Histopathology, St James's University Hospital, Leeds LS9 7TF
- 11.20 a.m. **PROTEOMIC DETECTION OF A MURINE LEUKAEMIA VIRUS IN HIGHER METASTATIC VARIANTS OF HUMAN PROSTATE CANCER CELLS ORTHOTOPICALLY CYCLED THROUGH NUDE MICE**
A. Glen^{a/b}, O. S. Yen^d, C. Eaton^a, F. C. Hamdy^c, P. C. Wright^d and I. Rehman^a
^aDepartment of Human Metabolism, Academic Unit of Bone Biology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX.
^bCentre for Stem Cell Biology, Department of Biomedical Science, University of Sheffield, Sheffield, S10 2TN
^cNuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU
^dBiological and Environmental Systems Group, ChELSI, Department of Chemical and Process Engineering, University of Sheffield, Mappin Street, Sheffield, S1 3JD
- 11.35 a.m. **COORDINATING CYCLIN FUNCTION ON THE NUCLEAR MATRIX DURING INITIATION OF MAMMALIAN DNA REPLICATION**
N. A. Copeland¹, F. A. Rahman¹, J. Munkley¹, K. M. Roper¹, H. E. Sercombe^{1,2}, J. F-X. Ainscough³ and D. Coverley^{1,2}.
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²Cizzle Biotechnology Ltd, The University of York, Heslington, York YO10 5YW
³Leeds Institute of Genetics, Health and Therapeutics, Leeds University Leeds LS2 9JT
- 11.50 a.m. **CROSSTALK BETWEEN SITE-SPECIFIC MODIFICATIONS ON P53 AND HISTONE H3**
L. Warnock, R. Adamson, C. Lynch and J. Milner
Yorkshire Cancer Research P53 Unit, Department of Biology, University of York, York YO10 5DD
- 12.05 a.m. **BUFFET LUNCH AND SCIENTIFIC EXHIBITION**
- 1.00 p.m. **POSTER VIEWING**

OPEN PAPERS – SCIENTIFIC SESSION 3

Chair: Dr D Gilham

- 2.00 p.m. **THE ROLE OF $\alpha 9\beta 1$ INTEGRIN AND HEPATOCYTE GROWTH FACTOR IN EPITHELIAL CELL BEHAVIOUR**
S. Roy, L. Bingle, J. F. Marshall[†], R. Bass*, V. Ellis*, P. M. Speight and S. A. Whawell
Oral and Maxillofacial Pathology, School of Clinical Dentistry, University of Sheffield, Claremont Crescent, Sheffield S10 2TA
[†]Centre for Tumour Biology, Institute of Cancer, Bart's and The London, Queen Mary's School of Medicine and Dentistry, Queen Mary, University of London, Charterhouse Square, London EC1M 6BQ
*School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ
- 2.15 p.m. **DEVELOPMENT OF NOVEL ANTI-ANGIOGENIC AND ANTI-TUMOURIGENIC PEPTIDE DERIVED FROM THE C-TERMINUS OF TIMP-3**
Y-Y. Chen¹, M. Muthana¹, N. Brown², C. Lewis¹ and M. Barker¹
¹Department of Infection & Immunity and ²Department of Oncology, University of Sheffield Medical School, Sheffield S10 2RX
- 2.30 p.m. **DEVELOPING A TISSUE ENGINEERED MODEL OF THE MOLECULAR PATHOGENESIS OF BARRETT'S METAPLASIA**
N. H. Green¹, Q. Huang¹, B. Corfe², G. Battaglia¹, S. MacNeil¹ and J. Bury²
¹Kroto Research Institute, North Campus, University of Sheffield, Broad Lane, Sheffield S3 7HQ
²University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX
- 2.45 p.m. **INITIATION AND PROGRESSION OF CARCINOGEN-INDUCED COLORECTAL CARCINOGENESIS FOLLOWING LOSS OF IL-4 RECEPTOR FUNCTION**
N. Ingram, C. W. S. Ko, S. L. Perry, P. L. Coletta and M. A. Hull
Section of Molecular Gastroenterology, Leeds Institute of Molecular Medicine, Wellcome Trust Brenner Building, St James's University Hospital Leeds LS9 7TF
- 3.00 p.m. **DNA DAMAGE IN PROSTATE CANCER STEM CELLS**
F. M. Frame, S. Klein, M. Fraser, M. Meuth, R. G. Bristow and N. J. Maitland
YCR Cancer Research Unit, Department of Biology, University of York, Heslington, York YO10 5DD
- 3.15 p.m. **TEA, POSTER SESSION AND SCIENTIFIC EXHIBITION**

OPEN PAPERS – SCIENTIFIC SESSION 3

Chair: Professor R Cartwright

3.45 p.m. **BUILDING BETTER MODELS OF COLON CANCER IN THE MOUSE**

Professor Alan Clarke, Cardiff School of Biosciences, Cardiff University,
Cardiff, CF10 3AX

4.45 p.m. **ANNOUNCEMENT OF POSTER AWARD**

4.50 p.m. **CLOSING ADDRESS**

Mrs Elaine King

5.00 p.m. **WINE AND CANAPES**

**ORAL
PRESENTATIONS**

OPEN PAPERS – SCIENTIFIC SESSION 1

Discovery of a novel chemokine receptor antagonist using molecular modelling and virtual high throughput screening

K. Afarinkia, C.W.G. Fishwick*, M. V. Vinader, L. H. Patterson

The Institute of Cancer Therapeutics, University of Bradford, West Yorkshire BD7 1DP.

*School of Chemistry, University of Leeds, Leeds LS2 9JT

The chemokines (*chemotactic cytokines*) have widespread involvement in the regulation of tumour cell functions, including growth, angiogenesis, survival, migration and metastasis. In particular, a role for the CXCL12 chemokine, the sole natural ligand of CXC chemokine receptor-4 (CXCR4), has been extensively demonstrated in cancer metastasis. Blocking CXCR4 expression using siRNA and mAB antagonism of CXCR4 are both shown to cause a significant decrease in tumour migration and invasion both *in vitro* and *in vivo*.

Here, we disclose the discovery of ICT-5040, a novel small molecule CXCR4 antagonist, and report on the progress towards optimisation of this lead.

We first constructed and validated a homology model of CXCR4 based on the crystal structure of bovine rhodopsin GPCR-7TM. The binding cavity within the receptor was identified computationally and was used as the focus of a virtual high-throughput screening (vHTS) of a library of 60,000 chemical compounds specifically selected for drug-likeness, water solubility, Lipinsky compliancy. At the same time we set up and validated a functional cell-based calcium flux assay. In vitro screening of the top 100 molecules from the virtual hit-set afforded seven compounds with IC₅₀'s below 50 µM, the most potent of which was ICT-5040 (IC₅₀ = 3.8 µM).

In silico modelling of Doxorubicin penetration through multicell layers.

C.J. Evans^a, P.F Jones^c, P.M. Loadman^b, B.D. Sleeman^d, C.J. Twelves^c, S.W. Smye^e, R.M. Phillips^b

^aDivision of Medical Physics, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, ^bInstitute of Cancer Therapeutics, University of Bradford, ^cSection of Oncology and Clinical Research, Leeds Institute of Molecular Medicine, University of Leeds, ^dDepartment of Applied Mathematics, University of Leeds, ^eDepartment of Medical Physics and Engineering, Leeds Teaching Hospitals, St James's University Hospital, Leeds.

Background: Inadequate delivery of chemotherapeutic agents to solid tumours is a significant factor that limits curative potential. The aim of this study was to develop an *in silico* model based on these measurements which will predict how far a drug will penetrate from a blood vessel within its PK lifespan. The specific objective of this study is to develop a mathematical model for doxorubicin transport through multicellular layers and to assess the potential impact that efflux via P-Glycoprotein (PgP) may have on drug penetration.

Materials and Methods: Three cell lines were employed; DLD-1 (human colon carcinoma), MCF7 (human breast carcinoma) and MCF7-ADR (Doxorubicin resistant and PgP overexpressing derivative of MCF7). Cells were cultured on Transwell culture inserts to various thicknesses and doxorubicin (100, 50 or 25 μM) was added to the top chamber of the Transwell. The concentration of drug appearing in the bottom chamber was determined as function of time using HPLC-MS/MS analysis.

Results: In all cell lines, the rate of drug penetration was inversely proportional to the thickness of the multicell layer and the presence of PgP (MCF7-ADR) did not alter the rate of doxorubicin penetration compared to the wild type MCF7 cells. Initial studies have established a mathematical model based upon the fact that the transport of doxorubicin across cell membrane bilayers occurs by a passive flip-flop mechanism of the drug between two membrane leaflets. The mathematical model treats the Transwell setup as a series of compartments and the multicell layer is treated as a series of cell layers, separated by small intercellular spaces.

Conclusions: This initial model demonstrates good agreement between predicted and actual drug penetration rates *in vitro*. Further studies designed to incorporate pharmacokinetic parameters (both real and simulated) into the model are underway.

Longitudinal and intratumoral heterogeneity of KRAS and BRAF mutation status in patients with advanced colorectal cancer (aCRC)

Susan D. Richman, Philip Chambers, Sophie Grant, Catherine Daly, Matthew Seymour and Philip Quirke. (All authors LIMM, University of Leeds)

Recent advances in chemotherapeutic treatments for aCRC have seen the introduction of drugs targeting the epidermal growth factor receptor (EGFr) and mutation status of KRAS is a predictive biomarker of response to such drugs. Testing mutation status is normally done on only one formalin-fixed, paraffin-embedded tumour block and treatment decision is based upon the outcome of this single test. We investigated KRAS and BRAF mutation status heterogeneity, longitudinally (primary tumour versus metastatic lymph node versus secondary tumour) and at the intratumoral level (several separate tumour blocks from each patient).

Blocks from consenting patients in both the MRC FOCUS and PICCOLO aCRC clinical trials were tested for KRAS codons 12/13 and 61 and BRAF codon 600 mutations by pyrosequencing.

Results:(a) Intratumoural study

Codon analysed	Total no. of cases assessed	No. of tumours displaying homogeneity	No of tumours displaying heterogeneity
KRAS codons 12&13	18	17	1*
KRAS codon 61	18	18	0
BRAF codon 600	18	17	1

*2 synchronous tumours in 1 patient

(b) Longitudinal study

Codon analysed	No. of complete triplets (primary, Lymph Node, secondary)	Homogenous	Heterogeneous
KRAS codons 12&13	18	16	2
KRAS codon 61	16	16	0
BRAF codon 600	18	18	0

Summary: In the intratumoral study 1/18 (5.5%) displayed heterogeneity. In the longitudinal study, 2 tumours (11%) displayed heterogeneity suggesting that a single block will suffice in most cases.

Measuring water T_2 and water:fat signal ratios with MR spectroscopy (TEA-PRESS) and chemical shift imaging (IDEAL): preliminary results in breast cancer neoadjuvant chemotherapy patients

D.J. Manton, Ph.D. ¹, G.P. Liney, Ph.D. ², P. Gibbs, Ph.D. ¹,
M. Lowry, D.Phil. ¹, M.D. Pickles, Ph.D. ¹, L.W. Turnbull, M.D. ¹

1. YCR Centre for MR Investigations, The University of Hull
2. Medical Physics Department, Hull and East Yorkshire Hospitals NHS Trust

Approximately 5% of breast cancer patients receive neoadjuvant chemotherapy (NAC) to improve surgical outcome, but this can be unsuccessful in up to 42% of cases.

Change in tumour size, as determined by palpation or magnetic resonance imaging (MRI), may be a relatively late manifestation of response, but quantitative MRI and magnetic resonance spectroscopy (MRS) can both provide non-invasive measurements which might indicate non-response after only one or two cycles which, in turn, might allow individualised treatment.

A study has, therefore, been set up to measure water:fat signal ratio (WFSR), and water and lipid T_2 values in NAC patients using multi-echo proton MRS, the proven technique, and also using multi-echo IDEAL chemical-shift imaging (CSI) to determine if the latter's greatly increased spatial resolution is clinically useful.

Data have so far been obtained in nine patients prior to NAC.

IDEAL was able to detect lesion heterogeneity, but its T_2 values were systematically higher than the MRS values (the gold standard) by a factor of 1.69. IDEAL WFSR values were overestimated for low values (<1.5) and underestimated for higher values (>4.0).

Investigations are underway to determine the underlying technical reasons for these errors which should permit a thorough, theoretical correction of the data.

OPEN PAPERS – SCIENTIFIC SESSION 2

Screening of Gastrointestinal Cancers for Adenovirus Receptors: a basis for targeted cancer gene therapy

N. Fox¹, S. Priestman¹, C. Verbeke² & G.E. Blair¹

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Human adenoviruses (Ads) are potentially useful vectors for cancer gene therapy. The 51 human adenovirus serotypes are classified into six species (A to F). Most Ads utilise the Coxsackie B and adenovirus receptor (CAR) to interact with cells, however loss of CAR expression has been noted in a number of tumour cell types, leading to a reduced ability of these cells to be transduced by species C Ad5, the serotype most frequently used in cancer gene therapy studies. In contrast, CD46, CD80 and CD86 are proposed attachment molecules for species B Ads in cultured human cells.

We previously found that CAR expression was absent in more than 50% of pancreatic ductal adenocarcinomas. Pancreatic and colorectal cell lines and tissue samples were therefore screened for expression of species B receptor molecules. All cell lines studied expressed variable levels of CD46, but did not express CD80 or CD86. The cell lines were then infected with wild-type species B Ads (3, 11 and 35) to determine their susceptibility to infection in comparison with Ad5, and results showed increased susceptibility to infection by species B serotypes. Studies of pancreatic adenocarcinoma tissues by immunohistochemistry showed membranous CD46 expression (88% moderate to high expression) and heterogeneous CD86 staining (91% moderate to high expression), while the majority (72%) lacked CD80 expression.

Overall, our results show that directing species B adenovirus to tumours that express CD46 has potential promise for colorectal and pancreatic cancer gene therapy.

'Proteomic detection of a Murine Leukaemia Virus in higher metastatic variants of human prostate cancer cells orthotopically cycled through nude mice'

Adam Glen^{a/b} Ow S. Yen^d, Colby Eaton^a, Freddie C. Hamdy^c, Phillip C. Wright^d, Ishtiaq Rehman^a.

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Prostate cancer ranks second amongst the most common cause of cancer-related deaths in men. Death from prostate cancer is largely the result of metastatic disease for which there is currently no effective treatment. Thus, Pettaway *et al.* (1996) generated a panel of higher metastatic variant prostate cancer cell lines (LNCaP-LN3 and LNCaP-Pro5) by orthotopic injection of the poorly metastatic parental LNCaP cell line in the prostate of nude mice. We hypothesized that proteomic comparisons between the poorly metastatic LNCaP and highly metastatic LNCaP-LN3 cells will identify critical proteins/pathways involved in prostate cancer progression. An unexpected finding of this study was the detection of a protein highly similar to a murine leukaemia virus (MLV) using 2D PAGE and MS/MS analysis. The expression of this protein was restricted to LNCaP-LN3 cells and was confirmed by searching our previously published shotgun proteomic dataset against a murine database. Reverse-Transcription PCR confirmed the presence of MLV DNA sequences in LNCaP-LN3 cells and in LNCaP-Pro5 cells. Sequence analysis of the PCR products followed by Blast searches, confirmed the presence of DNA homologous to several murine retroviruses. Furthermore, electron microscopy analysis indicated the presence of virus-like particles (~100nm diameter), seen to be budding from the nuclear membrane of LNCaP-LN3 cells but not LNCaP cells. Therefore, our data suggest the presence of an integrated MLV-related virus in the higher metastatic LNCaP-LN3 and LNCaP-Pro5 cells. The presence of a MLV-related virus may be associated with the higher metastatic potential of these cells and subsequently has wider implications for the key determinants of metastatic behaviour and *in vivo* tumour models.

Coordinating cyclin function on the nuclear matrix during initiation of mammalian DNA replication

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Cyclins E and A have specialized roles during the transition from G₀ to S phase. Cyclin E supports assembly of pre-replication complexes, while cyclin A-dependent kinase acts later to terminate assembly and activate the DNA replication machinery [1]. The mechanism by which these events are coordinated temporally and spatially within the nucleus is not known. We showed recently that the nuclear matrix-associated DNA replication factor Ciz1 [2-4] interacts with cyclins E and A sequentially and via distinct cyclin binding motifs. This provides a molecular link between the structures that organize DNA replication in nuclear space and the regulators that control initiation in the cell cycle. The data suggest that Ciz1 co-ordinates the functions of cyclins E and A during the switch from pre-replication complex assembly to DNA synthesis, ensuring that cyclin E and cyclin A-dependent events occur in the same place and in the correct order [5]. This function of Ciz1 is normally immobilized at specific sub-nuclear sites associated with the nuclear matrix. Evidence will be presented which shows that Ciz1 expression is frequently disrupted in cancer cells and which suggests a specific impairment of function.

1. Coverley, D., H. Laman, and R.A. Laskey, *Distinct roles for cyclins E and A during DNA replication complex assembly and activation*. Nat Cell Biol, 2002. **4**(7): p. 523-8.
2. Ainscough, J.F., et al., *C-terminal domains deliver the DNA replication factor Ciz1 to the nuclear matrix*. J Cell Sci, 2007. **120**(Pt 1): p. 115-24.
3. Coverley, D., J. Marr, and J. Ainscough, *Ciz1 promotes mammalian DNA replication*. J Cell Sci, 2005. **118**(Pt 1): p. 101-12.
4. Mitsui, K., et al., *Cloning and characterization of a novel p21cip1/waf1-interacting zinc finger protein, Ciz1*. Biochem. Biophys. Res. Com., 1999. **264**: p. 457-464.
5. Copeland, N.A., et al., *Ciz1 cooperates with cyclin A/CDK2 to activate mammalian DNA replication in vitro*. Submitted.

Crosstalk between site-specific modifications on p53 and histone H3

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Abstract

Previously we have observed a link between p53 expression and histone H3 post-translational modifications. Here we ask if specific post-translational modifications of p53 impact upon histone H3 modifications in a selective manner. We have also screened for internal co-operative effects within the repertoire of p53 modifications. Exogenous p53 constructs were expressed in HCT116 p53^{-/-} cells. Four mutant p53 constructs were used, with single 'phosphorylation' mutations at serines 15 and 37 (S15A, S15D, S37A and S37D) and compared with exogenously expressed wild type p53. The results showed that the replacement of serine 15 with either alanine (S15A) or aspartic acid (S15D) induced phosphorylation at S33P, S37P and S46P. In contrast, phosphorylation mutants p53(S37A) and p53(S37D) were not phosphorylated on S33. S46 phosphorylation appeared specifically enhanced by p53(S37D) relative to p53(S37A). Distal induction of S392 phosphorylation was observed for each of the p53 N-terminal phosphorylation mutants. Analysis of endogenous histone H3 (from the transfected cells) revealed loss of dimethylated K9 following expression of wild-type and mutant p53 constructs. Expression of p53 (S15A), (S15D) and (S37A) selectively induced acetylation at K9 and K14. In contrast wt p53 and p53(S37D) had no effect upon K9 or K14 acetylation. K18 acetylation status was unaffected throughout.

OPEN PAPERS – SCIENTIFIC SESSION 3

The role of $\alpha 9\beta 1$ integrin and hepatocyte growth factor in epithelial cell behaviour

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Integrins initiate signalling in response to the extracellular matrix (ECM) which is important in wound healing and in cancer where integrins such as $\alpha 9\beta 1$ and $\alpha v\beta 6$ are expressed by epithelial cells. Cell motility is also regulated by growth factors such as hepatocyte growth factor (HGF), possibly by modulating integrin function.

Virally transfected oral keratinocytes expressed functionally active $\alpha 9$ integrin which mediated specific upregulation of adhesion and migration towards tenascin-C. Matrix metalloproteinase-2 and 9 expression was increased upon ligand engagement and cell surface plasmin generation was also enhanced in $\alpha 9$ expressing cells. HGF treatment resulted in increased $\alpha 9$ integrin levels and enhanced migration. The appearance of splice variants of the $\alpha 9$ gene upon HGF treatment was observed.

The $\alpha 9\beta 1$ integrin may play a key role in modulation of cell motility and expression of matrix degrading proteases. HGF has the potential to initiate this process by stimulating expression of this integrin.

Development of a novel anti-angiogenic and anti-tumourigenic peptide derived from the C-terminus of TIMP-3

Yung-Yi Chen¹, Munita Muthana¹, Nicola Brown², Claire Lewis¹ & Michael Barker¹

*¹Department of Infection & Immunity and ²Department of Oncology,
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Tissue inhibitor of metalloproteinases-3 (TIMP-3), is one of a family of 4 inhibitors of the matrix metalloproteinases (MMPs). While TIMPs are known to regulate angiogenesis by inhibiting the function of MMPs, TIMP-3 has recently been shown to exert a unique anti-angiogenic activity by binding to and inhibiting vascular endothelial growth factor receptor-2 (VEGFR-2). We have shown that a 16 amino acid peptide, derived from the C-terminal domain (p700), also inhibited this interaction. The aim of this project was to characterise the anti-angiogenic effect of p700 on VEGF-mediated endothelial cell functional responses and signalling *in vitro* and investigate the potential of p700 as an inhibitor of angiogenesis *in vivo*.

The results showed that p700 was able to inhibit the VEGF-induced proliferation, migration and differentiation of endothelial cells *in vitro*. This reflected a reduction in VEGF-induced VEGFR-2, Erk1/2 and PI₃K phosphorylation. Moreover, using a syngeneic murine subcutaneous 4T1 mammary adenocarcinoma model, p700 was able to significantly inhibit tumour growth and vascularisation *in vivo*. Taken together these studies show that this novel peptide may have therapeutic potential for the treatment of cancer.

Developing a tissue engineered model of the molecular pathogenesis of Barrett's metaplasia
Nicola H Green¹, Qizhi Huang¹, Bernard Corfe², Giuseppe Battaglia¹, Sheila MacNeil¹, Jonathan Bury²

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Barrett's metaplasia (BM) is a common precursor of oesophageal adenocarcinoma. Exposure of oesophageal epithelium to gastroduodenal refluxate is considered to be a key factor in its development but it is difficult to study this *in vivo*.

Our aim was to use 2D and 3D cell based models to investigate this hypothesis and gain a better understanding of the mechanism of this condition. Studies with acid exposed 2D cultures of primary human oesophageal squames (HOSs) and the underlying fibroblasts (HOFs) demonstrated that acidic insults did not translocate NF- κ B in HOFs but following exposure to pH4 for 60-120 minutes NF- κ B was activated in HOSs. In addition subjecting HOSs to pulsatile pH5 exposure triggered paracrine NF- κ B activation in HOFs. This NF- κ B activation may represent a very early event in BM development.

We hypothesize that acid refluxate activates NF- κ B signalling in oesophageal cells and may initiate pro-inflammatory events in these cells eventually inducing CDX1/2 expression in HOSs leading to inappropriate differentiation .

A 3D tissue-engineered model oesophagus based on HOS and HOF cells and pig oesophageal matrix was then developed yielding a model comparable to normal epithelium, as shown by Ki67, CK4, CK14 and involucrin staining. This oesophageal model will now be used to investigate the effects of acid/bile exposure on NF- κ B signalling, CDX1/2 expression and HOS differentiation.

Initiation and progression of carcinogen-induced colorectal carcinogenesis following loss of IL-4 receptor function.

N. Ingram, C.W.S. Ko, S.L. Perry, P.L. Coletta, M.A. Hull

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Colorectal cancer (CRC) is the second most common cause of cancer death in the UK with 16,000 deaths and 36,500 new cases annually. The anatomical and molecular progression of CRC through defined stages from benign aberrant crypt foci (ACF) and adenomas to invasive carcinoma make it particularly amenable to preventative intervention.

We have been investigating the IL-4 and IL-13 Th2 cytokine signaling pathways in CRC with a view to developing novel chemopreventive and therapeutic agents for CRC. We have extended our previous work to show that Balb/c IL-4R α ^{-/-} mice had a significant increase in ACF multiplicity (median 8.5, interquartile range (IQR) 7.25-12; n = 8) than wild-type mice (median 3, IQR 1-3.5; n = 9, p=0.007 Mann Whitney U-test) in azoxymethane-induced colorectal carcinogenesis. There was no significant difference in blood cell number or composition and no difference in the proportion of splenic CD4⁺, CD8⁺ or regulatory T cells in each treatment group. This suggests that there is no systemic immune modulation at this stage. To determine whether loss of signalling through IL-4 R α is also pro-tumorigenic at the carcinoma more advanced stages, we are characterising tumour multiplicity and size in wild-type and IL-4R α ^{-/-} animals at 32 weeks post carcinogen treatment.

DNA DAMAGE IN PROSTATE CANCER STEM CELLS

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The paramount problem with prostate cancer is therapy-resistant secondary tumours. It is hypothesised that prostate cancer stem cells (PCSCs) are resistant to first-line treatments including radiotherapy, androgen ablation and chemotherapy, and so are responsible for growth of these secondary tumours. To test this hypothesis we have analysed PCSCs in isolation from other more differentiated tumour cells, the transit-amplifying (TA) and committed basal (CB) cell populations. Cells were cultured from benign prostatic hyperplasia (BPH) and prostate cancer patient samples, and DNA damage was administered by irradiation (IR) or chemotherapeutic agents. Immunofluorescence was used to detect DNA repair proteins, comet assays to measure DNA damage directly and FACS analysis to measure proliferation and apoptosis. When examining TA and CB cells, 70-90% of cells were positive for γ H2AX foci at 30min post-IR, indicative of DNA breaks. However, in all samples examined so far (both BPH (x2) and cancer (x5 of Gleason 6 or 7)) the stem cells showed a delay in irradiation response and always showed a lower percentage of foci-positive cells at 30min post-IR. This was also true for other DNA damage response proteins including 53BP1, Chk2^{Th68} and ATM/ATR substrates. Work has now been initiated to determine if the stem cells actually sustain less damage or if the signal transduction pathways are altered. In addition, it will be determined whether or not cell proliferation rates affect the response to DNA damaging agents in the PCSCs. By determining the cellular response to these DNA damaging treatments the mechanisms of resistance can be elucidated and new therapeutic strategies developed.

**POSTER
PRESENTATIONS**

POSTER 1

α -2,8-Polysialyltransferase: a target for the development of anti-metastatic agents

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Introduction

Polysialic acid (PSA) is selectively expressed on several tumour types, most notably small cell lung cancer (SCLC) and neuroblastoma. A linear α -2,8-linked polymer of up to 200 sialic acid residues, PSA specifically decorates the neural cell adhesion molecule (NCAM). PSA synthesis is regulated by two polysialyltransferases, PST (ST8SiaIV) and STX (ST8SiaII). Changes in PSA-NCAM expression are associated with invasion, migration and metastasis.

Methods and Results

We have designed and synthesised a series of potential inhibitors of PST/STX, informed by computational chemistry and based on the natural substrate for these enzymes. We have synthesised agents in which the carbohydrate, phosphate and/or nucleoside components are modified or replaced. These complex structures have demanded the development of novel chemistries for their synthesis. In addition, we are currently developing *in vitro* and *in vivo* models to study the effects of biological or chemical modulation of PSA. We have successfully cloned, purified and expressed STX, and have developed an *in vitro* assay to enable assessment of the ability of agents to inhibit the enzyme and therefore reduce PSA synthesis. We report that several agents demonstrate such activity. We have previously characterised PSA expression profiles in several cancer cell lines known to express both PST and STX, namely SCLC, neuroblastoma and glioma.

Conclusion

In summary, these data illustrate the opportunity to develop selective inhibitors of PST and STX to modulate PSA expression.

POSTER 2

Design and Synthesis of Membrane Type Matrix Metalloproteinase (MT-MMP) targeted anti-tumour agents

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Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are associated with the metastatic process. Membrane-type matrix metalloproteinases (MT-MMPs) are highly active in tumours, but absent or inactive in normal tissues. MT-MMP is known to be elevated in the majority of human tumours and to be central to tumour invasion and angiogenesis. Our objective has been to design inactive peptide conjugates that release a potent drug when activated by MMPs within the tumour microenvironment.

Methods and Results

We report the design and synthesis of a new series of peptide-based conjugates of a potent anti-tumour agent based on our lead agent ICT2588, with improved physicochemical properties. The anti-tumour agent is attached to the peptide C-terminus, while the N-terminus is protected from non-specific cleavage by exopeptidases through employment of an "endcap." We have demonstrated the cleavage of these agents to allow release of the active anti-tumour agent. These prodrugs are stable in plasma, and undergo rapid cleavage and cytotoxic release selectively in tumour tissue. *In vivo* the prodrugs demonstrated wide tissue distribution, with activation observed selectively in the tumour and relative stability in plasma and normal tissues. The prodrugs resulted in active drug levels in the tumour comparable to that achieved by parent drug alone, but with little or no active drug exposure in plasma or normal tissues. Prodrug delivery to the tumour resulted in anti-tumour activity and a significant delay in tumour growth. In combination with doxorubicin, tumour cures were observed.

Conclusion

We have designed and synthesised a series of peptide-based conjugates of a potent anti-tumour agent. Our biological data demonstrate the potential to safely deliver such highly toxic drugs directly to the tumour.

POSTER 3

CYP1A1 activation and pharmacokinetics of a novel chloromethylpyrrolindoline with potential as a tumour-selective prodrug.

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The expression in a wide range of cancers of selected isoforms of Cytochrome P450 (CYP) that have drug metabolising activity has important implications for CYP-mediated tumour selective chemotherapy. We show the activation of a novel chloromethylpyrrolindoline (ICT2700) that is inactive until metabolised into a highly potent ($IC_{50} < 1nM$) antitumour agent by CYP1A1 in a murine xenograft.

Mice bearing s.c. CHO xenografts overexpressing human CYP1A1, were administered ICT2700 at a non-toxic dose of 150mg/kg (i.p.). The pharmacokinetics of ICT2700 and formation of the active C5-hydroxy metabolite were studied in plasma and major organs including lungs, liver and tumour using LC/MS. Greater than 95% of ICT2700 was present as parent compound in tissues and plasma indicating the systemic stability of this potential prodrug in normal tissue. The remaining 5% was a complex mixture of metabolites which are non toxic in vitro. ICT2700 AUCs_(0-24h) and C_{max} values demonstrated excellent distribution throughout the host tissue. The toxic C5 hydroxy active metabolite was detected in xenograft tissue. AUC and C_{max} in tumour were consistent with the concentrations required to produce cytotoxicity in vitro.

The biological stability and CYP1A1 expressing xenograft –selective activation of ICT2700 demonstrates the potential of the chloromethylpyrrolindolines as tumour-activated therapies.

POSTER 4

Novel Inhibitors of Heparanase as Anti-Angiogenic and Anti-Metastatic Agents

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Many cancers are characterised by an increase in heparanase, an *endo*- β -D-glucuronidase which exclusively degrades cell surface heparansulfate (HS). This degradation releases shorter HS chains into the extracellular space and to general circulation. These circulating heparansulfate chains and the proteins which are associated with them significantly contribute to many of the pathophysiological effects associated with cancer; in particular promotion of vascularisation in the tumour body, and the promotion of metastasis through degradation of ECM. Indeed, the evidence for an important role by heparanase in tumour growth and metastasis is overwhelming.

Low molecular weight heparins (LMWHs) such as PI-88 inhibit heparanase, and have shown promise in clinical trials as cancer therapeutics. However, as a class they are also associated with extensive and severe side effects which has prevented their progress beyond Phase II studies.

We have recently undertaken a programme to discover novel classes of small molecule heparanase inhibitors which are distinct from the LMWH class, and thus will not suffer from their shortcomings. Here, we report on the preparation and heparanase inhibitory activity of rationally designed manno-carbasugars bearing a basic pyridyl group at the anomeric position. Design of these inhibitors was inspired by the mechanistic understanding of the glycolytic process in heparanase degradation. This involves protonation of the exocyclic oxygen atom in the polysaccharides. Thus, introduction of a basic group at that position, coupled to non-hydrolysability of a carbasugar manifold affords a series of substrate analogue which competitively inhibit heparanase.

POSTER 5

A Comparison of Proteins and their Expression Levels within Different Regions of Multi-cellular Tumour Spheroids (MCTS)

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Solid tumours often exhibit a large degree of heterogeneity, which occurs as a result of the differential environmental pressures faced by discrete populations of cells within the tumour. Like solid tumours, MCTS are characterized by the emergence of cellular heterogeneity. Methods were developed for the separation of the different regions spheroids formed from HT29 cells. A series of sequential trypsin treatments were used to remove layers of cells from each spheroid region, defined as (i) the proliferative outer rim, (ii) an intermediate region, (iii) the hypoxic cells within the inner region of the spheroid and (iv) the non-adherent cells within the necrotic core. Following lysis of the cells, recovered protein from each region was to trypsin digestion. An iTRAQ labelling approach in combination with 2D peptide separation (isoelectric focusing and reverse phase HPLC), MALDI MS/MS mass spectrometry (LC MALDI) and database searching was used to compare changes in protein expression between different spheroids regions. A total of 649 unique proteins were found with an average of 144 proteins identified by LC/MALDI from each isoelectric focusing fraction. An average of 3.5 peptides (equivalent to an average of 11.6% sequence coverage) contributed to the identification of the protein.

POSTER 6

Pseudo 3D DWI of the Female Pelvis: A Potential Means of Increasing Staging Accuracy

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Diffusion weighted imaging (DWI) is now increasingly used in body imaging. In our practice we routinely use DWI in the examination of the female pelvis. DWI helps to determine the extent of the primary lesion, local lymph node involvement and distance metastasis, thereby improving the accuracy of the FIGO stage

However, DWI is inherently a low signal-to-noise (SNR) technique consequently multiple averages are utilised to increase the available SNR with resulting increases in scan time. Traditionally, DWI is acquired axially since less geometric distortions are noted. However, to fully assess the uterus and cervix DWI acquired in the sagittal plane and perpendicular to the long axis of the uterus/cervix would be advantageous. Obviously, these additional sequences would lead to an increased total scan time.

In this feasibility study pseudo '3D' DWI was acquired. All MR examinations were performed on a GE Healthcare 3.0T HDx scanner in combination with an 8 channel receive only phased array coil. To enable a '3D' acquisition 2D 5mm slices were acquired with a negative 2.4mm slice spacing thereby providing a pseudo 3D sequence.

Pseudo '3D' DWI was successfully obtained in more than 20 patients in a clinically acceptable imaging time. This allowed the reformatting of DWI data into any desired plane. This methodology has led to a considerable time savings since one '3D' DWI sequence has replaced several 2D DWI sequences.

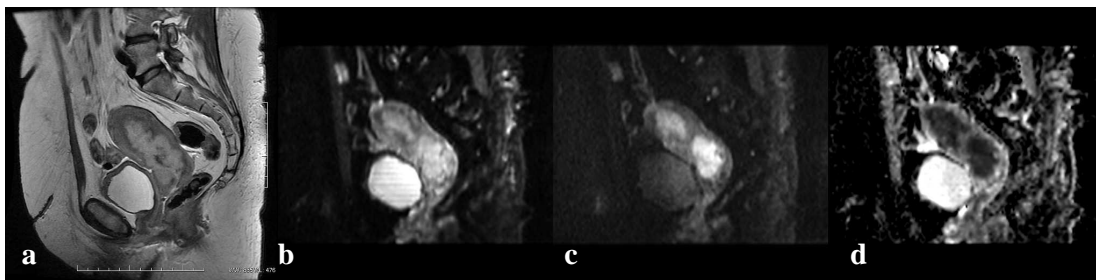


Fig. 1. Sagittal T2 FSE (a), axial diffusion data reformatted into the sagittal plane $b=0s/mm^2$ (b), $b=600s/mm^2$ (c) and ADC (d), low ADC dark, high ADC bright, images.

POSTER 7

Selective targeting of human lung cancer using virally-derived nuclear import peptides as adjuvants for liposome-mediated cancer gene therapy

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Liposome-mediated gene therapy holds great promise as a therapy for epithelial cancers such as bronchogenic carcinoma, particularly if detected at an early stage. However, current liposomes do not appear to be efficient in facilitating nuclear import and expression of transgenes, particularly in non-dividing cells. We have identified two peptides (Mu and VII[25-54]) derived from adenovirus core proteins which complex with the viral genome during nuclear import. These peptides significantly enhanced liposome-mediated plasmid DNA delivery to the bronchial epithelial carcinoma cell line A549; between 2-3 fold increase in reporter gene expression compared to liposome (DC-Chol/DOPE) and DNA alone. Previous work has been carried out on subconfluent submerged cells, which is not representative of the situation *in vivo*. We have therefore developed a tumour cell culture model that is more representative of the respiratory tract by culturing primary human polarised airway epithelial cells (AEC) derived from nasal brushings. AEC were seeded with A549 cells to determine the levels of targeted expression of transgenes in co-cultures of normal and tumour cells. To distinguish the A549 cells from AEC, A549 cells were pre-labelled with a Celltracker Green dye (CFDA) prior to co-culture which were then transfected with a DS Red expression plasmid complexed with peptide in DCChol/DOPE. Flow cytometry and fluorescence microscopy was used to distinguish cell populations and showed that unlabelled AEC could be distinguished from CFDA-labelled A549 cells. We are also analysing the immune response against each peptide to determine whether an immune response might limit re-administration of the liposome:peptide:DNA complex.

POSTER 8

The Role of CUB Domain Containing Protein 1 (CDCP1) in Colon Cancer

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CUB Domain Containing Protein 1 (CDCP1) is an 836 amino acid, approx. 130kDa type I transmembrane glycoprotein. Human CDCP1 has been shown to promote metastasis in cancer cell culture and mouse *in vivo* transplantation models. The expression level of CDCP1 is relatively high in colon cancer cell lines. We aim to elucidate the role that CDCP1 plays in colon cancer.

We have characterised the cell surface expression and total protein expression of CDCP1 in a panel of colon cancer cell lines. This identified two CDCP1 negative (Colo320 and Colo741) and three positive colon cancer cell lines (SW480, HCT116, HT-29). We have developed a Colo320-CDCP1-FLAG stable cell line. We are characterising phenotypic changes caused by altering CDCP1 protein expression using a CDCP1 expression vector and siRNA. We are interested in four main themes, the effect of CDCP1 on the cell cycle including apoptosis, the plasma membrane localisation of CDCP1, particularly whether it localises to lipid rafts and related domains, the effect of ligand binding on CDCP1 endocytosis (simulated by antibody binding) and the effect of CDCP1 on colon cancer cell migration. Preliminary data suggests that CDCP1 affects the cell cycle in colon cancer cell lines.

POSTER 9

The role of ASPM protein in cancer

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The development of aneuploidy is a trademark of many cancers. Aneuploidy arises when errors occur during mitosis leading to chromosomal misorganisation. A renewed interest in the role of aneuploidy in cancer has recently led to increased research into the involvement of mitosis-associated proteins in tumourigenesis. Primary microcephaly (MCPH) is a disorder of neurogenic mitosis. *MCPH5* encodes ASPM, a protein involved in mitotic spindle organisation. Our pilot data indicated that ASPM expression was upregulated at the protein level in breast and ovarian cancer, both of which exhibit high incidences of aneuploidy. We are currently expanding this research to investigate the role of ASPM in chromosomal instability in cancer. We have used immunohistochemistry on existing and newly assembled tissue microarrays to show that ASPM is variably expressed in breast and ovarian tumours, with high expression levels correlating with higher-grade ovarian tumours. We have performed immunofluorescence studies on cultured primary cell samples derived from ovarian tumour ascites and established ovarian cancer cell lines. These studies revealed the incidence and type of mitotic abnormalities in these cancers, which are being correlated to ASPM protein levels and the associated clinical data.

POSTER 10

The role of tumour cell syndecan-1 in conferring susceptibility to natural killer (NK) cells.

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Syndecan-1 (CD138) is a heparan-sulphate linked transmembrane proteoglycan expressed by a number of cell-types including plasma cells and their malignant counterpart, myeloma cells. In myeloma, elevated levels of syndecan-1 in the serum is a marker of poor prognosis. Several lines of evidence suggest that natural killer (NK) cells play an important role in controlling myeloma disease progression. Soluble proteoglycans can inhibit the NK cell mediated killing of tumour cells, but the mechanism by which this occurs is unclear. We tested the hypothesis that syndecan-1 expression on tumours might alter their susceptibility to NK cells *in vitro*. A leukaemic cell line lacking syndecan-1 expression was compared to transfected cells expressing either normal syndecan-1, or syndecan-1 lacking the attached heparan sulphate structures, for their susceptibility to NK cell mediated killing. Expression of syndecan-1 increased the susceptibility of the tumour to NK cells in a heparan sulphate dependent manner. Other proteoglycan molecules were unable to increase the susceptibility to NK cells, indicating a role for both protein and linked heparan sulphate molecules in this interaction. We are currently using siRNA-based approaches to test the role of myeloma cell syndecan-1 in determining susceptibility to NK cells and to elucidate the mechanism(s) by which syndecan-1 alters the susceptibility to NK cells.

POSTER 11

The effect of Transforming Growth Factor (TGF)- β on natural killer cell development and activity.

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Natural killer (NK) cells help to control myeloma progression but myeloma ultimately evades cellular immunity. Myeloma cells make TGF- β , an immunosuppressive cytokine. We show that TGF- β affects NK cell function at multiple levels, antagonising the action of IL-15. TGF- β blocks the IL-15 mediated increase in cell surface expression of numerous receptors known to be important in the NK cell mediated killing of tumour cells (NKG2D, NKp30, DNAM-1 and LFA-1) as well as blocking IL-15 mediated proliferation. In addition, TGF- β inhibits granzyme B expression, a key mediator of apoptosis induction in tumour cells. Inhibition of these molecules is due to reduced gene expression. Given these multiple levels of inhibition, it is not surprising that TGF- β inhibits the ability of NK cells to kill tumour targets. IL-15 also plays an essential role in the differentiation of NK cells from progenitor cells. Using an *in vitro* differentiation system, we show that the ability of TGF- β to block proliferation and NK cell receptor expression results in impaired NK cell development. Thus, TGF- β blocks the IL-15 mediated differentiation and proliferation of NK cells, as well as inhibiting their ability to kill tumour cells. These results suggest that TGF- β inhibition may help to restore productive immune responses to tumours.

POSTER 12

Radiosensitising effect of small molecule tyrosine kinase inhibitors in bladder cancer: Possible role of defective Ku DNA binding in bladder tumours in improving the therapeutic ratio

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Radical radiotherapy is as effective as cystectomy in treatment of muscle invasive bladder cancer and is increasingly used as the population ages. Elderly patients are unable to tolerate conventional chemotherapy agents when used as radiosensitisers. Recently, attempts to increase tumour radiosensitivity have begun to use a target-based approach; the small molecule tyrosine kinase inhibitors (TKIs), including imatinib, have fewer side effects than conventional chemotherapy agents. Imatinib reduces DNA repair following ionising radiation (IR) by targeting homologous recombination, through RAD51. As non-homologous end-joining (NHEJ) is still available for repair in normal cells, but is diminished in muscle-invasive bladder tumours, radiosensitisation should be more pronounced in tumours, thus increasing the therapeutic ratio of radiotherapy.

We are using isogenic bladder cancer cells, one of which has had NHEJ downregulated by Ku70 siRNA, to test this hypothesis. Cells are treated with imatinib, nilotinib, which acts similarly, or lapatinib, which inhibits the NHEJ pathway through DNA PK and is therefore being used as a control. We are looking for synergistic effects of TKIs with radiation using clonogenic assays, and effects on DNA repair protein expression and location within the cell and nuclear focus formation. FACS analysis is used to assess cell cycle and apoptosis effects.

POSTER 13

The use of microbeam confocal live imaging microscopy in the study of *XPC* gene variants in bladder cancer and their influence on *XPC* function

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Background: The human *XPC* gene encodes for a protein involved in nucleotide excision repair, essential for elimination of bulky DNA distortions and in repair of oxidative damage and double strand breaks, created by radiotherapy treatments. Rare genetic variants may contribute significantly to cancer predisposition. We have identified two new rare germline *XPC* variants (Phe302Ser and Arg393Trp) seen more commonly in 771 bladder cancer cases than 800 controls, but their functional effects have yet to be established. Also, the effects of *XPC* variant Trp690Ser, which leads to a decrease in protein function in skin fibroblast cells, in bladder tumour cells is unclear.

Aim: To investigate recruitment to focal DNA damage of the *XPC* proteins from the three rare gene variants in G1 and S phase in RT112 bladder cancer cells, to see if there is a functional difference compared to recruitment of protein from the wild-type gene.

Method: The *XPC* variants were created within a wild-type GFP-tagged plasmid using site-directed mutagenesis. Using RT112 cell lines transfected with fluorescently-tagged plasmids containing the *XPC* variants, cells are focally damaged using a 408nm diode laser beam, creating double strand breaks, and recruitment of *XPC* to the site of damage is quantified using live cell imaging and ImageJ analysis software.

Results: Results will be presented.

POSTER 14

Targeting the functions of Human Papilloma Virus 16 (HPV16) oncoproteins with RNA aptamers

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Human papilloma virus 16 (HPV16) is a high-risk papilloma virus associated with the development of cervical cancer due to the actions of the oncoproteins E6 and E7. The most well characterised role of E6 in oncogenesis is the degradation of the tumor suppressor p53, while E7 is most well known for its destabilisation of the cell cycle control protein pRb. Despite their small size, both of these oncoproteins bind a variety of additional cellular proteins, however, their functions in many of these interactions are largely unknown.

Aptamers are single-stranded RNA or DNA oligonucleotides that bind to target proteins with high specificity and affinity to rival that of antibodies. Aptamers can be generated to any exposed surface on a protein, and can often bind to targets inaccessible to antibodies. They can exert direct and localised effects on a protein-protein interaction within a cell and therefore (unlike siRNAs) they are not susceptible to off-target effects. In this study, RNA aptamers have been raised to both E6 and E7 with the aim of using these molecules as tools to modulate protein-protein interactions and therefore dissect the activities of these oncoproteins in cellular transformation.

Extra-levator abdominoperineal excision for low rectal cancer: results of a large observational European multicentre study

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Standard abdominoperineal excision (APE) for rectal cancer has a worse outcome than anterior resection (AR), partly due to the anatomy of the lower rectum. We have previously shown in a two-centre series that extra-levator APE removes more peritumoural tissue leading to reduced circumferential resection margin (CRM) involvement and intra-operative perforations (IOPs).

We collected specimen photographs along with clinical and pathological data from 176 extra-levator APEs from 11 European surgeons and compared them to 124 standard resections from 8 surgeons. Pathological dissection was performed using standard methods.

Extra-levator surgery removed more tissue per slice in the distal rectum (2317mm² vs. 1422mm², p<0.0001) leading to lower rates of CRM involvement (20% vs. 50%, p<0.0001) and IOPs (8% vs. 28%, p<0.0001). Extra-levator IOPs were significantly lower in those operated in the prone position compared to lithotomy (6% vs. 21%, p=0.03). However, extra-levator surgery was associated with an increase in perineal wound complications (38% vs. 20%, p=0.02), which were partly reduced by using myocutaneous flaps or Permacol™ mesh during reconstruction.

We have shown in a large European multicentre study that extra-levator APE in the prone position removes more tissue around low rectal tumours compared to standard surgery leading to a reduction in CRM involvement and IOPs. Widespread adoption of this technique could improve survival rates by around 10% and save an additional 200 lives per year in the United Kingdom.

POSTER 16

Complete mesocolic excision with central vascular ligation for colonic carcinoma: a reason for improved survival?

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There is wide variation in outcomes between different centres undertaking surgical resection of colonic adenocarcinoma, which may be related to the method and plane of surgery. We have previously shown a 15% 5-year survival advantage in mesocolic plane surgery compared to resections on the muscularis propria. Additionally, institutions offering complete mesocolic excision (CME) and central vascular ligation have reported 5-year survivals of up to 89%.

We collected the pathology reports and specimen photographs of 49 primary resections for colonic adenocarcinoma performed at an institution routinely offering CME and compared these to 40 standard specimens from another institution. Tissue morphometry was used to calculate the amount of tissue resected and the plane of surgery was graded by two independent assessors.

CME removed more tissue between the tumour and the major vascular resection margin (130mm vs. 87mm, $p < 0.0001$). The specimens were longer (332mm vs. 237mm, $p < 0.0001$) with a greater area of mesentery resected (19936mm² vs. 11389mm², $p < 0.0001$) and greater lymph node yield (33 vs. 19, $p < 0.0001$). CME surgery was meticulous with 92% of mesenteric resections in the mesocolic plane compared to just 40% with standard surgery.

CME surgery with central vascular ligation for colonic adenocarcinoma removes more tissue between the tumour and the vascular resection margin resulting in a maximal lymph node harvest. This, in addition to the better quality of surgery, may partly explain the differences in survival.

POSTER 17

Distinct target gene recognition by transcription factors within the Myc/Max/Mad network

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The Myc/Max/Mad transcription factor network is deregulated in most human cancers. Myc generally functions as a transcriptional activator by heterodimerisation with Max; it is expressed in proliferating cells, declines during differentiation, and is overexpressed in many human malignancies. In contrast, various Mad family proteins (Mad1, Mxi1, Mad3, Mad4 and Mnt) also heterodimerise with Max, but act as transcriptional repressors. Mnt is by far the most abundant of the Mad family proteins; it is expressed in both proliferating and differentiating cells, and is lost in specific cancers. It has generally been assumed that Myc:Max and Mnt:Max recognise the same target genes; the relative abundance of Myc:Max and Mnt:Max would thus determine target gene occupancy and expression during cellular proliferation and differentiation and in malignancies.

Using chromatin immunoprecipitation and tiled genomic microarrays (ChIP-CHIP), we have determined binding sites for Myc, Max and Mnt at 20,000 mouse promoters; this experiment was carried out in triplicate in both proliferating and differentiating mouse erythroleukemia cells. The reproducibility between replicates was extremely high, enabling an accurate ranking of the Myc, Max and Mnt target genes. Most of the Max target genes were also bound by either Myc or Mnt; surprisingly however, many of the Myc and Mnt targets did not overlap or had very different rankings. In addition, our microarray analysis indicated that Mnt recognises a subset of target genes in a Max-independent manner. We also identified target genes whose occupancy by Myc and Mnt is most sensitive to changes in the relative abundance of these proteins during proliferation and differentiation. This work will have relevance for understanding the mechanisms of tumourigenesis in cancers caused by overexpression of Myc or by loss of

Targeting POZ domain transcription factors as cancer therapeutics

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Many human cancers are characterised by the over-expression of transcription factors that normally regulate cellular proliferation and differentiation, and targeting the interactions of such proteins is a potential therapeutic strategy.

The POZ domain is a protein-protein interaction motif found in approximately 40 transcription factors (POZ-TFs). POZ domains direct the recruitment of transcriptional co-repressors to the POZ-TFs, and also mediate the specific interactions of different POZ-TFs with each other. POZ-TFs are involved in several human malignancies: for example, BCL6 is over-expressed in specific B-cell lymphomas, and Nac1 is over-expressed in ovarian cancer. In addition, the POZ-TF Miz-1 is implicated in tumours by virtue of its interaction both with BCL6 and with the c-Myc oncogene product.

Using a variety of biochemical and biophysical approaches, we have characterised the interaction network whereby POZ domains direct specific heteromeric interactions of the POZ-TFs with each other. We have identified a novel interface involved in POZ domain interactions, and have determined the crystal structure of four POZ domain proteins that are involved in cancers. This will be directly relevant for the design of therapeutics that target POZ-TFs in human malignancies.

POSTER 19

Exploiting culture adaptation of human embryonic stem cells as a paradigm for germ cell tumorigenesis

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Human embryonic stem (ES) cells acquire genetic changes during prolonged maintenance *in vitro*, which can provide them with a growth advantage over their genetically normal counterparts. As such, these cells are considered culture adapted. In the case of stem cells, culture adaptation must involve an increased propensity for self-renewal and a decreased propensity for death and/or differentiation, similar to the changes in fate which occur during tumorigenesis. Indeed, the genetic changes most frequently reported in culture adapted human ES cells are the same as those observed in embryonal carcinoma (EC) cells, the stem cells of testicular germ cell tumours. Thus, in an attempt to identify those genes critical in culture adaptation, we have compared gene expression in genetically normal and abnormal human ES cells, in addition to EC cells grown under the same conditions. We have utilised an exon array, and as such have been able to identify not only overall changes in gene expression, but also changes in expression at particular exons, revealing potential splice variants. We have identified a limited number of genes upregulated in both culture adapted ES cells and EC cells, providing oncogenic candidates in germ cell tumorigenesis.

POSTER 20

BRCA2 dependent homologous recombination is required for repair of Arsenite-induced replication lesions in mammalian cells.

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Arsenic exposure constitutes one of the most wide-spread environmental carcinogens, and is associated with increased risk of many different types of cancers. Here we report that arsenite (As[III]) can induce both replication dependent DNA double-strand breaks (DSB) and homologous recombination (HR) at doses as low as 5 μ M (0.65 mg/L), which are within the typical doses often found in drinking water in contaminated areas. We show that the production of DSBs is dependent on active replication and is likely to be the result of conversion of a DNA single-strand break (SSB) into a toxic DSB when encountered by a replication fork. We demonstrate that HR is required for the repair of these breaks and show that a functional HR pathway protects against As[III]-induced cytotoxicity. In addition, BRCA2 deficient cells are sensitive to As[III] and we suggest that As[III] could be exploited as a therapy for HR deficient tumours such as BRCA1 and BRCA2 mutated breast and ovarian cancers.

POSTER 21

RecQ5 helicase overcomes thymidine induced replication arrest.

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RecQ5 is a member of the RecQ family which consists of BLM, WRN and RecQ4. It is a 3' to 5' helicase with strand annealing activity and its activity is proposed to be associated with replication forks and homologous recombination. *In vitro* it can bind to synthetic replication forks and facilitate regression of these forks to form holliday junctions, while *in vivo* it binds to and co-localises with PCNA. RecQ5 depletion in *C.elegans* leads to a reduced life span whereas KO mice have an increased incidence of tumours and an increase in SCE. Here we investigate the function of RecQ5 in human cells.

We show that overexpression of RecQ5 allows cells to overcome thymidine induced replication arrest. Normal cell cycle check points are not triggered and cells can replicate and survive in higher doses of thymidine. We will also describe the protein interactions of RecQ5 which may facilitate this function.

POSTER 22

DNA damage response and repair in Uveal Melanoma

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At the molecular level primary cultures of uveal melanoma and established cell lines have been found to have reduced levels of sister chromatid exchange compared to matched patient bloods, cutaneous and other human cell lines (See abstract by L.Hoh). SCE is linked to DNA repair and defects in DNA repair pathways are associated to altered levels of SCE. Here we investigate the integrity of various repair pathways in uveal melanoma cell lines in order to understand better the process of SCE and the pathogenesis and chemoresistance of Uveal melanoma. There are a number of genetic disorders which have increased spontaneous SCE such as Bloom syndrome (BS) caused by mutations in the protein, BLM, or Fanconi's Anaemia (FA) caused by mutations in the FANC proteins which exhibit normal levels of spontaneous SCE but a lack of SCE in response to cross-linking agents. In addition many sporadic cancers exhibit increased SCE. Uveal melanoma however, is the only condition to date to exhibit decreased levels. It is thus a unique system in which to study the phenomenon of SCE and it's link to DNA repair.

Sister Chromatid Exchange in Uveal Melanoma

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During Sister Chromatid Exchange (SCE), two sister chromatids break and rejoin, resulting in the exchange of genetic information. Irregularities in SCE levels are associated with some cancer prone genetic syndromes, and increased SCE is reported in a range of tumours. Although SCE is routinely used to measure genetic instability, the mechanism and pathways that regulate it are not fully understood. In this pilot study we have determined and characterised SCE levels in uveal melanomas. SCE is associated with homologous recombination (HR) thus we hypothesis that uveal melanomas will have an alteration in HR (see abstract by Polly Gravels). The significance of a decrease in SCE is unclear, but as the initial genetic changes underlying tumourigenesis of uveal melanomas are unknown, alterations in HR/SCE rates may contribute to their progression or development.

**Developing Biomimetic Polymer Vesicles (BPV) for Intracellular Delivery
of Chemotherapeutic Agents to Head & Neck Cancer Cells**

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Biomimetic polymer vesicles (BPV) are synthetic block co-polymers that self-assemble in water to form membrane-enclosed nano-vesicles. BPV have the potential to encapsulate and carry chemotherapeutic drugs into cells thereby reducing the off target toxicity that often compromises anti-cancer treatment.

Here, we assess the *in vitro* efficiency of BPV to penetrate and deliver their load to head and neck cancer cells (HNSCC) cultured as both monolayers and as tumour spheroids (small solid tumour masses). Using BPV loaded with fluorescent rhodamine we found that BPV are internalised by HNSCC within 2 minutes of administration and maximal delivery of BPV load is achieved within 30 minutes. In addition, our preliminary experiments show that BPV loaded with only 20 % paclitaxel demonstrated similar killing of HNSCC grown as monolayers compared to the same concentration of free drug alone. We are currently trying to increase the amount of paclitaxel encapsulated by BPV and are also attempting to load BPV with paclitaxel and cisplatin for combination therapy. BPV could provide a safe and efficient method of delivering drugs for the treatment of head and neck cancers, offering significant advances in drug delivery with the goal to improving treatment of cancer patients.

POSTER 25

The Functional Role of CASP8 D302H and Other Apoptosis Gene Variants in Breast Cancer

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It is well established that perturbations in high penetrance genes such as BRCA1 and BRCA2 predispose to breast cancer. However, low penetrance genes are still under investigation. We recently reported that a coding single nucleotide polymorphism (SNP) in the caspase 8 gene (CASP8 D302H) is associated with a reduced risk of breast cancer. More recently we identified a CASP8 4-SNP haplotype associated with an increased risk of breast cancer. Our hypothesis is that CASP8 and other apoptotic genes influence breast cancer susceptibility via effects on the apoptotic response.

Our objectives are to study the functional effects of CASP8 D302H and the 4-SNP haplotype on apoptosis induction in peripheral blood lymphocytes (PBLs).

We have recruited women attending mammography screening and measured the ability of their PBLs to undergo drug-induced apoptosis. Levels of apoptosis and caspase-8 activity were determined by FACS analysis.

Based on data from 35 samples, apoptosis levels range from 50% to 92% (median 78%, SD 10.5) and CASP8 protein levels 46% to 92% (median 67%, SD 11.8). We have successfully detected variations in apoptotic/CASP8 response and aim to determine whether these variations correlate with CASP8 genotype, to help us understand the mechanism of the association with breast cancer.

POSTER 26

Sequencing breast cancer risk haplotype carriers in the Caspase 8 gene.

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Highly-penetrant mutations of the BRCA1 and BRCA2 genes were found over 10 years ago to confer a lifetime risk of breast cancer of over 80%. Since that time, medium and low penetrance genetic variants predisposing to breast cancer have been discovered, such as the caspase 8 D302H single nucleotide polymorphism (SNP) risk variant. More recently, a 4 SNP CASP8 haplotype, 1-1-2-1, was found to be linked to an increased risk of breast cancer. Our hypothesis is that the 1-1-2-1 risk haplotype might carry further interesting, perhaps causative variants in the caspase 8 gene.

Our main objective is to sequence the whole of the CASP8 gene in 47 DNA samples, 24 of which are homozygote for the 1-1-2-1 risk haplotype and 23 homozygote for the alternative 1-2 protective haplotype to identify further variants.

Primers and corresponding PCR protocols for the caspase 8 exons and introns were designed. The 47 samples were sequenced using standard fluorescent dideoxy nucleotide chemistry with analysis on the ABI 3730. Results were analysed using Sequencher and Finch TV. A PCR-based assay was developed to examine two large insertion/deletion polymorphisms.

A heterozygote 115bp deletion in exon 3 was identified in one risk haplotype carrier, and polymorphic copy number variants were found in intron 11. One copy number variant is strongly associated with the risk haplotype.

Modulation of Fanconi Anaemia Pathway in Terminally Differentiated Cells

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The Fanconi anaemia (FA) pathway is a versatile DNA repair pathway that is required for S-phase progression and the repair of inter-strand crosslinks and double strand breaks. The FA proteins consist of an ubiquitin ligase core protein complex (FANCA, FANCB/FAAP95, FANCC, FANCE, FANCF, FANCG/XRCC9, FANCL, FANCM, FAAP100 and FAAP24), two monoubiquitinated proteins (FANCD2 and FANCI), a helicase (FANCI/BRIP1) and two breast cancer susceptibility gene (BRCA2/FANCD1 and PALB2/FANCN). Upon DNA damage, such as ionizing radiation (IR) or UV light, FANCD2 is activated by monoubiquitylation. Since downregulation of other DNA pathways controlled by ubiquitylation, such as NER, was reported in terminally differentiated cells, we were interested in investigating whether this also happens for the FA pathway.

The ability to activate the FA pathway after DNA damage was compared in naïve and differentiated human acute monocytic leukaemia cells (THP-1) by immunoprecipitation and western blot of FANCD2. It was observed that monoubiquitylation of FANCD2, after either UV or IR, was abolished in terminally differentiated cells. In addition, the cellular levels of FANCA, FANCI and FANCD2 proteins were decreased upon differentiation. Also, the mRNA level of FANCD2, FANCI, FANCA and the E2 enzyme of the pathway, UBE2T were downregulated to less than 20% of their original level upon terminal differentiation. Interestingly, the E3 subunit FANCL, the deubiquitylase USP1, and FANCE were less downregulated than the genes mentioned above, at 40% of their original level. Surprisingly, the mRNA level of FANCC was hardly modified at the terminal differentiation stage. The lack of FANCD2 monoubiquitylation upon DNA damage was further investigated with co-immunoprecipitation studies. Strikingly, interaction between MRE11, FANCI and FANCD2 (which fails to monoubiquitylate) was observed in terminally differentiated samples, regardless whether samples have been subjected to IR. Moreover, this interaction was only observed, in a lesser extent, in naïve THP1 cells challenged with IR. Combining the results obtained, we suggest that inactivity of FA pathway upon differentiation of THP1 may not only be affected by differential downregulation of FA genes during differentiation, but also by stoichiometry change in turnover rate between FA core complex and FANCD2. Whether the stoichiometry change is due to stalled FA core complex, or introduction of a more potent deubiquitylation enzyme that favours reverse required further investigation.

Relative Genetic Imbalance (RGI) between chromosome 8 and c-Myc copy number as an indication of survival in uveal melanoma

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Amplification of the long arm of chromosome 8 has been correlated to metastatic death in uveal melanoma; and patients with only one additional copy of 8q have a better prognosis than those with higher amplification. The most commonly identified shortest region of overlap (SRO) within this region is 8q21-qter; within which can be found the locus of the oncogene *MYC* at 8q24.1. *MYC* amplification is of interest, as deregulation of the nuclear transcription factor has been correlated with increased cellular growth, proliferation and self-renewal, in addition to a poor level of differentiation. The objective of this study was to determine if increased *MYC* copy number compared to centromeric 8 (Relative Genetic Imbalance or RGI) was a better indicator of prognosis.

Fluorescence in situ hybridisation (FISH) on 76 archival primary uveal melanoma samples was performed for CEP8 and *MYC* copy number. 60% of samples showed a RGI, confirming *MYC* amplification and indicating that high levels of amplification for 8q will be missed using CEP 8 alone. RGI for *MYC* was found not to correlate with patient survival, in the absence of RGI for chromosome 3 and 8, but where RGI between chromosomes 3 and 8 was present, amplification of *MYC* further exacerbated the prognosis.

Differential detection of Ciz1 splice variants in normal and cancer cells using a custom exon-junction microarray

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Ciz1 stimulates initiation of DNA replication (1) and is part of nuclear matrix associated DNA replication factories (2). Ciz1 is estrogen-responsive and is thought to play a role in the development of oestrogen responsive tumours (3). It has also been linked with certain paediatric cancers (4, 5). We showed that an alternatively spliced human Ciz1 variant, lacking exon 4 is mis-expressed in Ewings Tumour cell lines. Variant protein retains replication activity, but fails to form sub-nuclear foci (5). Similarly, a second variant is prevalent in some types of lung cancer (in preparation). Ciz1 variants appear to offer selective targets for therapeutic intervention. Therefore, we aim is to systematically survey Ciz1 variant expression in order to validate previously reported alternative splicing events and to discover new ones. To do this we have developed an exon junction-array focused on the human Ciz1 gene that is capable of detecting all known variants and hypothetical variants of Ciz1. We present results, initially using a set of paediatric cancer cell lines that show the array to be capable of reproducible and differential detection of Ciz1 splice variants. From this analysis we have so far identified one novel Ciz1 splice variant whose expression appears to be restricted to cancer cell lines.

(1) Ciz1 promotes mammalian DNA replication

Dawn Coverley, Jackie Marr and Justin Ainscough *J Cell Sci.* 2005 118:101-12

(2) C-terminal domains deliver the DNA replication factor Ciz1 to the nuclear matrix

Justin F.-X. Ainscough, Faisal Abdel Rahman, Heather Sercombe, Alicia Sedo, Bjorn Gerlach and Dawn Coverley, *J Cell Sci.* 2007 120:115-24

(3) Ciz1, a Novel DNA-binding coactivator of the estrogen receptor alpha, confers hypersensitivity to estrogen action

Petra den Hollander, Suresh K. Rayala, Dawn Coverley and Rakesh Kumar *Cancer Res.* 2006 15;66(22):11021-9

(4) Ciz1, Cip1 interacting zinc finger protein 1 binds the consensus DNA sequence ARYSR(0-2)YYAC

Warder DE and Keherly MJ. *J Biomed Sci.* 2003 10(4):406-17

(5) Cancer-associated mis-splicing of exon 4 influences the subnuclear distribution of the DNA replication factor

CIZ1 Faisal Abdel Rahman, Justin F.-X. Ainscough, Nikki Copeland and Dawn Coverley *Hum Mutat.* 2007 28(10):993-1004

POSTER 30

Identifying a role for Ciz1 in the DNA damage response

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Ciz1 is involved in DNA replication, where it acts to co-ordinate the actions of cyclins E and A in association with the nuclear matrix. Ciz1 contains a number of SQ/TQ motifs, which are targeted by mediators of the proliferating cell's response to DNA damage (DDR), ATM and ATR, and has been implicated as part of this pathway. We have developed a cell-free system with which to study components of the DDR, using Histone H2AX phosphorylation and DNA synthesis as direct and indirect markers of DDR activation, respectively. Using this approach we have uncoupled the cell's response to DNA damage from the damage itself, so that effects of an activated response on an undamaged genome can be monitored. In addition to demonstrating this method's potential as a screening tool for inhibitors of the response, we have used it to confirm that Ciz1 is phosphorylated during the DDR. We present data demonstrating the capacity of isolated nuclei and cytosolic extracts from synchronised cells to reconstitute and induce the DDR in vitro, and show that DDR-activated cellular extracts phosphorylate SQ/TQ motifs present within Ciz1. This suggests that phosphorylation of Ciz1 may play a role in the restraint of DNA replication that cells experience in response to double strand breaks.

The nuclear matrix protein Ciz1 cooperates with cyclin A/CDK2 to activate mammalian DNA replication *in vitro*

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Ciz1 is a nuclear matrix associated protein that is differentially spliced in a number of cancers. Furthermore, over-expression of Ciz1 in breast cancer cell lines appears to enhance proliferation, invasion and tumourigenic properties. In NIH3T3 cell lines, over-expression of Ciz1 promotes DNA replication and, conversely, depletion of Ciz1 by siRNA reduces the number of cells that replicate. Similarly in cell-free DNA replication experiments using isolated G1-phase nuclei, addition of recombinant Ciz1 promotes DNA replication. The mechanism by which Ciz1 promotes DNA replication is not completely understood. Here we show that Ciz1 interacts sequentially with cyclins E and A via distinct cyclin binding motifs that are required for DNA replication. In competition assays, cyclin A displaces cyclin E from its binding site on Ciz1. In addition, Ciz1 cooperates with Cyclin A to promote initiation of DNA replication in 'competent' G1-phase nuclei most likely by immobilising cyclin A within the nucleus. These results suggest that Ciz1 co-ordinates the functions of cyclins E and A during the switch from pre-replication complex assembly to DNA synthesis, ensuring that cyclin E and cyclin A-dependent events occur in the same place and in the correct order.

Cyclin E is a Conditional Nuclear Matrix Protein

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Cyclins E and A play distinct and sequential roles in initiation of mammalian DNA replication. To further understand the functions of cyclin E we have explored its spatial and temporal organization in a range of mammalian cells. We have shown that in some cells cyclin E resists salt/nuclease extraction indicating an association with the nuclear matrix. This is the first time a cyclin has been shown to be part of the nuclear matrix, although determinants of cell cycle dependent initiation are known to be associated. In mouse 3T3 cells a proportion of cyclin E is immobilized in nuclear matrix-associated sub-nuclear foci throughout interphase of the cell cycle. However, in mouse embryonic stem (ES) cells none of the cyclin E resisted extraction with salt/nuclease. Notably, after ES cells were induced to differentiate, a sub-population of cyclin E became salt/nuclease resistant. A similar transition onto the nuclear matrix was observed in normal human urothelial cells induced to differentiate *in vitro*. Cyclin E expression is altered in some types of cancer cell, and the nuclear matrix also appears to be commonly disrupted. We studied a range of cancer cell lines and found that in these cells cyclin E showed a similar localization to that found in undifferentiated cells.

MODELLING PROSTATE CANCER STEM CELLS IN VIVO

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The prostate is renewed throughout adult life and this depends on a subpopulation of epithelial stem cells. Stem cells have the ability to perpetuate themselves through self-renewal and their longevity makes them excellent candidates for tumour-initiation.

Research from our laboratory identified the cancer stem cell (CSC) from human prostate tumours as phenotypically $\alpha_2\beta_1^{\text{hi}}/\text{CD133}^+$. The CSC population possess a capacity for self-renewal (in contrast to the androgen-dependent secretory luminal population) and differentiation to a secretory luminal lineage. Further work has focused on the 'gold standard' assay for cancer stem cells which is tumour-initiation *in vivo*. However, modelling tumour-initiation, from selected patient's cell populations, *in vivo* has proved difficult. This may be due to i) the mouse model, ii) the tumour microenvironment, iii) the hormonal status of the host and iv) surgical technique. Recently we have developed a procedure for successfully grafting and serially transplanting primary human prostate cancer tissue in testosterone-supplemented Rag2^{-/-} Gamma C^{-/-} mice. The 042/07 xenograft has a latency of 40 days when grafted subcutaneously and metastases are observed in multiple organs. Orthotopic grafting also results in high tumour take (>95%) with resultant metastasis to multiple sites. In contrast, grafting isolated cell populations reduces tumour take dramatically, from all phenotypes tested, which may be due to the loss of tumour stroma.

EPIGENETIC REGULATION OF THE STEM CELL MARKER CD133 IN PROSTATE CANCER

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Epigenetic disruption represents a crucial point in prostate cancer development and progression, suggesting the potential for new therapeutic approaches. Moreover, it has recently been proposed that epigenetic disruption of progenitor cells may be an initial step in cancer development. However, at present there is no direct evidence of epigenetic deregulation of prostate cancer stem cells.

Recent publications (*Yi et al., 2008, Cancer Res*) have shown that expression of the stem cell surface marker CD133 can be regulated by epigenetic mechanisms. In the present study we used methylation specific PCR and pyrosequencing in order to test whether the expression of CD133 is differentially regulated by DNA methylation in benign versus prostate cancer samples, as has recently been shown for brain and colon.

The CD133 promoter was hypermethylated in most of the prostate cancer cell lines analysed, when compared with non-malignant cell lines. In contrast, these sequences were unmethylated in most of the primary tissues analysed, without distinction between benign and cancer. This discrepancy indicates that cell lines might not represent a valid model for DNA methylation studies. Furthermore, these data suggest that other mechanisms, rather than DNA methylation, might regulate CD133 expression in prostate tissues. Importantly, this analysis represent the first DNA methylation study carried out in prostate stem cells.

CD44 EXPRESSION IN PROSTATE CANCER STEM CELLS

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CD44 is a transmembrane glycoprotein with multiple isoforms generated by alternative splicing. It has been implicated in a number of disease processes, including tumour metastasis. Several splice variants of CD44 have been reported in prostate carcinomas, but there are conflicting reports on its relation to carcinoma stage. According to the cancer stem-cell (CSC) hypothesis a small number of CSCs with tumour-initiating and tumour-maintaining capacity exist within the mass of more differentiated cancer cells.

CSCs can be isolated on the basis of their expression of $\alpha 2\beta 1$ integrins, CD44 and the cell surface antigen CD133. However, little is known about CD44 isoform expression in these cells. Therefore CD44 expression was investigated in prostate cell lines, and cancer and benign primary patient samples.

RT-PCR and real time PCR showed that prostate cell lines expressed the commonest short isoform of CD44 (CD44S) together with larger forms containing variant exons: v2-v10, v6-v10 and v8-v10. Analysis of benign and cancer primary samples showed a significant reduction of expression of splice variant expression in cancer progenitor cells. Variability of expression was observed in CD133-positive stem cells from both cancer and benign tissues.

Immunofluorescence staining confirmed CD44 expression in selected cell populations. Differential expression of CD44 isoforms between prostate CSCs and more differentiated cancer cells could be helpful in identifying new therapeutic targets for improved treatment of prostate cancer.

IDENTIFICATION OF GENES INVOLVED IN STROMAL ANDROGEN RECEPTOR SIGNALLING: IMPORTANCE OF CALCIUM SIGNALLING.

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Androgens have a vital role in prostate development and pathogenesis of benign prostatic hyperplasia (BPH) and prostate cancer. Stroma may mediate androgenic effects upon epithelium, with a key regulatory role in stem cell fate and cancer progression. Here, we evaluate androgen-regulated stromal genes that may influence stem cell fate or differentiation.

BPH stromal cells treated with dihydrotestosterone were fractionated for the androgen receptor population. Microarray analysis determined androgen induced differentially expressed genes. Chromatin-immunoprecipitation assay (chIP) assessed functional androgen response elements (ARE) within candidate androgen receptor (AR) interacting transcription factors.

Differential gene expression of 165 probes (6h) and 193 probes (14h, $P \leq 0.05$) determined SRF, MUC-1 and STIM1 (transmembrane glycoprotein that can monitor intracellular calcium levels) as androgen-regulated, confirmed by qRT-PCR. Two ARE in the STIM1 promoter bound AR. Gene Set Enrichment software detected a further 20 genes from the calcium signalling pathway including calmodulins and calcium binding proteins.

The identification of this set of genes underlines the importance of calcium signalling for the stromal androgen receptor pathway. Targeting growth receptor pathway proteins may allow cancer stem cell and prostate-specific targeting of therapies and of androgen-responsive proteins. STIM1 is critical for migration of breast cancer tumour cells, and STIM1 blockade may potentially be therapeutic for tumour metastasis.

GENE EXPRESSION PATTERNS IN HUMAN PROSTATE CANCER STEM CELLS

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The tumour-initiating and sustaining capabilities of many cancers are believed to reside in a small subpopulation of cells termed cancer stem cells. Rare prostate epithelial cells with a CD44⁺/α₂β₁^{hi}/CD133⁺ phenotype have the properties of prostate cancer stem cells and have previously been isolated from human prostate tumours.

Recent Affymetrix gene-expression array data has identified two closely-related genes that are significantly down-regulated in stem cells compared to committed cells in both benign and malignant tissue in human prostate. Where expressed, these potential 'stem cell control' genes are believed to work by inhibiting carboxypeptidase A4 function.

Quantitative RT-PCR was initially used to analyse mRNA expression of the 3 genes in benign and malignant primary prostate cancer tissue. To investigate the relationship between differentiation, mRNA expression after treatment of prostate cell lines with a differentiation agent (sodium butyrate), histone deacetylation inhibitor (trichostatin A) and DNA methylation inhibitor (2'-deoxy-5-azacytidine) was also analysed.

These initial experiments suggest that these genes do indeed play a role in differentiation and may be part of the same differentiation pathway. The mechanisms which achieve the necessary tight control of expression of fate-determining genes are currently under investigation.

IL-6 EXPRESSION IN PROSTATE CANCER STEM CELLS AND STAT3 SIGNALING PATHWAY

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Prostate cancer is the most common cancer in men in the UK and a major health problem. Currently there is no effective therapy for the recurring form of prostate cancer. Treatment failure may be due to the existence of cancer stem cells (CSCs), which is the focus of our research. Gene expression profiling of prostate CSCs showed over-representation of the JAK-STAT signalling pathway and interleukin-6 (IL-6) (Birnbaum et al., 2008), which activates the JAK-STAT pathway. Studies have shown that this pathway is important for self-renewal of human embryonic stem cells and maintenance of their undifferentiated state, with STAT3, the main component of the JAK-STAT pathway, to be central for maintenance of stemness.

Initial studies have centred on IL-6 expression, which has also been shown to be over expressed in high grade prostate tumours (Twillie et al., 1995). Analysis of mRNA levels using qRT-PCR, from primary prostate tissue (4 BPH; 4 prostate cancer: consisting of one Gleason 7 (relapsed after hormonal therapy), three Gleason grade > 8), confirmed that IL-6 is indeed expressed at higher levels in the stem cell population (CD133⁺ $\alpha_2\beta_1^{\text{hi}}$) compared to the committed progenitor population ($\alpha_2\beta_1^{\text{lo}}$). Preliminary work showing phosphorylation of Stat3 suggests that the JAK-STAT signalling pathway is constitutively active in prostate cancer stem cells, although we have yet to determine whether the IL6-receptor is expressed in these cells. Future work will concentrate on what the effect of blocking this pathway has on the cancer stem cell function, such as their potential to self-renew.

**Basal survival of human cancer cells involves JNK2 suppression
of a novel pro-apoptotic pathway**

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In mammalian cells the induction of apoptosis under stress conditions is well studied. However, regulation of apoptosis under basal physiological conditions is poorly understood but is nonetheless important since under these conditions abnormal suppression of apoptosis leads to cancer. Here we have employed RNAi to study the regulation of apoptosis in human cancer cells under basal conditions of cell culture. Our results demonstrate that JNK2 silencing releases a pro-apoptotic pathway mediated by JNK1, c-Jun, E3 ligase Fbw7, kinases GSK3 and ERK and culminating in activation of caspase 8. This pro-apoptotic pathway appears to be selectively active in cancer cells since non-cancer cells are refractory to JNK2 silencing. Moreover, in cancer cells the pro-apoptotic mediators JNK1 and c-Jun are active in the absence of 'activating' phosphorylations typically observed in response to stress. We propose that intrinsic oncogenic stress results in constitutive activation of a novel JNK1-dependent pro-apoptotic pathway and that JNK2 functions as a cancer-specific survival factor by suppressing this pathway.

POSTER 40

Oncogenic viral protein HPV E7 up-regulates the SIRT1 longevity protein in human cervical cancer cells

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Senescence is blocked in human cervical keratinocytes infected with high risk human papillomavirus (e.g. HPV type16). Viral oncoproteins HPV E6 and HPV E7 access the cell cycle via cellular p53 and retinoblastoma proteins respectively. Previously we have shown that HPV E7, not HPV E6, is also responsible for cervical cancer cell survival (SiHa cells; HPV type16). We now present evidence that SIRT1, an aging-related NAD-dependent deacetylase, mediates HPV E7 survival function in SiHa cervical cancer cells. Moreover, HPV E7 up-regulates SIRT1 protein when expressed in primary human keratinocytes. Conversely, SIRT1 levels decrease following RNAi-mediated silencing of HPV E7 in SiHa cells. Silencing HPV E6 has no effect on SIRT1 but, as expected, causes marked accumulation of p53 protein accompanied by p53-mediated up-regulation of p21. However p53 acetylation (K382Ac) was barely detectable. Since p53 is a known SIRT1 substrate we propose that elevated SIRT1 levels (induced by HPV E7) attenuate p53 pro-apoptotic capacity via its de-acetylation. Our discovery that HPV E7 up-regulates SIRT1 links a clinically important oncogenic virus with the multi-functional SIRT1 protein. This link may open the way for a more in-depth understanding of the process of HPV-induced malignant transformation and also of the inter-relationships between aging and cancer.

POSTER 41

JNK2-dependent regulation of SIRT1 protein stability

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Mammalian SIRT1 is an NAD-dependent deacetylase with critical roles in the maintenance of homeostasis and cell survival. Elevated levels of SIRT1 protein are evident in cancer in which SIRT1 can function as a cancer-specific survival factor. Here we demonstrate that elevated SIRT1 protein in human cells is not attributable to increased SIRT1 mRNA levels but, instead, reflects SIRT1 protein stability. RNAi-mediated depletion of JNK2 reduced the half-life of SIRT1 protein from >9 h to <2 h and this correlated with lack of SIRT1 protein phosphorylation at serine 27. In contrast, depletion of JNK1 had no effect upon SIRT1 protein stability and SIRT1 phosphorylation at serine 47 showed no correlation with SIRT1 protein stability. Thus we show that JNK2 is linked, directly or indirectly, with SIRT1 protein stability and that this function is coupled with SIRT1 phosphorylation at serine 27. Our observations identify a route for therapeutic modulation of SIRT1 protein levels in SIRT1-linked diseases including cancer, neurodegeneration and diabetes.

POSTER 42

Is enhanced suppression of p53 via [SIRT1/AROS] a cancer-related event that enables cancer cell survival?

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SIRT1 is an NAD-dependent deacetylase that induces cancer specific cell survival. Differences in SIRT1 activity observed in cancer cell lines compared to non-cancer lines may be attributable to alterations in SIRT1 regulation. Active regulator of SIRT1 (AROS) regulates SIRT1 activity at the post-translational level, directly binding to SIRT1 distal to its deacetylation domain. AROS enhances SIRT1 repression of the tumour suppressor p53 in cancer cell lines, resulting in reduced cell cycle arrest and greater proliferation. Thus AROS contributes to heightened resistance to cell death in cancer cells, a hallmark of cancerous growth.

AROS depletion by RNAi will characterise the role of AROS in cancer specific SIRT1 up regulation. Depletion of AROS in HCT116 colorectal cancer cells results in apoptosis; ARPE19 non-cancer cells are refractory to identical treatment. Cancer specific apoptosis induced by AROS RNAi is comparable to the apoptosis produced by SIRT1 depletion. However the impact of AROS versus SIRT1 RNAi on p53 protein levels shows a contrast. Ongoing work will resolve the cancer specific anti-apoptotic function of AROS. AROS may be an essential factor for SIRT1 function, without which SIRT1 cannot prolong cancer cell survival. However AROS may also act independently of SIRT1 to decrease cancer cell death.

POSTER 43

P53 auto-regulation via novel SIRT1 splice variation

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P53 expression levels undergo exquisite regulation since they dictate its influence as a stress-responsive transcription factor and our paramount tumour suppressor. We report a feedback loop whereby p53 constitutively auto-regulates its expression levels via novel splice-variation of SIRT1, a pivotal NAD⁺-dependent protein de-acetylase that coordinates energy metabolism with stress-tolerance, multiple homeostatic processes and modulation of lifespan. We describe the first alternative SIRT1 splice-variant, SIRT1-ΔExon8, which is p53-dependent, stress-inducible, expressed ubiquitously in humans, and deregulated in cancer cells. Importantly, constitutive and reciprocal regulation exists between p53 and the SIRT1 splice-variant SIRT1-ΔExon8, revealing extensive inter-dependency between these master regulators of multiple phenomena.

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