Irish Association for Cancer Research

Annual Meeting

3-4 March 2011

Rochestown Park Hotel, Cork

Incorporating:

Satellite Meeting of the IACR – 2nd March 2011

Cancer Therapeutics: Small Molecule and Biological Strategies in the Molecular-Targeted Era

Supported by:
The Department for Employment and Learning
through its "Strengthening the all-Island Research Base" initiative

Department for Employment and Learning
www.delni.gov.uk

IACR COMMITTEE MEMBERS 2011

President Elect, Professor Elaine Kay

David Waugh, Honorary Secretary

Sharon McKenna, Honorary Treasurer

Grace Callagy

Dean Fennell

Ann Hopkins

Karen Keeshan

Daniel Longley

Ciaran Morrison

Paul Mullan

Lorraine O'Driscoll

Jacintha O'Sullivan

Leonie Young

CONTENTS

2nd Annual 2011 Meeting of the Society for Translational Oncology / 3rd International Cancer Symposium of the Centre for Cancer Research and Cell Biology, Queen's University Belfast

September 7-8, 2011
WATERFRONT HALL, BELFAST, NORTHERN IRELAND

CHALLENGES IN CANCER: ANSWERING THE DIFFICULT QUESTIONS

Join us for presentations and collaborative dialogue about the major challenges in cancer, including usefulness of animal models, drug resistance, patient stratification, adaptive trials and how academia can interact with industry for the betterment of cancer treatment

Attend this leading international conference to:

- Gain insight that will shape the course of clinical oncology practice
- Apply the strategic and scientific information gathered to research methodology/clinical practice
- Create opportunities for face-to-face meetings with leading innovators in cancer research

The **Keynote lecture** will be given by the esteemed recipient of the **2011 Pinedo Cancer Care Prize** (to be announced)

The **McClay Foundation Lecture** will be given by ***Dr Richard M Goldberg of the UNC Lineberger Comprehensive Cancer Center***

(CME details to be finalised)

REGISTER NOW ***http://www.qub.ac.uk/ccrcb*** ***(registration deadline: July 29th, 2011)***

For further information please contact: sto-ccrcbconf@qub.ac.uk

in partnership with

The Oncologist®
The official journal of the Society for Translational Oncology

IRISH ASSOCIATION FOR CANCER RESEARCH

All Correspondence to: Sinead Cassidy
Conference Secretariat
9A Coolkill, Sandyford, Dublin 18
Tel: (01) 295 8859
Email: sinead@sineadcassidy.com

ANNUAL MEETING - ROCHESTOWN PARK HOTEL, CORK, 2ND / 3RD / 4TH MARCH 2011

Dear Colleagues,

It is my pleasure to welcome you to our Annual Meeting in Cork. Our Annual Meeting is the focal point of the Association's activities. Looking through the long list of abstracts that have been submitted to the meeting it is great to see many familiar names returning to our meeting. Equally encouraging is the number of first-time registrants to the meeting; we hope that you find the meeting stimulating, rewarding and like your peers consider this to be the "must-attend" local meeting in the annual calendar.

In registering for the meeting, you automatically assume the role of a Member of the Irish Association for Cancer Research (IACR), which also qualifies you as a Member of the wider European Association (EACR). Importantly, as Members, this is your association and I would encourage all of you to attend the Annual General Meeting of the Association. This provides you with an opportunity to hear what the Council is planning for subsequent years and for you to influence the direction that the Association takes in moving forward.

This will be a very poignant meeting for IACR as we close a very significant chapter of our history. With significant regret I have to inform you that Professor Mark Lawler tendered his resignation as President of the Association in September. Many of you will know that Mark has given long and dedicated service to the Association and that in concert with his various other responsibilities, he has had a very strong influence in stimulating the growth of cancer research in Ireland and has brought a wider international awareness of the island's research activities. I know that I speak on behalf of you all and my colleagues on the Council in wishing Mark every success in his future pursuits and offer our sincere thanks and appreciation for all that he has done for this Association. It is certainly true that while we acknowledge the loss of his scientific contribution to the meeting, we will also miss those wonderful moments when Mark's "stage-twin" gave infamous performances at the Gala Dinner. I am delighted to inform you that Professor Elaine Kay has agreed to accept the Council's nomination as our new President. Elaine will be a strong advocate for strengthening research investment and will be the ideal person to represent the clinical and scientific cancer research community to government and charitable bodies alike.

Coming back to this current Annual Meeting, we have been able to incorporate a Satellite Symposium entitled "Cancer Therapeutics: Small Molecule and Biological Strategies in the Molecular-Targeted Era", thanks to funding from the Department of Employment and Learning and from the Centre for Cancer Research and Cell Biology at Queen's University Belfast. We are delighted to host this Satellite Meeting as IACR has been a long-standing advocate of strengthening the All-Island research base and extend our thanks to DEL for its significant contribution to stimulate Cancer Therapeutics research throughout Ireland.

On behalf of the Council, I would also like to take this opportunity to thank all of the speakers for accepting our invitation to this meeting and for the clinical insights and basic science that they will share with all of us. Many of the speakers have travelled a significant distance to join us in Cork. We are very appreciative of the time they have taken from their busy schedules and the contribution that they will make to the success of this meeting. This meeting would also not be possible without generous financial assistance from our many sponsors. In particular, we would like to extend our sincere thanks to the Irish Cancer Society who continue to be a tremendous partner for IACR and provide strong advocacy for the research that IACR members conduct in Ireland. In addition, we are appreciative of the financial assistance provided by each of our "Gold" and "Silver" Level sponsors. We trust that all of our delegates will take the opportunity to visit all the trade stands during the programme.

Finally, I would like to thank all the members of the Council for their contribution to supporting IACR activities throughout the year and extend a special thanks to our Treasurer Dr Sharon McKenna and Mrs Sinead Cassidy for the significant efforts that they have put into the organization of this meeting.

With best wishes,

Sincerely,

David Waugh

Dr. David Waugh
Hon. Secretary

2nd March, 2011

Cancer Therapeutics: Small Molecule and Biological Strategies in the Molecular-Targeted Era

SATELLITE MEETING OF THE IACR – ROCHESTOWN PARK HOTEL, CORK

ONCOLOGY DRUG DISCOVERY OVERVIEW AND SMALL MOLECULE DEVELOPMENT
Chair: Prof David Haigh, Queen's University Belfast

10.30 – 11.00 **Oncology Drugs of the Future - Trends and highlights from new molecularly targeted drugs / drug candidates in clinical evaluation**
Andrew Thomas (AstraZeneca)

11:00 – 11:30 **Fragment Based Hit Identification and Structure Based Approaches to Cancer Drug Discovery**
Martin Drysdale (Beatson Institute for Cancer Research, University of Glasgow)

11.30- 12.00 **Drugging the DNA Damage Response pathways**
Dr Niall Martin (ex-Kudos Pharma)

12.00-12.30 **Chemical Biology Research at the Broad**
Michael A. Foley, Director, Broad Institute, Boston

12.30-13.30 **LUNCH**

KEYNOTE PRESENTATION
Chair: Prof Anita Maguire, University College Cork

13:30 – 14:15 **Cancer Drug Discovery Using Fragment-Based Methods**
Prof Steve Fesik (Vanderbilt University)

SHORT RESEARCH PRESENTATIONS

14:15–14.35 **Development of Novel and highly potent legumain Inhibitors for the treatment of Poor Prognosis Breast Cancer**
Dr Rich Williams (Queen's University Belfast)

14.35-14.55 **Design and synthesis of novel indolocarbazole derivatives as anti-cancer agents**
Dr. Florence McCarthy (University College Cork)

14.55-15.15 **Development of pyrrolo-1,5-benzoxazepines (PBOXs) as novel anti-cancer agents**
Dr Daniela Zisterer (Trinity College Dublin)

15:15 – 15:45 **TEA/COFFEE BREAK**

2nd March, 2011

Cancer Therapeutics: Small Molecule and Biological Strategies in the Molecular-Targeted Era

SATELLITE MEETING OF THE IACR – ROCHESTOWN PARK HOTEL, CORK

ANTIBODY AND BIOLOGICAL BASED-THERAPEUTICS
Chair: to be confirmed

16:00 – 16.30	**Strategies for biological targeting in head and neck cancer** *Dr Kevin Harrington (Institute of Cancer Research, London)*
16:30 – 17:00	**A novel strategy for targeting metalloproteinases in cancer** *Prof Gillian Murphy (University of Cambridge)*
17:00 - 17:30	***Targeting of Cathepsin S in Tumourigenesis*** *Dr Chris Scott (Queen's University Belfast)*
17:30 - 18:00	**The anti-tumour efficacy of the novel peptide inhibitor of angiogenesis ALM-201** *Dr Martin O'Rourke (Almac Discovery)*
18:00 - 18.15	**A G Murphy**, Education and Research Centre, St Vincent's University Hospital **Development of novel anti-angiogenic compounds for colorectal cancer using a tumour explant model and zebrafish.**
18.15 - 18.30	**M Mullooly**, St. Vincent's University Hospital, St. Vincent's University Hospital **Adams as new therapeutic targets for triple negative breast cancer**

Supported by:
The Department for Employment and Learning
through its "Strengthening the all-Island Research Base" initiative

IRISH ASSOCIATION FOR CANCER RESEARCH – 3rd March 2011

ROCHESTOWN PARK HOTEL, CORK

08.30 – 08.45 ***Welcome: Dr David Waugh, Honorary Secretary***

08.45 - 10.30 ***Proffered Papers Session:***

08.45 - 09.00 **M Cronin**, Cork Cancer Research Centre, University College Cork, Cork, Ireland
Systemic Tumour Targeting With Orally Administered Non-Pathogenic Bacteria

09.00 - 09.15 **J Lynch,** [1]Cancer Genetics, Royal College of Surgeons, Dublin
Mir-335 Suppresses The Migratory And Invasive Capacity Of Neuroblastoma Cells

09.15 - 09.30 **A Redmond,** Nuclear Receptor Transcription, Cancer Research UK Cambridge Research Institute, Cambridge, UK
The role of the chromatin protein HMGB2 in estrogen driven breast cancer

09.30 - 09.45 **S English**, UCD Conway Institute of Biomolecular and Biomedical Research,University College Dublin,Belfield, Dublin 4
Hypoxia in Epithelial Ovarian Cancer: Remodelling the Epigenome and Taxol® response.

09.45 - 10.00 **A Michielsen**, Centre for Colorectal Disease, St. Vincent's University Hospital, Dublin
Bevacizumab Causes Inhibition Of Dendritic Cells And Is Associated With Patient Survival In Colorectal Cancer

10.00 - 10.15 **NT Crawford**, CCRCB, Queens University Belfast, Belfast, N.Ireland
Identification Of The Transcriptional Complex Through Which Tbx2 Drives The Proliferation Of Breast Cancer Cells

10.-15 - 10.30 **IS Babina,** Department of Surgery, Royal College of Surgeons in Ireland, Dublin
CD44 Affiliation With Lipid Rafts Is Associated With Decreased Motility Of Breast Cancer Cells

10.30-11.00 ***COFFEE/TEA/EXHIBITS***

11.00-12.30 **PLENARY SESSION I - CHROMATIN AND GENOMIC STABILITY**

Chairpersons: Dr. Paul Mullan (CCRCB) and Dr. Leonie Young (RCSI)

11.00-11.30 **Activation of the checkpoint kinase, Chk1, by cyclin-dependent kinase, CDK, and the Rad9 mediator protein**
Professor Noel F. Lowndes, Professor and Head of Department, National University of Ireland, Galway

11.30-12.00 **Mechanisms of Estrogen Receptor transcription in breast cancer**
Dr. Jason Carroll, Nuclear Receptor Transcription Laboratory, Cancer Research UK Cambridge Research Institute, UK

IRISH ASSOCIATION FOR CANCER RESEARCH – 3rd March 2011

ROCHESTOWN PARK HOTEL, CORK

12.00-12.30 **PLENARY SESSION I - CHROMATIN AND GENOMIC STABILITY Cont'd**

Maintenance of Genomic Stability by Human Deubiquitinating Enzyme Complexes
Dr. Martin Cohn, Department of Biochemistry, University of Oxford, UK

12.30 - 13.40 **Posters and Exhibits (Finger Buffet Lunch)**

13.40 - 14.10 **IACR AGM**

14.10 - 15.00 ***EACR Young Scientists Presentations***

14.10 – 14.30 **SS McDade**,Centre for Cancer Research and Cell Biology, QUB, Belfast, UK
ChIP-seq identifies p63 target genes involved in palatal fusion and EMT

14.30 – 14.50 **A. Pickard**,CCRCB, QUB, Belfast, United Kingdom
The Stromal Function Of The Retinoblastoma Protein In Controlling Differentiation And Cancer Cell Invasion

15.00-16.00 **Oral Posters Presentations (8 talks x 7 mins)**

AC Cichon, Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland
The Cell Non-Autonomous Effects Of Pten

LC Oram, Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, UK
BRCA1 is a master regulator of breast cell fate through co-ordinated activation of a Notch signalling program.

C Walsh, Endocrine Oncology Research Group, Royal College of Surgeons in Ireland, Dublin 2
The role of the developmental protein HOXC11 in breast cancer tumour adaptability

S Dasgupta,Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, N. Ireland
ADAM-17 is a key regulator of ErbB survival response in colorectal cancer.

P Dunne, CCRCB, Queen's University Belfast, Belfast, Northern Ireland
Oncogenic Kras Controls Chemotherapy-Induced Growth Factor Shedding By Inducing Mek And Erk-Mediated Activation Of Adam17 In Colorectal Cancer

M Cotter, Breast Cancer Research Group, St. Vincent's University Hospital, Dublin
Poly (ADP-Ribose) Polymerase 1: A New Target for the Treatment of Triple Negative Breast Cancer

IRISH ASSOCIATION FOR CANCER RESEARCH – 3rd March 2011

ROCHESTOWN PARK HOTEL, CORK

15.00-16.00 **Oral Posters Presentations (8 talks x 7 mins) Cont'd**

J Lysaght, Surgery, Trinity College Dublin, Dublin, Ireland
The human omentum is a rich source of activated inflammatory CD4$^+$ and CD8$^+$ T cells, with a potential pathological role in oesophageal adenocarcinoma development.

M Prencipe, UCD School of Medicine and Medical Science, UCD Conway Institute of Biomolecular and Biomedical Research and Mater Misericordiae University Hospital, University College Dublin
Mechanisms of docetaxel resistance in prostate cancer: is NFkB involved?

16.00-16.30 ***COFFEE/TEA***

16.30-18.00 **PLENARY SESSION II**

INFLAMMATION, CYTOKINES AND THE MICROENVIRONMENT

Chairpersons: Dr. Ann Hopkins (RCSI) and Prof Gerry O'Sullivan (Cork Cancer Research Centre)

16.30-17.00 **TMEM: A new direction in the pathological assessment of metastatic risk in breast cancer**
Dr. Joan Jones, Weill College of Medicine, Cornell University, New York

17.00-17.30 ***'Macrophages without SOCS block inflammation but drive aggressive cancers***
Professor Jim Johnston, Centre for Infection and Immunity, Queen's University Belfast

17.30-18.00 ***EACR STATE OF THE ART LECTURE***

"Drosophila models for tumour-related inflammation and Colorectal Cancer"
Dr. Marcos Vidal, The Beatson Institute, University of Glasgow

18.05-19.30 **Main Poster Session/Exhibits with Sponsored Wine/Canapé Reception**
(Kindly sponsored by Labplan)

20.00 **Buffet Dinner**
(Kindly Sponsored by Irish Cancer Society)

IRISH ASSOCIATION FOR CANCER RESEARCH – 4th March 2011

ROCHESTOWN PARK HOTEL, CORK

08.15-10.00 **Proffered Papers Session**

08.15-08.30 <u>N Lynam-Lennon</u>, Department of Surgery, Trinity College Dublin, Dublin, Ireland
MicroRNA-31 modulates tumour sensitivity to radiation in oesophageal cancer

08.30-08.45 <u>Donley C</u>, School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast
The Role of FKBPL and RBCK1 in Oestrogen Receptor Signalling

08.45-09.00 <u>AJ Eustace</u>, Molecular Therapeutics for Cancer Ireland, National Institute for Cellular Biotechnology, Dublin 9
Dasatinib overcomes acquired temozolomide resistance in metastatic melanoma cell lines.

09.00-09.15 <u>J O'Keeffe</u>, [1]Biochemistry Dept, University College Cork
HRG-1, a Heme-Binding Protein with Enhanced Expression in Colorectaladenocarcinoma and Essential for Endosomal Trafficking of Nutrient Transporters

09.15-09.30 <u>GM Kelly</u>, Department of Biochemistry, University College Cork, Cork, Ireland
Insulin-like Growth Factor I Receptor Tyrosine Kinase activation is regulated by GSK-3β mediated phosphorylation of serine 1248 in the C terminal tail.

09.30-09.45 <u>M McDermott</u>, National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
Eukaryotic elongation factor 2 in acquired lapatinib resistance in HER2 positive breast cancer cells

09.45-10.00 <u>LR Barkley</u>, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Galway, Ireland
Direct Control Of CDC7 and DBF4 by MIR-29

10.00-10.30 ***Coffee/Exhibits***

10.30-12.30 <u>Plenary Session III</u>
SIGNALLING CUES AND THE REGULATION OF CELL FATE

Chairpersons: Dr. Sharon McKenna (UCC) and Dr Dan Longley (QUB)

10.30-11.00 **Molecular Insights into Mammalian Autophagy**
Dr. Sharon Tooze, Secretory Pathways Laboratory, Cancer Research UK London Research Institute, London, UK

11.00-11.30 **Lysosomal control of cancer cell invasion and survival**
Marja Jäättelä, Professor and Head of Department, Institute of Cancer Biology, Copenhagen, Denmark

11.30-12.00 **Many ways to TRAIL receptor signalling regulation by chemotherapeutic drugs**
Olivier Micheau, Oncology Research Group Leader, INSERM, Dijon, France

IRISH ASSOCIATION FOR CANCER RESEARCH – 4th March 2011

ROCHESTOWN PARK HOTEL, CORK

12.00-12.30 **2 proffered papers (2 x 15 minutes)**

12.00-12.15 L O' Leary, Department of Biochemistry, National University of Ireland, Galway,
Generation Of Decoy Receptor Insensitive Trail Variants

12.15-12.30 F Furlong,UCD School of Medicine and Medical Science, University College Dublin
Stabilisation of MAD2 Expression Through Inhibition Of MIR-433; A Novel Mechanism For Restoring Chemo-Responsiveness In Ovarian Tumours

12.30-13.30 **Posters/Exhibits & Finger Buffet Lunch**

13.30-15.30 **Plenary Session IV**

BIOMARKERS FOR CANCER DIAGNOSIS AND TARGETED THERAPY

Chairpersons: Grace Callagy (NUIG) and Lorraine O'Driscoll (TCD)

13.30-14.00 "**Genomic studies of drug resistance in ovarian cancer"**
Dr. James Brenton, Functional Genomics of Ovarian Cancer Laboratory, Cancer Research UK Cambridge Research Institute, University of Cambridge

14.00-14.30 **Aberrations of the miRNome in human cancer: Causes and Consequences**
Dr. Muller Fabbri, Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, Ohio State University, Columbus

14.30-15.00 **MicroRNAs and the DNA damage response: the relevance for cancer**
Professor Erik Wiemer, Department of Medical Oncology, Josephine Nefkens Institute, Erasmus University, Rotterdam, The Netherlands

15.00-15.30 **Biomarker Discovery and Validation for Prostate Cancer Grade and Stage: Addressing the Clinical Dilemma**
Professor William Watson, Conway Research Institute, University College Dublin

15.30-16.00 **TEA/COFFEE**

16.00-16.30 **BIOMARKERS FOR CANCER DIAGNOSIS AND TARGETED THERAPY CONT'D**

"Cancer Biomarkers: From Pilot Studies to Clinical Utility"
Professor Joe Duffy, Department of Biochemistry, St Vincent's Hospital, Dublin

IRISH ASSOCIATION FOR CANCER RESEARCH – 4th March 2011

ROCHESTOWN PARK HOTEL, CORK

16.30-17.30 **PLENARY SESSION V**

ENERGY, METABOLISM AND CANCER SESSION

Chairperson: Jacintha O'Sullivan (TCD)

16.30-17.00 **Professor Giuseppe Matarese, Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Napoli, Italy**

17.00-17.30 **"Molecular factors linking obesity with cancer"**
Dr Graham Pidgeon,Institute of Molecular Medicine, Trinity College Dublin

17.35 **IRISH CANCER SOCIETY LECTURE**

Introduced by: **Mr John McCormick, Chief Executive Officer, Irish Cancer Society**

Deconstructing Tumour Suppression and Oncogenesis to Find Effective Ways to Kill Cancer Cells
Professor Gerard Evan, Head of Department and Sir William Dunn Professor, Department of Biochemistry, University of Cambridge

20.00 **Gala Dinner**

Including Award Ceremony:

Presentation of Merck-Serono Graduate Travel Awards

EACR Young Scientist Awards and IACR Young Scientist Awards

Followed by Band and Late Bar

PLENARY SPEAKER BIOGRAPHICAL SKETCHES

ANDREW THOMAS, AstraZeneca

Andy Thomas gained his PhD in Organic Chemistry with Professor Ian Fleming at the University of Cambridge, followed by post-doctoral studies as a NATO fellow with Professor Barry Trost at the University of Wisconsin, Madison and at Stanford University.

He joined ICI Pharmaceuticals in 1988, initially working on anti-hypertensive projects, and has worked at the Alderley Park site, Cheshire UK, since that time; sequentially for ICI, Zeneca and since 2000 for AstraZeneca. In 1992, Andy transferred to cancer research and during the past 19 years, he has worked predominantly on kinase targets. He has played a key role in several project teams that have delivered candidate drugs which have been evaluated in clinical trials. These include the EGFR TKI programme that led to the discovery of IRESSA, the VEGFR TKI programme that discovered ZACTIMA/ZICTIFA, and more recently CDK2 and FGFR kinase inhibitor programmes that led to AZD5438 and AZD4547 respectively.

Andy has authored more than 60 publications and patents and is currently a Senior Principal Scientist in the Oncology iMed at AstraZeneca.

PROFESSOR MARTIN DRYSDALE, The Beatson Institute for Cancer Research, Glasgow

Martin Drysdale received his B.Sc. (1986) and Ph.D. (1990) in chemistry from St. Andrews University in Scotland. From the end of 1989 to 1991 he was a Parke-Davis Neuroscience Post-Doctoral Research Fellow at the PD site in Cambridge, UK and then spent 6 years at Wellcome and GlaxoWellcome in the UK working on projects in the CNS and inflammatory therapeutic areas. In 1997 he joined RiboTargets back in Cambridge UK, then a Biotech start-up company, to head up and develop the chemistry group there working on anti-infective targets. After surviving several rounds of mergers, acquisitions and company name changes he became Director of Chemistry & Structural Science and finally Deputy Director of Research at Vernalis working in the oncology and CNS areas, with a particular interest in Structure Based Drug Design and Fragment Based Methods of hit identification. In January 2009 he moved to his current position as Head of Drug Discovery at the Beatson Institute for Cancer Research in Glasgow, Scotland.

DR NIALL MARTIN, Mission Therapeutics (ex-Kudos Pharma)
Head of Research/Operations at Mission Therapeutics

- Head of KuDOS (2008 to Oct 1, 2010)
- Ran KuDOS Drug Discovery 1999-2010
- Initiated and headed the PARP programme that identified olaparib and subsequent CD back-up compounds (~25 clinical trials incl. Phase II)
- Key role in KuDOS acquisition (2006)
- In Pharma industry since 1990 (Zeneca and Fournier)

MICHAEL A. FOLEY, Director of the Chemical Biology Platform at the Broad Institute of MIT and Harvard

Michael Foley joined the Broad Institute in 2006 as director of the Broad's Chemical Biology Platform. He brings extensive knowledge of chemistry and chemical technology, including techniques based on diversity-oriented synthesis (DOS) and medicinal chemistry. Under his leadership, the platform oversees all aspects of chemical libraries and high-throughput chemical screening at the Broad. The platform works to systematically create DOS libraries and develop new approaches to target identification for cell-based screens.

Foley was a co-founder of Infinity Pharmaceuticals and served as Vice President of Chemistry from 2001 to 2006. He was also a co-founder of CombinatoRx Inc., and previously worked at Bristol-Myers Squibb and GlaxoSmithKline. He obtained his Ph.D. at Harvard, and helped establish the Harvard Institute of Chemistry and Cell Biology.

Foley received a B.S. in chemistry from St. Norbert College, an M.S. in chemistry from Utah State University, and a Ph.D. in chemistry from Harvard University.

PROFESSOR STEVE FESIK (Vanderbilt University)

Stephen W. Fesik, Ph.D. is the Orrin H. Ingram, II Chair in Cancer Research and a Professor of Biochemistry, Pharmacology, and Chemistry in Vanderbilt University School of Medicine. In addition, he is a member of the Vanderbilt Ingram Cancer Center (VICC), the Vanderbilt Institute of Chemical Biology (VICB), and the Center for Structural Biology (CSB). The focus of his research is on cancer drug discovery using fragment-based approaches and structure-based drug design. Prior to joining Vanderbilt in May, 2009, Dr. Fesik was the Divisional Vice President of Cancer Research at Abbott (2000-2009) where he built a pipeline of compounds that are showing promising anti-cancer activities in early stage clinical trials. In addition, while he was at Abbott, he developed several new NMR methods, determined the three-dimensional structures of several proteins and protein/ligand complexes, pioneered a method for drug discovery called SAR by NMR, and applied this method to identify and optimize ligands for binding to many protein drug targets. His research has also involved the use of siRNA for target identification and target validation. Dr. Fesik has published 238 papers, trained 35 postdoctoral fellows, and has served as a member of the Editorial Boards of the *Journal of Medicinal Chemistry, Journal of Biomolecular NMR, Biophysical Journal, Molecular Cell*, *Chemical Biology & Drug Design, ChemMedChem, Molecular Cancer Therapeutics, Oncogene, Combinatorial Chemistry and High Throughput Screening,* and the Highlights Advisory Panel for *Nature Reviews Cancer.* He has served on the Keystone Scientific Advisory Board and Board of Directors and currently is a member of the Scientific Advisory Board of the UPenn Abramson Cancer Center, Aileron Therapeutics, Ansaris, and the Bruker Board of Directors. He has obtained several awards, including the Chairman's Award (1996), Outstanding Researcher of the Year Award (1997), and the Researcher of the Year Team Award (2008) from Abbott, the Servier Lecturer Award (1998) from the University of Montreal, the ASBMB-Fritz Lipmann Award (1999), the Lifetime Achievement Award in Nuclear Magnetic Resonance from EAS (2003), the SBS Technology Innovation Award (2010), and the NIH Director's Pioneer Award (2010). Recently he has been elected Fellow of the American Association for the Advancement of Science (AAAS).

RICH WILLIAMS, Queen's University of Belfast

Rich obtained his PhD in Organic chemistry at the University of Sheffield under the supervision of Professor Varinder Aggarwal. PhD included the several sub projects including screening methodology of enhancing the rate of the notoriously slow Baylis-Hillman reaction. Another part of his research was the synthesis of chiral catalysts for the Baylis-Hillman reaction via combinatorial screening platforms.

Upon completion of his PhD in 2001 moved to the States to work for Albany Molecular Research, Inc., (AMRI) in upstate New York. During his time at AMRI was heavily involved in the development of BMS-708163 a gamma-secretase inhibitor for the treatment of Alzheimer's disease. BMS-708163 is currently progressing through phase III clinical trials. Also developed key synthetic methodology of large synthesis of (S,S)-astaxanthin for Hawaii biotechnology.

In late 2005 moved to Vanderbilt University to work for Professor Jeff Conn in the newly formed CNS drug discovery group. In early 2006 became a part of the TES medicinal chemistry working with Dr Craig Lindsley. For the next three years worked on the development on a series of allosteric modulators programmes for the treatment of CNS disorders, such as Schizophrenia, Parkinson's disease and fragile X based autism. In early 2007 two patents for mGluR5 positive allosteric modulators were generated and were licensed to J&J. This series was further developed to overcome metabolic issues and generated a novel series of compounds that have progressed into pre-clinic development. During his time at Vanderbilt I published 8 patents (3 for mGluR5 PAMs and 5 for GlyT1) and over 20 peer reviewed publications. Two of the publications revolved around the development of the first highly selective CNS active muscarinic subtype 1 antagonist for the treatment of seizures.

In 2009 I took a position as a lecturer in Medicinal chemistry within the CCRCB. Within the CCRCB I have been involved in the development of several drug discovery projects from target validation through to two hit to lead development programmes. The first of these programmes is the development of novel and highly potent Legumain inhibitors for the treatment of poor prognosis breast cancers.

FLORENCE McCARTHY, University College Cork

Florence McCarthy graduated from the School of Pharmacy, University of Sunderland, UK. His PhD was undertaken in Medicinal Chemistry with Prof. Paul Groundwater at the University of Sunderland entitled "Synthesis of Novel Ellipticines as Molecular Probes and DNA Binders". He then held a Post-doctoral position with Prof. Bill Denny, Auckland Cancer Society Research Centre, University of Auckland, New Zealand in conjunction with Pfizer Global Research and Development where he worked on inhibitors of the Wee1 and Chk1 checkpoint kinases and an ErbB kinase inhibitor programme. He joined UCC as a Lecturer in the School of Pharmacy and the Department of Chemistry in 2003, being made permanent in 2005. Teaching duties involve a variety of topics in Organic and Pharmaceutical Chemistry to programmes including Chemistry, Chemistry of Pharmaceutical Compounds (CPC), Pharmacy, Food and Nutritional Sciences and Nursing. Dr. McCarthy is also the Academic-in-charge of the Waters Mass Spectrometry Laboratory.

DANIELA ZISTERER, Trinity College Dublin

Daniela Zisterer has been a Lecturer in the School of Biochemistry and Immunology in Trinity College Dublin, since 2001. She was made a TCD Fellow and a Senior Lecturer in 2007. She is also a graduate of Trinity. She is currently the Coordinator of the Moderatorship in Biochemistry with Cell Biology.

Her main research programme seeks to understand the molecular mechanisms underlying apoptotic cell death and how deregulated apoptosis leads to cancer. She is currently involved in developing novel anti-cancer agents with a number of international research collaborators. One of her ongoing projects is the development of a novel series of pyrrolo-1,5-benzoxazepine (PBOX) compounds as potential anti-cancer agents. She has identified that these PBOX compounds potently induce apoptotic cell death in many resistant human cancer cells while eliciting minimal toxicity on normal cells and she has recently been granted a patent for their use as anti-cancer agents.

She is the author of over 50 publications in some of the best international scientific journals including Cancer Research, the Journal of Pharmacology and Experimental Therapeutics, Molecular Pharmacology and the Journal of Biological Chemistry. Her research is supported by Science Foundation Ireland, Enterprise Ireland, the Health Research Board and the Children's Leukaemia Research Project.

KEVIN HARRINGTON, Institute of Cancer Research, London

Dr Kevin Harrington specialises in developing new treatments using viruses that selectively destroy cancer cells. He is a Senior Lecturer at The Institute of Cancer Research and an Honorary Consultant Clinical Oncologist at The Royal Marsden NHS Foundation Trust.

Dr Harrington studied medicine at St Bartholomew's Hospital, London, and began focusing on head and neck cancer while a PhD student at Hammersmith Hospital. He completed post doctoral research in molecular medicine at the Mayo Clinic, Minnesota, before joining the ICR in 2001 as Targeted Therapy Team Leader within the Section of Cell and Molecular Biology.

He is currently working with a range of viruses that are able to grow in - and kill - cancer cells, but not normal healthy cells. Some of these viruses have naturally evolved to grow preferentially in cancer cells because of the cells' specific genetic defects; others have been genetically engineered to grow selectively in cancer cells. Dr Harrington hopes new treatments using these viruses will improve patients' cure rates and have fewer side-effects compared to current therapies.

He says his specialisation area of head and neck cancers is in particular need of extensive further research, as it represents a diverse group of diseases with varied challenges for treatment and generally poor survival rates. "There is a real need to improve treatment options," Dr Harrington says.

Much of Dr Harrington's laboratory work is immediately translated into clinical trials at The Royal Marsden, most often in patients with head and neck cancers and melanomas. Dr Harrington says the ICR's partnership with The Royal Marsden allows him to conduct innovative laboratory research and apply it in the clinical setting, achieving "real patient benefit".

The virus therapies developed are generally given in combination with standard anti-cancer treatments, such as chemotherapy and radiotherapy. Dr Harrington's research has shown that some viruses can make cancer cells more sensitive to radiation; while the radiation may also favourably alter the effect of some viruses on cancer cells.

One promising treatment is a reovirus (a tumour-specific virus) which Phase I and II trials demonstrated was targeting tumours after intravenous injection. In 2009, the Federal Drug Administration approved a Phase III trial of this virus in the USA.

"One of the greatest challenges we have faced is working out how to inject these viruses into patients so they are able to reach the tumours and kill them efficiently, before being inactivated by the immune system," Dr Harrington says.

A second avenue of research involves a genetically-modified herpes simplex virus (cold sore virus) given by direct injection into the tumour. As well as killing tumour cells, this modified virus has the added benefit of expressing a protein that stimulates the immune system. A number of trials are underway involving this virus in combination with other treatments for patients with several cancer types including breast, head and neck and melanoma.

Dr Harrington is a Fellow of the Royal College of Physicians and a Fellow of the Royal College of Radiologists. In his infrequent spare time, he enjoys gardening, playing and watching football, and reading.

PROF GILLIAN MURPHY, Deputy Head of Dept of Oncology, University of Cambridge

Professor Gillian Murphy is currently Chair of Cancer Cell Biology and deputy Head of the Dept. of Oncology, University of Cambridge.

Research interests: the function of metalloproteinases, including their structure-function relationships and extracellular regulation, including the function of the TIMP inhibitors.

Work is currently focused on cell surface associated forms including the membrane type matrix metalloproteinases (MT MMPs) and the disintegrin-type metalloproteinases (ADAMs). She has published over 250 original research papers, book chapters and reviews and was made a Fellow of the Academy of Medical Sciences in 2005.

CHRIS SCOTT, Queen's University of Belfast

Chris obtained a primary Biochemistry degree (1996) and a PhD in protease biochemistry at Queens University Belfast in 2000. He then undertook a McClay Trust-sponsored post doc in bacterial proteases within the School of Pharmacy, QUB, before becoming one of the founder scientific team members at the start-up biotech company Fusion Antibodies. Chris then returned to the School of Pharmacy at QUB as a lecturer in 2003. He is now Director of Research in Molecular Therapeutics within the school, and his team is focused on the study of cysteine proteases in disease. He has collaborated extensively with industry to develop a number of lead biologic molecules targeted towards cathepsin proteases in cancer progression

MARTIN O'ROURKE, (Almac Discovery)

Martin received his degree in Applied Biochemical Sciences in 1998 and his PhD in vascular biology from the same institution in 2001. Martin completed two post doctoral positions at Queen's University Belfast in the School of Pharmacy, initially investigating the functional role of PARP inhibitors in tumour vasculature, before focusing on anti-angiogenic drug discovery with Prof's Chris Shaw and David Hirst. This post-doc identified the several novel anti-angiogenics and vasoactive agents which were subsequently patented or published.

Martin joined Almac Discovery in 2008 and was appointed Group Leader in 2010; he is currently leading project teams investigating a small molecule kinase inhibitor and a novel anti-angiogenic peptide.

NOEL F. LOWNDES BA (Mod), PhD
Chair of Biochemistry & Director of the Centre for Chromsome Biology, NUI Galway

Professor Lowndes graduated with a degree in Genetics from Trinity College Dublin in 1983. His post-graduate studies were completed at the Beatson Institute for Cancer Research, Glasgow and Columbia University, New York. He was awarded a PhD in Molecular Pathology by the University of Glasgow in 1987 for his work on the transcriptional regulation of the c-Ha-*ras* proto-oncogene. His post-doctoral work on the regulation of cell cycle regulated gene transcription was conducted in Lee Johnston's laboratory at the National Institute for Medical Research (NIMR) in London. In 1993 Professor Lowndes established his own laboratory at the Cancer Research UK (CRUK) Clare Hall laboratories, widely regarded as world-class in the fields of DNA repair and cell cycle research. In 2001, Professor Lowndes returned to Ireland to take up the Chair of Biochemistry at NUI Galway. Since that time the Department of Biochemistry at NUI Galway has significantly developed its research capacity. In particular, he is the founding director of the Centre for Chromosome Biology (http://www.chromosome.ie/), where his laboratory focuses on genetic and biochemical analyses of genome stability pathways.

In 2003, in recognition of his research achievements, Professor Lowndes was elected to the membership of the European Molecular Biology Organisation, one of just five EMBO members in Ireland.

JASON CARROLL, Nuclear Receptor Transcription Laboratory, Cancer Research UK, Cambridge Research Institute UK

Dr Jason Carroll is based at the Cambridge Research Institute is exploring the role of the oestrogen receptor (ER) in breast cancer development and treatment. In particular, he is studying how the hormone oestrogen fuels the growth of breast cancer cells by binding to the ER. And his work is focusing on how tamoxifen, a commonly used breast cancer drug, works by blocking the ER. This pioneering work is providing important insights into how some breast cancers become resistant to treatment.

Understanding the oestrogen receptor

In the presence of oestrogen, the ER switches on certain genes that trigger the growth of cells. Dr Carroll is using cutting-edge techniques to investigate exactly how the ER brings about these changes.

More specifically, he is interested in how the ER binds to DNA and cooperates with the many other proteins involved to determine whether genes are switched on or off. This has important implications for breast cancer development.

Investigating tamoxifen and resistance to treatment

Dr Carroll is investigating the effect that the drug tamoxifen has on the ER. In particular, he is finding out in greater detail how tamoxifen blocks the growth of breast cancer cells and why some women eventually stop responding well to this treatment.

He has already made a real breakthrough in this field by showing that tamoxifen acts through the ER to directly switch off another key protein, ERBB2, in breast cancer cells. Importantly, the team found that cancer cells can become resistant to tamoxifen by overriding this control switch and turning ERBB2 back on. This exciting discovery provides new opportunities to tackle drug resistance and improve breast cancer treatment in the future.

MARTIN COHN, Department of Biochemistry, University of Oxford, UK

2000 Ph.D. from the University of Copenhagen, Denmark
2000-2009 Postdoctoral fellow at the Dana-Farber Cancer Institute, Harvard Medical School, USA, with Professors Pat Nakatani and Alan D'Andrea
2009- Group Leader in the Department of Biochemistry, University of Oxford.

JOAN JONES, Weill College of Medicine, Cornell University, New York

Dr. Jones' research interest is **TMEM**, the **T**umor **M**icro**E**nvironment of **M**etastasis. Observations from intravital imaging in animal models of breast cancer have revealed local and transitory migratory events that facilitate the hematogenous spread of tumor cells. Dr. Jones' work has shown that this microenvironment can be identified in routine surgical pathology sections and quantitated to predict metastatic risk. This work is ongoing with population studies in breast cancer, and is expanding to other organ systems including lung and kidney. Dr. Jones is also Director of Anatomic Pathology at NYP/WCMC in New York City.

Specialties

Anatomic Pathology (Board Certified)
Anatomic Pathology and Clinical Pathology
Pathology

Education/Training

Medical School - Albert Einstein College of Medicine of Yeshiva University, Bronx, NY
Internship - Bronx Municipal Hospital Center
Internship - Albert Einstein College of Medicine of Yeshiva University, Bronx, NY
Residency - Bronx Municipal Hospital Center
Residency - Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

Areas of expertise

Gynecologic Pathology
Breast Pathology

PROF JIM JOHNSTON, Queen's University of Belfast

Professor Jim Johnston is the first Chair of Immunology and is the Deputy Director of the centre for Infection and Immunity within the School of Medicine, Queen's University Belfast. He was postdoctoral Fellow and later a Senior Staff Fellow, Lymphocyte Cell Biology Section, Arthritis and Rheumatism Branch, NIAMS, NIH Bethesda, from 1991 -1996. While there he made a number of key discoveries including the identification of JAK3 as the kinase responsible for IL-2 signalling in T cells and the overall mechanism responsible for Severe Combined Immunodeficiency (SCID) in patients with mutations of this gene. These findings were published in Nature and Science. In 1996 he started his own group in DNAX Research Institute in Palo Alto, California where his research focused on cytokine biology. More recently his research has focused on the cytokine signalling cascades, which rely on intracellular molecules such as Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). These pathways are regulated, in part, by the suppressor of cytokine signalling (SOCS) protein family. The work of his group and others have suggested that SOCS attenuate cytokine signal transduction by binding to phosphorylated tyrosine residues on signalling intermediates such as receptor chains. This work was published in Nature Cell Biology. He was later part of the collaborative research that lead to this discovery of the role of SOCS3 in atopic disease and was published in Nature Medicine and Nature Immunology.In the past number of years his group have reported that SOCS3 interacts abnormally with JAK2 V617F and shown that this regulates the development of myeloproliferative disease. Moreover, the group has also studied the role of SOCS in Atopic disease.
Jim Johnston is teh recipient of the 2010 Irish Society of Immunology public lecture medal. He was awarded the Hajime award at DNAX 1999 and the Most Valuable Scientist award at the NIH, Bethesda in 1996. He has been a member of the Journal of Biological Chemistry Editorial Board from 2003-2009 and has been a member of the MRC Panel of Experts 2004-2010. He has been Chairman of the SFI Grant Panel and HRB Pathology & Immunology Grant Panel in 2004-2008. He has also been an invited speaker at many international meetings.

He was the founder and was Chief Scientist of Fusion Antibodies Ltd, a Biotechnology spinout company from Queen's University. The company develops human Monoclonal Antibody therapeutics for the cancer treatment. Fusion Antibodies Ltd has raised over £6 million in venture funding, employs 25 people and has won a host of awards for its leading-edge technology platform FET (Fusion Expression Technology). Professor Johnston acts as a member of the board of this company. Jim Johnston has published over 100 per reviewed scientific manuscripts in the immunology discipline. This research is supported by sustained funding from a range of organisations such as the The Wellcome Trust, BBSRC, CRUK, Leukaemia Research Fund, EU-FP7 and the Action Cancer.

MARCOS VIDAL, The Beatson Institute, University of Glasgow

Education and qualifications:

PhD	2003	National University of Rosario (UNR), Argentina.
MSc	1999	'Licenciatura en Biotecnología' (equivalent to Master in Biotechnology), UNR, Argentina.

Appointments:

Starting on March 2009	Group Leader, BICR, Glasgow, UK
2003-2009 USA (2003-2007)	Post-doctoral Fellow with Ross Cagan. Washington University, MO, Mount Sinai School of Medicine (2007-2009).

RESEARCH

Summary of group's interests:

The spread of malignant cells from the primary tumour to distant sites, or metastasis, is the major cause of death in cancer patients. The biology of metastasis is, however, still poorly understood. Recent investigations suggest a paradigm shift: cellular invasion and migration could occur early during tumorigenesis and with a predominant role of the normal, untransformed tissue surrounding the tumour, the 'tumour microenvironment'.

The contributions of the microenvironment are best studied *in vivo*. The fruit fly *Drosophila melanogaster* offers a unique research opportunity due its amenable genetics, short life cycle and conserved signaling pathways with low genetic redundancy. Remarkably, and despite the apparent caveat of a long evolutionary distance from humans, many aspects of cancer biology have been successfully modeled in *Drosophila*.

Our laboratory utilizes *Drosophila* and aims to better understand the cellular and molecular events that lead to the invasion and migration of epithelial cells away from their site of origin. In particular, we have discovered recently that there is an inflammatory response to tumours in Drosophila, and defined the genetic contexts in which this inflammatory response acts as a tumor suppressor or a tumor promoter.

DR. SHARON TOOZE, London Research Institute, Cancer Research UK

Dr. Tooze's research focuses on understanding organelle biogenesis and homeostasis at the level of protein modification, protein targeting, protein-protein interactions, thereby obtaining new information about how cells function. Since 2005 her laboratory has been studying mammalian autophagy with the aim to understand how the process occurs after amino acid deprivation. The focus of her work is on understanding the early events on the formation of autophagosomes.

Dr. Sharon A. Tooze received her Ph.D. from the European Molecular Biology Laboratory, and completed her postdoctoral work at the European Molecular Biology Laboratory. Since 1993 she has served as Head of the Secretory Pathways Laboratory at the Imperial Cancer Research Fund (now known as Cancer Research UK) in London

PROF. MARJA HELENA JÄÄTTELÄ, Head Of Deprtment, Institute Of Cancer Biology, Copenhagen, Denmark

Research Professor in Cancer Biology and Head of the Department,
Apoptosis Department, Institute of Cancer Biology, Danish Cancer Society
Web: www.cancer.dk/apoptosis

Previous Career

1997-2003	Principal Investigator, Apoptosis Laboratory, Danish Cancer Society
1993-94	Visiting scientist, Dr. Visva Dixit, Univ. of Michigan, Ann Arbor, MI
1991	Visiting scientist, Dr. Gloria Li, Sloan-Kettering-Memorial Cancer Center, New York, NY
1991-96	Junior/Senior Research fellow, Danish Cancer Society, Copenhagen
1990	Lecturer, Dept. of Pathology, Univ. of Helsinki, Finland

Higher Education / Degrees

2003	Professor in Cancer Biology, Copenhagen University
1996	Docent in Cancer Biology, Univ. of Helsinki Medical School, Finland
1993	Certified Physician
1990	Doctor in Medical Sciences (PhD in Cancer Biology), Univ. of Helsinki, Finland
1989	Doctor in Medicine (MD), Univ. of Helsinki, Finland

Current Research Interests

Lysosomes and autophagosomes in cancer progression
Alternative cell death pathways in cancer
Survival mechanisms (especially Hsp70 and sphingolipid metabolism) in cancer
Genotoxic stress

OLIVIER MICHEAU, Oncology Research Group Leader, INSERM, Dijon, France

Olivier Micheau is an INSERM researcher in the INSERM research Center U866 «Lipids, nutrition, cancer» in Dijon, France. (since 2003)
1991-1995 BSc Biochemistry UCL (Univ Central Lancashire, UK) - Master genetics Toulouse, France
1995-1999 PhD in Molecular and cellular Biochemistry INSERM U517 in Dijon
1999-2003 Post-doc in Jurg Tschopp's Lab (Switzerland - Lausanne)
2003- Permanent position at the INSERM U866 in Dijon

JAMES BRENTON, Functional Genomics of Ovarian Cancer Laboratory, Cancer Research UK Cambridge Research Institute, University of Cambridge

James D. Brenton qualified in medicine from University College London in 1988 and trained in medical oncology at the Royal Marsden Hospital, Princess Margaret Hospital and the Department of Oncology, University of Cambridge. His PhD work was carried out at the Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology. He was a Cancer Research UK Senior Clinical Research Fellow from 2001–2006 and has been a group leader at the Cambridge Research Institute from 2006. His research focuses on the identification of prognostic and predictive markers in ovarian cancer, with particular emphasis on genomic profiling of clinical samples and bioinformatic analysis. He is Vice-Chair of CR-UK Biomarkers and Imaging Discovery and Development Committee, member NCRI ovarian cancer subgroup and a member of CR-UK Clinical Fellows Mentor Panel.

Research Goals
To identify molecular mechanisms of treatment resistance in ovarian cancer.

Qualifications and Personal History
1993–1996 PhD Wellcome/Gurdon Institute
1999–2000 George Knudson Memorial Fellowship and Department of Medicine Fellow, Ontario Cancer Institute/Princess Margaret Hospital
2001–2006 Cancer Research UK Senior Clinical Research Fellow and Honorary Consultant in Medical Oncology

MULLER FABBRI, Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Centre, Ohio State University, Columbus

Dr. Fabbri is a Research Scientist at the Department of Molecular Virology, Immunology and Medical Genetics at the Ohio State University, Columbus, OH. He also is Director of the Gene Therapy Unit at the Scientific Institute for Cancer Research (IRST) in Meldola, Italy. He got his MD degree in Italy, where he has spent 7 years working as a practicing medical oncologist. In 2003 he has joined Dr. Croce's lab at Thomas Jefferson University in Philadelphia, PA, where he has worked on cancer gene therapy with onco-suppressor genes located within fragile sites (FHIT, WWOX, PARKIN). In 2004, Dr. Fabbri has followed Dr. Croce when he moved to the Ohio State University, where he has contributed to the most relevant discoveries made by Croce's group in the field of microRNAs and cancer. In 2008 he became Research Scientist with Principal Investigation status, and focused his research on the effects of microRNAs on the epigenetic machinery. He was the first to discover that a specific group of microRNAs (miR-29 family) directly targets two key methyltransferases (DNMT3A and 3B) responsible for gene methylation, and induce re-expression of methylation-silenced tumor suppressor genes. He has recently identified a new pathogenetic mechanism which correlates microRNAs and two protein coding genes (namely, Tp53 and ZAP70) in the pathogenesis and prognosis of human chronic lymphocytic leukemia, a discovery that has been recently published in JAMA.

ERIK WIEMER, Department of Medical Oncology, Josephine Nefkins Institute, Erasmus University, Rotterdam, The Netherlands

Erik Wiemer obtained his Ph.D. degree in (bio)chemistry from the University of Amsterdam in 1991. His thesis dealt with the biogenesis of peroxisomes in relation to disorders of peroxisome assembly. From 1990 – 1992 he worked as postdoctoral fellow at the Christian de Duve Institute of Cellular and Molecular Pathology of the Université Catholique de Louvain in Brussels on the intracellular routing of glycosomal proteins and metabolite transport in trypanosmes. A second postdoctoral period was spent at the department of Biology of the University of California at San Diego. Here he used yeast as a tool to identify genes involved in the assembly of peroxisomes and was the first to identify the molecular defect in a group of inherited peroxisomal disorders. In 1995 he returned to the Netherlands and joined the department of Hematology of the Erasmus Medical Center where he investigated drug resistance in hematological malignancies. In 2002 he moved to the department of Medical Oncology, became a staff member and group leader in 2004 and currently holds the position of associate professor. His research interests are the molecular basis of drug resistance, the role of non-protein coding RNAs - in particular miRNAs - in cancer and the pharmacodynamic and pharmacokinetic characteristics of novel anti-cancer drugs.

PROF. WILLIAM WATSON, Conway Institute, University College Dublin, Dublin

Research Interests:

My research focus has been in the area of "Apoptosis", the mechanism by which cells undergo programmed cell death, and I have investigated its role in the development of: Urological Cancers, Urological Fibrosis and Inflammation. This has lead to an understanding by which prostate cancer cells develop resistance to apoptosis during their development and progression leading to better mechanisms of detection and the identification of targets for therapeutic manipulation. My research has also identified a link between apoptosis and the process of fibrosis in kidney and Crohn's disease, as well as the resolution of inflammation in inflammatory disorders such as Sepsis. Prostate cancer represents the focus of my research where we continues to look at resistance to apoptosis and the role of hypoxia in the signalling process. In collaboration with the Proteomic Unit we have developed a biomarker discovery project in urine and serum. The programme of research is underpinned by the development of the Prostate Cancer Research Consortium which is a membership of the International Cancer Biomarker Consortium- www.fhcrc.org/science/international_biomarker/teams.

Education:

Year 1991 **Institution:** University College Dublin
Qualification: BSc **Subject:**
Year 1995 **Institution:** Royal Coll Surgeons in Ireland
Qualification: PhD **Subject:**
Year 1992 **Institution:** Dublin Institute of Technology (DIT), IRL
Qualification: GRSC **Subject:**

PROF JOE DUFFY, School of Medicine & Medical Science, Dept of Pathology and Laboratory Medicine, St Vincents University Hospital, Dublin

Research Interests:
Molecular biomarkers in breast cancer: use of novel biomarkers for aiding the early diagnosis of breast cancer, determining prognosis and predicting reponse to therapy.
Molecular mechanisms of cancer metastasis

Teaching Interests:
Cancer biology to BSc Hons course in Biochemistry and Pharmacology.
Tumor biomarkers to postgraduate Nursing students

Research Projects:
Sponsor : Science Foundation Ireland (SFI)
Title : Molecular therapeutics for cancer: translational research to individualise therapy with targeted agents
Start Date / End Date : 01-OCT-09 / 01-OCT-12

PROF GIUSEPPE MATARESE, Laboratorio de Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Richerche, Napoli, Italy

GRAHAM PIDGEON, Institute of Molecular Medicine, Trinity College Dublin

Dr. Graham Pidgeon graduated from DCU with a degree in Analytical Science and obtained a Ph.D. in Cancer Research from the Dept. Surgery, RCSI/DCU in 2000. Awarded an American Cancer Foundation Fellowship, he worked as research fellow at Wayne State University, Michigan with Prof. Kenneth Honn on the regulation of prostate cancer survival by bioactive lipids. In 2002 he returned to Ireland as a senior postdoctoral fellow in the Dept. Clinical Pharmacology at RCSI. He was awarded a HRB postdoctoral fellowship in 2004, and joined Dr. Ken O'Byrne in the Thoracic Oncology Research Group at the Institute of Molecular Medicine at TCD/St. James's Hospital as Research Lecturer. There he established his own research group focused on the regulation of VEGF-mediated survival pathways by COX and LOX in lung cancer. He was appointed Senior Lecturer in Dept. Surgery at TCD/St.James's in 2007 and expanded his research into other prevalent solid malignancies, including oesophageal, colorectal and breast cancer. He has published in a number of high impact journals including Cancer Research and Circulation, and was awarded the IJS doctor award 2005 for Cardiology. His current research is focused on the molecular pathways linking excess adipose tissue, obesity and the metabolic syndrome with tumour development and progression. He established the obesity research group and Ireland's first adipose tissue biobank in 2007. The group are examining molecular pathways activated in cancer cells following culture with human adipose tissue, and investigating the activation of immune cell subsets in visceral adipose tissue. Other areas of interest include the role of bioactive lipid enzymes, including cycloxygenase and lipoxygenase, in thrombosis and metastasis of oesophageal, colorectal and lung cancer patients. The ultimate aim of this research program is to identify novel therapeutic targets that may prevent the development and/or progression of multiple cancers, through understanding the molecular mechanisms behind the disease.

PROF GERARD EVAN, Head of Department and Sir William Dunn Professor, Department of Biochemistry, University of Cambridge

Gerard Evan received his BA in Biochemistry from the University of Oxford (St. Peter's College) in 1977 and his Ph.D in Molecular Immunology in 1981 from the University of Cambridge (King's College). He was then first an MRC Post-Doctoral Fellow and second a Science and Engineering Research Council post doctoral fellow in the laboratory of J. Michael Bishop at UCSF from 1982-84. In 1984 he returned to the UK to become an Assistant Member of the Cambridge Branch of the Ludwig Institute for Cancer Research and a Research Fellow of Downing College, Cambridge. In 1988 he joined the Imperial Cancer Research Fund (ICRF) Laboratories in London as a Senior Scientist (1988-90) and then Principal Scientist (1990-1999). He was awarded the Pfizer prize in Biology in 1995 and in 1996 was elected as the Royal Society's Napier Professor of Cancer Research. In 1999 he was elected a Fellow of the UK Academy of Medical Sciences and later that year appointed to the Gerson and Barbara Bass Bakar Distinguished Professor of Cancer Biology at the University of California, San Francisco. He was elected to the Royal Society in 2004, to the Neal Levitan Research Chair of the Brain Tumor Society and, in 2006, became a Senior Scholar of the Ellison Medical Research Foundation for Aging. In 2009, he was elected to the Sir William Dunn chair of Biochemistry in the University of Cambridge.

PLENARY SPEAKER ABSTRACTS

Oncology Drugs of the Future
Trends and highlights from new molecularly targeted drugs / drug candidates in clinical evaluation
Andrew P Thomas, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire

Summary of presentation:

Recent experience over the last decade would indicate that successful discovery and development of oncology drugs is becoming more difficult. The challenges faced in finding anti-cancer drugs today and the current approaches be taken will be discussed and analysed. Leading drug candidates and approaches in current clinical development will be highlighted and trends suggested.

This overview will be made predominantly from a small molecule perspective, but will also include biological examples within a broader view of the science and issues of oncology drug discovery and development.

Fragment Based Hit Identification and Structure Based Approaches to Cancer Drug Discovery
Martin J Drysdale, Head Drug Discovery Programme, The Beatson Institute for Cancer Research, Glasgow

The use of weak binding "fragments" of molecules is now recognised as an efficient and robust method of hit identification in the drug discovery process. The use and integration of fragment hits into successful lead optimisation is the critical determinant of whether this technology will become accepted as a significant tool in drug discovery. A number of compounds which have evolved using fragment based hit identification are now in phase I-III clinical trials suggesting that this is a technology which will find a permanent place in the armory of the Drug Discovery Scientist.

At the newly established Drug Discovery Programme at the Beatson Institute for Cancer Research we are exploiting the basic biology strengths within the Beatson Institute and wider Cancer Research UK network, to investigate some of the most exciting and challenging cancer targets. Central to our strategy is Fragment-Based Drug Discovery methods and we will use NMR and Surface Plasmon Resonance as primary tools for fragment-based hit identification. I will discuss some results around our initial forays into some of these areas.

Drugging the DNA Damage Response pathways
Dr Niall Martin (ex-Kudos Pharma)

DNA damage response (DDR) processes have developed to protect the genome from harmful damage that leads to DNA mutations and/or cell death. However, these protective mechanisms have been identified to play an increasing role in tumourogenesis following pathway mutations in which function is lost or in some circumstances over activated. Drug resistance to many chemotherapies is also recognised to be due in part to DNA repair mechanisms, where efficient repair can negate cytotoxic activity. As such identifying viable clinical inhibitors of DDR proteins is of increasing high interest to oncology. Moreover, the recent data for synthetic lethality of PARP inhibitors (olaparib) in BRCA1 and 2 deficient tumours shows the power of inhibiting DDR enzymes in selective genetic backgrounds. In this talk I will discuss the challenges of drugging the DDR and the potential rewards of identifying potent inhibitors.

Cancer Drug Discovery Using Fragment-Based Methods
Prof. Steve Fesik (Vanderbilt University)

Cancer is a devastating disease that affects the lives of almost everyone, and its effective treatment still remains an important unmet medical need. In order to discover new cancer drugs, we are applying fragment-based methods and structure-based design to identify and optimize small molecules that inhibit highly validated cancer targets. In this presentation, examples will be given of how this methodology can be used to discover small molecules that bind to highly validated but technically challenging cancer targets.

Development of Novel and highly potent legumain Inhibitors for the treatment of Poor Prognosis Breast Cancer

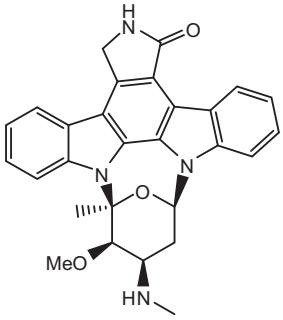
Rich Williams, CCRCB, Queen's University Belfast

AEP1 (legumain) was first identified in humans as a putative cysteine protease with homology to parasitic haemoglobinase. It was first linked to cancer as a novel asparagines end peptidase by gene expression profiling and tumour tissue array analyses where it was found to be over-expressed and to promote the invasion and metastases of a range of solid tumours. Our interest in Legumain as a therapeutic target arose from a microarray study where we identified the naturally occurring inhibitor of legumain, Cystatin 6 (CST6), as a repressed target of the oncogenic transcription factor T-box 2 (TBX2). We observed that TBX2 drove the proliferation of a subset of breast cancers through its ability to repress multiple growth control genes including CST6. CST6 (also called cystatin E/M) is a cysteine protease inhibitor and a putative breast tumour suppressor gene whose expression is down-regulated in a number of cancer types including breast, cervical, glioma, prostate and gastric. CST6 functions as a dual inhibitor of cysteine proteases (including several cathepsins) and the AEP class of enzymes including legumain. Through a process of site-directed mutagenesis of both the CST6 cysteine protease- and legumain-inhibitory domains, as well as specific siRNA knockdowns, we have identified the protease activity responsible for this proliferation phenotype in breast cancer cells as legumain. Due to high expression levels of legumain in breast cancer, and its selective role in promoting the cell growth and proliferation of breast cancer as opposed to normal mammary epithelial cells, it represents a good drug target. To date, two groups have developed a series of highly selective legumain inhibitors using these approaches. Both groups have taken advantage of the substrate binding motif, either Ala-Ala-Asn or Pro-Asn, and coupled this recognition sequence to an electrophilic warhead, such as a Michael acceptor or an epoxide. These legumain inhibitors demonstrate good potency and selectivity (nanomolar activity) in cell-free assays. The use of these types of irreversible inhibitors can be limited due off-target activity (reactivity of electrophilic warhead) and controlling the dosing regimen to avoid toxicity. To this end we have designed, synthesized and screened a series of reversible Legumain inhibitors with sub-micromolar potency.

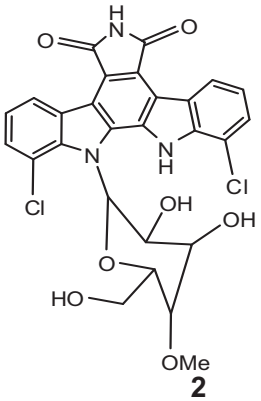
Design and synthesis of novel indolocarbazole derivatives as anti-cancer agents

Florence O. McCarthy, *Department of Chemistry and Analytical and Biological Chemistry Research Facility, University College Cork, Western Road, Cork, Ireland.*
e-mail f.mccarthy@ucc.ie

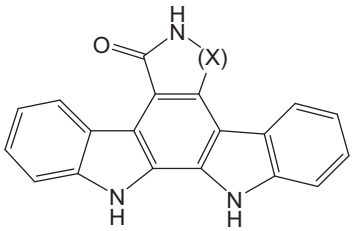
Initial interest in the possible use of indolocarbazole antibiotics as clinical anti-tumour agents began as a result of the discovery of a natural product, Staurosporine **1**, found to be a nanomolar inhibitor of the enzyme PKC *in vitro*.[1] Rebeccamycin **2**, which also possesses the conserved indolo[2,3-*a*]carbazole framework, exhibits an IC_{50} value of 1.75 μM against Topoisomerase I, yet is virtually inactive towards Topoisomerase II and PKC.[2]



1



2



3

Our current research involves modification of the pyrrolidine-2,5-dione heterocyclic component of the polycyclic ring system, engineering its bioisosteric replacement with uracil and other 5- and 6-membered analogues (**3**) in order to investigate the potential synergistic multi-modal activity against protein kinases and supercoiling enzymes (Topo I/ II).[3]

References:

1. M. Prudhomme, Current Pharmaceutical Design, 1997, **3**, 265
2. M. Prudhomme, Current Medicinal Chemistry, 2000, **7**, 1189
3. L. T. Pierce, M. M. Cahill and F. O. McCarthy (2010) 'Design And Synthesis Of Novel 5,6-Bisindolylpyrimidin-4-Ones Structurally Related To Ruboxistaurin (LY333531)'. *Tetrahedron*, 66 (51):9754-9761

Development of pyrrolo-1,5-benzoxazepines (PBOXs) as novel anti-cancer agents

Daniela M. Zisterer, *School of Biochemistry & Immunology, Trinity College Dublin*

We have previously synthesised, screened and identified lead pyrrolo-1,5-benzoxazepine (PBOX) compounds which potently induce apoptotic cell death in a wide variety of human cancerous cells with minimal toxicity to normal cells suggesting their potential as anti-cancer therapeutics. The PBOXs also induce apoptosis in ex vivo *chronic lymphocytic leukaemia and chronic myeloid leukaemia (CML) patient samples including those that are resistant to imatinib (the current frontline treatment for CML). Furthermore, we have shown that PBOXs impair the growth of tumours in breast carcinoma and CML tumour models. The development of multi-drug resistance (MDR) is a major obstacle in the successful treatment of cancer and a predominant cause of MDR is due to expression of members of ABC transporter family such as P-glycoprotein or breast cancer resistance protein. We have shown that lead PBOXs do not appear to be substrates of these MDR proteins. Thus, prominent resistance mechanisms like high expression of ABC transporters or p53 loss of function mutation/deletions do not substantially affect cancer cell sensitivity to the PBOXs. We have also demonstrated anti-angiogenic effects of the PBOXs. Collectively these results suggest that the PBOXs are a promising group of potential anti-cancer agents.*

Tubulin has been identified as the molecular target of the PBOXs hence the compounds are a novel series of microtubule-targeting agent. Work is ongoing to identify the PBOX binding site on tubulin through x-ray crystallographic studies and to examine the signalling pathway(s) leading from PBOX binding to tubulin to apoptotic cell death.

Strategies for biological targeting in head and neck cancer

Kevin Harrington, Targeted Therapy Team, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB. E-mail: Kevin.Harrington@icr.ac.uk

For many tumour types (including head and neck cancers), the outcome of treatment is largely dominated by the success or failure of attempts to control the primary lesion and its locoregional extent. Radiotherapy (RT) plays a key role in this process, but frequently fails to achieve locoregional control. Even so, when it is successful, RT can leave the patient with severe life-long side effects. In recent years, there have been significant technical advances (3-D conformal, intensity-modulated and image-guided RT) in RT delivery, opening up the possibility of using RT more effectively. Technical RT can be used either to escalate the RT dose in tumour tissue (while keeping normal tissue doses within acceptable standard levels) or to reduce the dose to normal tissues (while keeping tumour doses at the standard level). Each of these approaches is currently being tested in the clinic. For the former strategy, the expectation is that gains in tumour control probability (TCP) will be worthwhile, but may be modest (<10%). By combining chemotherapy with RT, we can improve TCP but this comes at the cost of increased toxicity from non-specific sensitisation of normal tissues. Indeed, it is widely accepted that most combination chemoradiation regimens are already delivered at or close to the limits of normal tissue tolerance and this limits further development of this strategy. The greatest opportunity for using RT more effectively in the future lies with the development of targeted drugs to achieve tumour-selective radiosensitisation. A framework by which putative targeted radiosensitisers may be evaluated will be discussed and preclinical and clinical examples of promising strategies based on HSP90 inhibition and Chk1 inhibition will be presented. In addition, the potential for rational patient selection (based on HPV status) for treatment intensification and de-intensification strategies will be discussed.

A novel strategy for targeting metalloproteinases in cancer

Gillian Murphy
Dept of Oncology, University of Cambridge, Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Cambridge CB2 0RE UK

Epithelial tumours evolve in a multi-step manner, involving both inflammatory and mesenchymal cells. Although intrinsic factors drive malignant progression, the micro-environment of neoplastic cells is a major feature of tumorigenesis. Extracellular proteinases, notably the metalloproteinases, are key players in the regulation of this cellular environment, acting as major effectors of both cell-cell and cell-extracellular matrix (ECM) interactions. They are involved in modifying ECM integrity, growth factor availability and the function of cell surface signalling systems, with consequent effects on cellular differentiation, proliferation and apoptosis.This has made metalloproteinases important targets for therapeutic interventions in cancer and small molecule inhibitors focussed on chelation of the active site zinc and binding within the immediate active site pocket were developed. These were not successful in early clinical trials due to the relative lack of specificity or of precise knowledge of the target proteinase(s) in specific cancers. We can now appreciate that it is essential that we understand the relative roles of the different enzymes (of which there are over 60) in terms of their pro and anti tumour activity and their precise sites of expression The next generations of metalloproteinase inhibitors need the added specificity that might be gained from an understanding of the structure of individual active sites, the role of extra catalytic motifs in substrate binding and other aspects of their biology. We have exploited scFv technology to target the extra-catalytic domains of key membrane metallporoteinases for the development of unique inhibitory antibodies. The biochemical and cell biological evaluation of this approach has been evaluated for MMP-14 and ADAM17, before we proceed to in vivo studies of tumorigenesis.

Targeting of Cathepsin S in Tumorigenesis
Christopher Scott, Molecular Therapeutics Cluster, School of Pharmacy, Queen's University Belfast

A growing body of data has highlighted the role of various lysosomal enzymes in promoting tumour progression. Cathepsin S is one of eleven cysteine proteases found in the human genome that normally has restricted expression in leukocytes and professional antigen presenting cells. A growing body of evidence is now showing that its deregulation can promote the development of tumours, highlighting it as a therapeutic target. Using a combination of genetic and mechanistic approaches we are examining the sources and role of this protease in models of colorectal cancer carcinomas. Furthermore, we have applied antibodies as selective inhibitors to target secreted Cathepsin S as a therapeutic strategy, demonstrating their ability to inhibit tumour angiogenesis and growth. We have also shown that this approach can augment chemotherapeutic and anti-angiogenic treatments and may therefore have clinical utility in combination therapies to target invasive tumours.

The anti-tumour efficacy of the novel peptide inhibitor of angiogenesis ALM-201
Dr Martin O'Rourke (Almac Discovery)

Neovascularization is critical to tumour growth and metastasis and this has led to the development of a number of marketed anti-angiogenic agents which target VEGF/VEGFR2 receptors, for clinical use in specific types of cancer. However, incomplete responses and/or resistance to these therapies has highlighted the need for new agents targeting alternative pathways. Here we describe the characterisation of a novel peptide derived from the natural protein FKBP-like binding protein (FKBPL), which has extremely potent anti-angiogenic activity, is very effective in mouse xenograft models at low doses, and exerts its effects through microtubule binding using CD44 as a cell-entry mechanism.

ALM-201 has been profiled in a range of human microvascular endothelial cell (HMEC-1) assays and potently inhibited migration, tubule formation and microvessel formation *in vitro* and *in vivo*. Although the peptide has a marked effect on migration, ALM-201does not inhibit proliferation in a range of growth factor stimulated proliferation assays. Importantly, there is a significant disconnect between the pharmacokinetic and pharmacodynamic profiles of ALM-201 which allows for q.d. dosing in mouse xenograft models. The peptide is well tolerated with no signs of toxicity observed in mouse xenograft models up to 80 days of dosing. The mechanism by which ALM-201 inhibits angiogenesis involves the cell surface receptor CD44, as determined by siRNA depletion of the receptor in migration assays. Furthermore, the peptide potently inhibits microtubule assembly and downstream signalling, thus promoting the anti-migratory phenotype.

In summary, ALM-201 is a novel, targeted microtubule binding agent which exhibits potent anti-angiogenic activity *in vitro* and *in vivo*. Pre-clinical development is in progress with a Phase 1 clinical study planned for 2011.

Activation of the checkpoint kinase, Chk1, by cyclin-dependent kinase, CDK, and the Rad9 mediator protein

Ramesh Kumar, Carla Abreu, Muriel Grenon and **Noel F. Lowndes**
Centre for Chromosome Biology, School of Natural Sciences, NUI Galway, Ireland

Chk1 is an evolutionarily conserved protein kinase that is activated by replication stress and DNA damage. Once activated it regulates phosphorylation of downstream substrates required for the correct biological responses to the above stresses. The molecular mechanisms behind activation of Chk1 are unclear. Using the yeast model system we have shown a role for the Rad9 protein, a so-called mediator or adaptor protein, in the activation of Chk1. This role is dependent on the prior phosphorylation by B-type cyclin-dependent kinase complexes of a domain in Rad9 termed the Chk1 Activation Domain (CAD). CDK-dependent phosphorylation of the Rad9 CAD is required for a direct molecular interaction between phosphorylated Rad9 and Chk1. Our latest results and a model for Chk1 activation and its relevance for activation of human CHK1 will be presented.

MECHANISMS OF ESTROGEN RECEPTOR TRANSCRIPTION IN BREAST CANCER

Toni Hurtado, Kelly A. Holmes and Jason S. Carroll
Cancer Research UK, Cambridge Research Institute and University of Cambridge, Robinson Way, Cambridge, UK, CB2 ORE

Estrogen Receptor (ER) is the defining feature of luminal breast cancers, where is functions as a transcription factor. Recent genomic analyses of ER-chromatin interaction regions, revealed the enrichment of DNA motifs for Forkhead factors. The Forkhead protein FOXA1 was subsequently shown to bind to ~half of the ER binding events in the genome of the MCF-7 breast cancer cell line. We have extended on these findings to map ER binding events in primary breast cancers and liver metastases and find conservation of the FOXA1-mediated programming of ER binding. We find context dependent ER *cis*-regulatory elements that give insight into underlying transcriptional networks influencing clinical outcome. We have also explored the mechanistic basis for the FOXA1-ER interaction and show that FOXA1 is the sole defining factor that governs differential ER-chromatin interactions. This dependency is also observed in the tamoxifen-ER mediated response in breast cancer cells. Furthermore, expression of FOXA1 in non-breast cancer cells is sufficient to induce global ER binding and response to the anti-proliferative effects of the breast cancer drug tamoxifen. As such, FOXA1 is the primary determinant that regulates estrogen-ER activity and endocrine response in breast cancer cells.

MAINTENANCE OF GENOMIC STABILITY BY HUMAN DEUBIQUITINATING ENZYME COMPLEXES

Dr Martin Cohn, Department of Biochemistry, University of Oxford, UK

The Fanconi Anemia (FA) DNA repair pathway is critical for the cellular response to DNA cross-linking agents. Deregulation of the pathway results in genomic instability and increased mutagenesis. A key player in the pathway is the FANCD2 protein, which is monoubiquitinated upon DNA damage. This critical modification is in part regulated by the USP1/UAF1 deubiquitinating enzyme complex, ensuring a tight balance between the ubiquitinated and un-modified state of FANCD2, helping to keep the FA pathway in either its on or off state depending on the extent of genotoxic stress. Recent data demonstrate that the regulatory subunit of the USP1/UAF1 complex, UAF1, also regulates the enzymatic activities of other deubiquitinating enzyme complexes in human cells. A possible general mechanism of regulating deubiquitinating enzymes will be discussed.

TMEM: A new direction in the pathological assessment of metastatic risk in breast cancer

Joan Jones, Weill College of Medicine, Cornell University, New York

Most breast cancer patients carry a lifelong risk for development of metastatic disease. Although about 80% of breast cancer patients are treated with adjuvant chemotherapy, a significant proportion of these are treated unnecessarily. Currently established clinical prognostic criteria such as histopathologic grade or tumor size do not successfully predict systemic metastatic potential. Even angiolymphatic invasion and regional lymph node metastases do not always correlate with subsequent distant spread. This may be because the mechanisms of hematogenous spread are different from those for lymphatic spread. Hence new methods to identify tumors likely to metastasize are needed. In this regard, we have developed a novel method for predicting risk of metastasis. The method derives from multiphoton-based intravital imaging, which has demonstrated that intravasation of mammary carcinoma cells requires a macrophage to be in direct contact with an endothelial cell, and an invasive carcinoma cell. We call the direct apposition of these three cell types “TMEM,” for Tumor MicroEnvironment of Metastasis. The test is applicable to routine FFPE breast cancer tissue samples. What distinguishes this test is that it is based on a mechanism for tumor cell dissemination. We are in the process of testing the ability of this test to predict risk of metastasis in a large cohort of breast cancer patients, and in future studies will look to compare the performance of this test with that of the Onctotype DX test. The goal is to develop a clinically useful test that permits tailoring of treatment according to TMEM score.

DROSOPHILA MODELS FOR TUMOUR-RELATED INFLAMMATION AND COLORECTAL CANCER

Dr. Marcos Vidal, The Beatson Institute, University of Glasgow

Since Coley's discovery over a century ago that sepsis or bacterial toxins could result in tumour necrosis, the role of inflammatory cytokines and the inflammatory response in cancer progression remains paradoxical. In the case of TNF, there is undisputed evidence indicating both pro- and anti-tumour activities for the cytokine. Consequently, TNF is under consideration as both a target and a therapy for cancer patients, emphasizing our need for a better understanding of these contrasting context-dependent roles. In the fruit fly, a TNF-dependent mechanism results in the elimination by apoptosis of cells deficient for the polarity tumor suppressors *dlg* or *scrib*. This anti-tumour TNF activity improves host survival. However, that in tumours deficient for *scribble* that also express the Ras oncoprotein, the TNF signal was diverted to become a pro-tumour signal that enhances tumour growth and stimulates invasive migration. In this case, TNF promotes malignancy and is detrimental to host survival. I will discuss our current work suggesting that this TNF signaling is originated by the innate immune system.

These results indicate that the role of TNF as both a promoter and suppressor of tumours is highly conserved in metazoa, and suggest that tumours can evolve into malignancy by Ras activation and hijacking of TNF signaling. They also provide the first genetically defined model for the role of TNF as a tumor promoter, uncover a key role of oncogenic Ras, and bring *Drosophila* to the field of tumor inflammation."

"Molecular insights into mammalian autophagy"

Sharon A. Tooze, Secretary Pathways Laboratory, Cancer Research UK, London Research Institute, London, UK

Autophagy occurs in most cells and performs an essential housekeeping function to remove damaged organelles, in particular mitochondria. Autophagy occurs through the formation of a double membrane that sequesters or engulfs cytosolic proteins, membranes, and organelles. The autophagosome then fuses with endosomes and lysosomes, and delivers the sequestered material for degradation. Autophagy can also be induced by external stress including amino acid starvation. A better molecular understanding of autophagy is an important goal as autophagy is implicated in a number of human diseases, many of which can either be characterized by an imbalance in protein, organelle or cellular homeostasis, ultimately resulting in an alteration of the autophagic response. We have been studying a set of Atg (autophagy related proteins) involved in autophagosome formation, in addition to novel proteins recently implicated in autophagy in our lab. We have shown that ULK1, a serine-threonine kinase, and mAtg9, a multi-spanning membrane protein, are required for autophagy. We now know that WIPI2, a PtdIns-3 P binding protein is also required for autophagy, and is recruited to autophagsomal membranes at early stages. Furthermore, our recent data suggests that autophagosome formation requires contributions from both the Golgi and endosomes, and trafficking from these organelles contributes to autophagosome formation induced by amino acid starvation.

REFERENCES

Chan, E.Y.W., Longatti, A., McKnight, N.C., Tooze, S.A. (2009) Kinase-inactivated ULK proteins inhibit autophagy via their conserved C-terminal domain using an Atg13-independent mechanism. **Molecular and Cellular Biology**, 29:157-171.

Razi, M., Chan, E.Y.W., Tooze, S.A. (2009) Early Endosomes and Endosomal Coatomer are required for Autophagy. **Journal of Cell Biology**, 185:305-321.

Morvan, J., Köchl, R., Watson, R. , Lucy M. Collinson, L.M., Jefferies, H.B.J., Tooze, S.A. (2009) *In vitro* reconstitution of fusion between Immature Autophagosomes and Endosomes. **Autophagy**, 5: 676-689.

de Lartigue, J., Polson, H., Feldman, M. , Shokat, K., **Tooze, S.A.,** Urbé, S. Clague, M.J. (2009) PIKfyve regulation of endosome-linked pathways. **Traffic**, 10:883-893.

Webber, J.L. and Tooze, S.A. (2010) Co-ordinated regulation of autophagy by p38 MAPK through mAtg9 and p38IP. **EMBO Journal**, 29:27-40.

Polson, H.E.J., de Lartigue, J., Rigden, D.J., Reedijk, M., Urbé, S., Clague, M.J. and Tooze, S.A. (2010) Mammalian Atg18 (WIPI2) localises to omegasome-anchored phagophores and positively regulates LC3 lipidation. **Autophagy**, 6: 506-522.

Many ways to TRAIL receptor signalling regulation by chemotherapeutic drugs
Olivier Micheau, Oncology Research Group Leader, INSERM, Dijon, France

The cytokine TRAIL belongs to the TNF super familly and is considered as a promising anti-cancer agent. Recombinant preparations of TRAIL or derivatives induce apoptosis selectively in cancer cells by activating the membrane death receptors TRAIL-R1 and/or TRAIL-R2 also coined DR4 and DR5. Tumor cell resistance to TRAIL-induced cell death mainly results from the dysregulation of a large panel of inhibitors, including TRAIL antagonistic receptors, as well as cellular inhibitors such as c-FLIP, Bcl-2 family members or regulators of effector caspases. Resistance to TRAIL-induced cell death can nontheless, in most instances, be overcomed by chemotherapeutic drugs. I will present our last findings and discuss the possible ways to restore TRAIL-induced cell death by these compounds.

Genomic studies of drug resistance in ovarian cancer
Dr James D. Brenton PhD FRCP, Group Leader and Honorary Consultant in Medical Oncology
Functional Genomics of Ovarian Cancer Laboratory
Cancer Research UK Cambridge Research Institute, Cambridge, UK

Our understanding of the molecular basis of ovarian cancer has rapidly changed over the last three years. The recognition that different histological subtypes of ovarian cancer are different disease entities has lead to significant progress in identifying the cell of origin, precursor lesions and key driver mutations for each type. High-grade serous ovarian cancer (HGSOC) is the commonest type and is responsible for the majority of ovarian cancer mortality. HGSOC is initially extraordinairily sensitive to platinum-based chemotherapy but retreatment of relapsed disease results in the development of progressive drug resistance.

The most important clinical research question is to identify biomarkers of drug response and resistance in HGSOC. I will contrast emerging clinical and genomic data for platinum and paclitaxel resistance pathways in HGSOC with data from other cancers in which drug resistance can be explained by intra-tumour genetic heterogeneity, evolutionary theory and the cancer stem cell hypothesis. I will present recent work using array comparative genomic hybridization and next-generation sequencing that suggests that differences in initial response to platinum chemotherapy and the subsequent patterns of resistance in HGSOC may reflect genetic heterogeneity and selective pressure from chemotherapy.

Aberrations of the miRNome in human cancer: Causes and Consequences
Muller Fabbri, MD

MicroRNAs (miRNAs) are small, non-coding RNAs with gene regulatory functions. Several studies have demonstrated that their expression is altered in human tumors with respect to the normal tissue counterpart, and is involved in human carcinogenesis. Several levels of evidence are showing that miRNAs undergo the same regulatory mechanisms of any other protein coding gene, including transcription factor regulation and epigenetic modifications. More recently, it has been discovered that another category of non-coding RNAs (the transcribed ultraconserved regions) is de-regulated in cancer and miRNAs interact with these non-coding RNAs, affecting their expression. This interaction between two different groups of non-coding RNAs represents a new layer of gene expression regulation, whose implications are currently under investigation. This lecture will focus on what is currently known on the factors that alter the miRNome (defined as the full spectrum of miRNA expression in a genome), and on what these aberrations mean for the development of the malignant phenotype.

Biomarker Discovery and Validation for Prostate Cancer Grade and Stage: Addressing the Clinical Dilemma
William Watson, Associate Professor of Cancer Biology
UCD School of Medicine and Medical Science, Conway Institute, University College Dublin

Prostate cancer remains the most common form of male cancer in the US and Europe. Despite PSA screening decreasing cancer mortality it has been associate with the over detection and over treatment of the disease, impacting on patients quality of life. Determining the most appropriate treatment strategy represents a significant dilemma for the patient and clinician. Our focus of research has been driven by the clinical need to identify biomarkers that will inform appropriate treatment options, specifically focusing on tumour grade and stage.

Preclinical serum samples were collected from men with different grades and stages of prostate cancer as part of the Prostate Cancer Research Consortium Bioresource. Their analysis by 2-D DIGE and label-free LC-MS/MS and the use of novel statistical software has identified panels of proteins with acceptable predictability for further validation. Using multiplex antibody assays and multiple reaction monitoring (MRM) assays using triple quadruple mass spectrometry these panels are being validated in independent samples from the Prostate Cancer Research Consortium Bioresource and our international collaborators and show AUC values in line for clinical utility. With careful validation these panels, in combination with current clinical tools, could improve diagnosis and thus patient outcome through the selection of appropriate treatment options.

MOLECULAR FACTORS LINKING OBESITY AND CANCER
Graham Pidgeon, Institute of Molecular Medicine, Trinity College Dublin

Obesity is linked to increased incidence and mortality of many cancer types. Of all cancers, gastrointestinal cancers, particularly oesophageal adenocarcinoma (OAD), display some of the strongest epidemiological associations with obesity, making them excellent models to investigate this link at a molecular level. Interestingly, excess adiposity is understood to be a causal factor in Barrett's oesophagus, a clinical precursor of oesophageal adenocarcinoma defined by squamous to columnar cell metaplasia in the oesophagus. In 2007, a unique adipose tissue bioresource from patients undergoing resectional GI surgery was established in our unit to complement the well maintained tumour biobanks and clinical databanks. We aimed to determine the pro-tumour properties of visceral adipose tissue in pre-mailignant inflammatory conditions and in established cancers. Obesity, measured by visceral fat area, was associated with a pro-inflammatory and pro-angiogenic gene signature in central visceral fat. A circulating pro-inflammatory phenotype, common to visceral obesity, male sex and cancer, was mediated through reduction in levels of IL-4 and adiponectin and elevation in levels of leptin, IL-6, IL-8, MCP-1, IFNγ and VEGF. Visceral adipose tissue increased glycolytic flux and up regulated focal adhesion pathways in OAD cells, promoting cytoskeletal reorganisation and epithelial mesenchymal transition (EMT), culminating in increased proliferative, migratory and invasive capacity of OAD. In patient biopsies, elevated expression of markers of invasion and metastasis MMP9, PAI-1 and SNAI2 together with reduced expression of tumour suppressor p53 and epithelial marker E-cadherin, were associated with visceral obesity. Increased expression of MMP9 and PAI-1 and decreased expression of tumour suppressor p53 correlated with aggressive tumour biology and PAI-1 and SNAI2 were found to be independent prognostic factors in OAD. This work highlights a novel role for visceral obesity in up regulation of pro-tumour pathways characteristic of OAD. In separate work, the co-culture of non-neoplastic Barrett's epithelial cells with adipose tissue explants, altered the expression of several well established oncogenes. MMP-1 and IL8 mRNA expression were significantly up-regulated compared to non obese patients, potentially contributing to the carcinogenenic progression of Barrett's oesophagus. The molecular dissection of the complex role of obesity in the process of tumour initiation and promotion will assist in the understanding of obesity associated cancer and lead to the development of stratified therapies for OAD patients with visceral obesity

DECONSTRUCTING TUMOUR SUPPRESSION AND ONCOGENESIS TO FIND EFFECTIVE WAYS TO KILL CANCER CELLS

Gerard I. Evan, Lamorna Brown Swigart, Laura Soucek, Nicole Sodir, Melissa Junttila, Roderick Kortlever & Daniel Garcia, Dept.
Biochemistry, University of Cambridge, UK. and Dept. Pathology, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, USA

At present we can only target pharmacologically a small proportion of the molecular effectors that drive and maintain cancers but novel technologies are rapidly expanding the “druggable” repertoire. We are developing switchable genetic technologies that allow the transient and reversible inhibition of expression of any adult gene, either systemically or in a tissue-specific manner. In this way, we can model, in pre-clinical in vivo models, the therapeutic index of inhibiting any specific molecular target. Thus far, we have focused on p53 and Myc, pleiotropic transcription factors that, respectively, suppress and drive cancer. The p53 tumour suppressor pathway is functionally inactivated in most human cancers, so we have directly assessed the therapeutic impact of restoring p53 in established tumours in mice. Our studies indicate that p53’s checkered evolutionary legacy, evolving initially as a transcriptional coordinator of stress and damage responses that was only lately commandeered into the role of tumour suppression, has compromised its efficacy as a tumour suppressor. These data have profound implications since they set inherent limits both to the efficacy of p53 as a tumour suppressor and to the therapeutic utility of p53-restoration as a cancer therapy. The Myc oncoprotein is functionally deregulated in almost all human cancers, even though the gene is not, of itself, frequently mutated. To investigate the therapeutic potential of Myc inhibition we have constructed switchable genetic mouse models in which endogenous Myc can be systemically and reversibly inhibited in normal and tumour tissues in vivo. Our data indicate that inhibiting Myc has a remarkably efficacious and durable therapeutic impact on multiple cancer types, triggering widespread tumour cell apoptosis while eliciting only mild, reversible and non-cytotoxic side effects in normal tissues.

ChIP-seq identifies p63 target genes involved in palatal fusion and EMT

SS McDade[1], A Henry[1], G Pivato[1], C Mitsopoulos[2], K Fenwick[2], A Ashworth[2], C Lord[2], D McCance[2]

[1]Centre for Cancer Research and Cell Biology, QUB, Belfast, UK

[2]Breakthrough Breast Cancer Research Centre, ICR, London, UK

p63 knockout mice and patients with p63 mutations exhibit limb, epidermal and craniofacial abnormalities including cleft palate. Palatal fusion is of particular interest since it involves co-ordinate regulation of proliferation, migration, adhesion, apoptosis and epithelial to mesenchymal transition (EMT), processes which are subverted during tumorigenesis. We recently identified p63 as a regulator of TGFB1 induced EMT breast cells, however despite extensive efforts and the identification of a wide range of direct transcriptional targets the precise transcriptional role of p63 in development and differentiation is still unclear.

We used ChIP-seq to identify p63 target genes on a genome wide basis and identified >8000 high stringency p63 binding sites in primary human foreskin keratinocytes (HFKs). We took an integrative approach to identify constitutively regulated p63 target genes using publicly available microarray data for p63 depletion in 3 epithelial cell types. Gene ontology analysis and database mining of these gene sets indicated significant enrichment of genes genetically linked to cleft palate, a phenotype also associated with p63 mutation. We confirmed binding and regulation of a number of these genes by q-PCR ChIP and showed that expression of these genes is decreased in HFKs depleted for p63.

Transcription factor binding site enrichment analysis of the p63 binding regions, identified the AP-2α transcription factor. AP-2α mutation is also associated with craniofacial abnormalities and cleft palate. Binding to a number of these sites was confirmed by ChIP and re-ChIP assays and AP-2α enhanced p63 mediated activation of the IRF6 enhancer.

The identification of p63 as a key regulator of genes involved in palatal fusion and the processes involved will provide the basis for further studies into its role in carcinogenesis. Furthermore, its relationship with AP-2α may help us better understand how these frequently de-regulated transcription factors function in tumorigenesis.

THE STROMAL FUNCTION OF THE RETINOBLASTOMA PROTEIN IN CONTROLLING DIFFERENTIATION AND CANCER CELL INVASION

A. Pickard[1], A-C. Cichon[1], D. J. McCance[1]
[1]CCRCB, QUB, Belfast, United Kingdom

The retinoblastoma protein (pRb) is known to regulate cell growth in a cell non-autonomus fashion. How pRb controls the growth of neighbouring cells is unclear and is the focus of this study. Using three-dimensional skin cultures we have examined the non-cell autonomous functions of pRb. Depletion of pRb levels in stromal fibroblasts resulted in increased proliferation and loss of differentiation of the epithelium, suggesting that the cross-talk between the two cell types is altered. Using cytokine arrays, RT-PCR and ELISA we identified elevated expression of KGF (keratinocyte growth factor) in pRb-depleted fibroblasts. Knock-down of KGF in pRb-depleted fibroblasts allowed the epithelium to differentiate normally suggesting KGF inhibits differentiation. Culturing non-invasive transformed epithelial cells with pRb-depleted fibroblasts promoted invasion. The number of invasions could be reduced by blocking KGF binding to its receptor, using heparin, or by depleting KGF in the fibroblasts. To further examine the role of KGF in mediating invasion, the expression of matrix metalloproteases (MMPs) in epithelial cells were measured following KGF treatment. MMP1, a known regulator of invasion, as well as transcription factors known to elevate MMP1 expression (Ets2) were induced by KGF. Knockdown of either the KGF receptor (FGFR2b) or Ets2 in epithelial cells reduced invasions caused by pRb-depleted fibroblasts, further implying that invasions are mediated via KGF-dependent induction of Ets2 and MMP1. In order to investigate the significance of these findings in the tumour microenvironment the phosphorylation status of pRb was assessed in the stromal compartment of head and neck tumours. Increased pRb phosphorylation was observed in dyplastic regions compared to normal tissues suggesting that pRb is inactivated in these regions. Together our data indicate a key role for pRb controlled pathways in the stromal compartment, both in regulating growth and differentiation of normal epithelia but also in preventing invasion of transformed epithelial cells.

PROFFERED PAPERS - ABSTRACTS

DEVELOPMENT OF NOVEL ANTI-ANGIOGENIC COMPOUNDS FOR COLORECTAL CANCER USING A TUMOUR EXPLANT MODEL AND ZEBRAFISH.

AG Murphy[1,2], D Fennelly[1], A Reynolds[2], M Tosetto[1], J Hyland[1], K Sheahan[1], D O'Donoghue[1], H Mulcahy[1], B Kennedy[2], J O'Sullivan[3]

[1]Education and Research Centre, St Vincent's University Hospital, Dublin, Ireland

[2]Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

[3]Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland

INTRODUCTION: The treatment of metastatic colorectal cancer has significantly improved with the development of novel molecular targeted drugs such as Bevacizumab (Avastin); however response rates are modest (40%). There is an urgent need for the development of newer drugs which target angiogenesis and inflammation more effectively. Historically, initial drug screening has been performed using monolayer cell cultures, but these do not effectively present the entire tumour microenvironment. Novel models are required to investigate newer target lead drugs.

METHODS: Chemical screens of randomised drug libraries were performed in zebrafish to identify drugs that inhibit developmental angiogenesis. Two lead drugs were then tested using human ex vivo colorectal explants to examine their potential to inhibit angiogenic and inflammatory protein secretions for the following factors VEGF, MCP-1, GRO-alpha, IL-6, TNF, IL-8 and IL-1β.

Human explants from 5 patients were cultured for 72 hours, and levels of the above factors were assessed using ELISA.

RESULTS: Compound B and F significantly inhibit intersegmental vessel development in zebrafish.

Compound B (1 μM) reduced the expression of IL-6 ($p=0.02$) and the 10 μM concentration reduced the expression of VEGF ($p=0.047$), IL-6 ($p=0.02$) and IL-1β ($p=0.01$). Compound F (1 μM) reduced the expression of VEGF ($P=0.025$) and the 10 μM concentration reduced the expression of VEGF ($p=0.01$) and IL-1β ($p=0.01$).

Avastin (1 μM) reduced the expression of VEGF ($p=0.01$) and IL-6 ($p=0.02$) and the 10 μM concentration reduced the expression of VEGF ($p=0.003$) and IL-6 ($p=0.02$).

Avastin or the other drugs did not affect the expression of MCP-1, GRO-alpha, TNF and IL-8.

CONCLUSION: These studies have characterised two novel compounds with the potential to become important anti-angiogenic drugs in colorectal cancer.

ADAMS AS NEW THERAPEUTIC TARGETS FOR TRIPLE NEGATIVE BREAST CANCER

M Mullooly[1,2], PM McGowan[1,2], S Sukor[1,2], SF Madden[3], E McDermott[1], J Crown[1,3], N O'Donovan[3], MJ Duffy[1,2]

[1]St. Vincent's University Hospital, St. Vincent's University Hospital, Dublin, Ireland
[2]School of Medicine and Medical Science, University College Dublin, Dublin, Ireland
[3]National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland

Background: Triple negative breast cancers (TNBC) lack expression of ER, PR and HER2. Consequently, targeted therapy is currently unavailable for these patients. At least some TNBCs however, possess high levels of EGFR and are dependent on EGFR signaling. Activation of EGFR signaling is triggered by the release of ligands such as TGFα, EGF and HB-EGF. Release of these ligands is catalysed by the proteases, ADAM10 and ADAM17. The aim of this study was to test the hypothesis that inhibition of ADAM10/17 was a new approach for treating patients with TNBC.

Methods: ADAM10 and 17 were measured at protein and mRNA levels in extracts of TN and non-TNBC. Pooled data from 22 published gene expression databases (N=approx 4000 patients) was used to relate ADAM and ADAM17 mRNA to patient outcome. The effect of the dual ADAM10/17 inhibitor, GW280264X (GSK) on the proliferation of 7 TN and 6 non-TN breast cancer cell lines was investigated using the acid phosphatase assay and Calcusyn software.

Results: Using extracts of breast cancer (n=185), the active form of ADAM17 was found in 98 % of TN and 95 % of non-TN samples. Importantly, significantly higher levels were present in TN than non-TN samples ($p = 0.025$, Mann Whitney U). Similarly, the active form of ADAM10 was detected in the majority (82%) of TN samples. Using pooled data from published datasets, high ADAM10 expression was associated with adverse prognosis in patients with basal-type (most of which were TN) breast cancer. Using the dual ADAM10/17 inhibitor GW280264X, we found up to 90 % reduction in cell growth (36 to 89 % growth reduction) across a panel of TNBC lines with IC50 concentrations ranging from 0.43 μM to 2.8 μM. In contrast, non TNBC lines responded with a range of 10 to 73 % growth inhibition and IC50 concentrations of 2.8 to >20 μM.

Conclusions: Our results indicate that ADAM10 and 17 are expressed in the majority of TNBCs. Furthermore, ADAM inhibition resulted in impaired cell growth, migration and invasion of TNBC cells in vitro. Taken together, we propose that ADAM10 and ADAM17 may be novel targets for therapeutic intervention in TNBC.

The authors would like to thank SFI (SRC award, 08/SRC/B1410 to MTCI) for funding this work.

SYSTEMIC TUMOUR TARGETING WITH ORALLY ADMINISTERED NON-PATHOGENIC BACTERIA

M Cronin[1], C Baban[1], S Collins[1], D van Sinderen[2], G C O'Sullivan[1], M Tangney[1]
[1]Cork Cancer Research Centre, University College Cork, Cork, Ireland
[2]Department of Microbiology , University College Cork, Cork, Ireland

Background/aims: Specific properties of different bacterial genera can be exploited in their use as gene and cell therapy vectors. One such strategy involves delivery of non-invasive bacteria to the tumour site with bacterial expression of genes locally (external to the tumour cell). Tumour-specific bacterial colonisation, while originally believed to be due to preferential growth of anaerobic bacteria in hypoxic tumours, has more recently been demonstrated to involve leaky vasculature and lack of immune surveillance within tumours. By transfection with plasmids suitable for bacterial expression of heterologous genes, these bacteria can home to tumours, replicate within them and locally express therapeutic proteins.

Methods: Lux-labelled B. breve fed to mice bearing subcutaneous tumours (B16, LLC or MCF7) were detected by whole body imaging specifically in tumours. Findings were confirmed by bacterial culture and mice remained healthy throughout experiments.

Results: We report the novel finding that oral administration of bifidobacteria is equally as effective as IV administration in achieving specific targeting of distal tumours. These bacteria also colonised lungs bearing small B16 melanoma metastases suggesting that hypoxia is not required for tumour-specific growth. With regards the mechanism of this bifidobacterial translocation, cytokine analyses indicated up-regulation of IFN in the GIT, which is associated with increases in epithelial permeability. Tumour colonization was reduced in immune-competent mice compared with athymic, and ELISA demonstrated low levels of serum IgG antibodies against the vector 2 weeks post administration.

Conclusion: These findings indicate potential for safe and efficient gene based treatment and/or detection of tumours via ingestion of non-pathogenic bacteria expressing therapeutic or reporter genes.

MIR-335 SUPPRESSES THE MIGRATORY AND INVASIVE CAPACITY OF NEUROBLASTOMA CELLS.

J Lynch[1,2], J Fay[1,2], K Bryan[1], KM Watters[1], M Meehan[1,2], J Ryan[1,2], I Bray[1], RL Stallings[1,2]
[1]Cancer Genetics, Royal College of Surgeons, Dublin, Ireland
[2]National Chilgren's Research Centre, Our Lady's Childrens Hospital Crumlin, Dublin, Ireland

MicroRNAs are post-transcriptional gene regulators that play a ubiquitous role in cellular processing and functioning. Aberrant expression of miRNAs has been correlated with tumourigenesis in many cancer types, including neuroblastoma. Neuroblastoma is an often fatal paediatric cancer derived from precursor cells of the sympathetic nervous system and is defined by many different genetic subtypes. One of the more clinically aggressive subtypes is characterized by high level amplification of the MYCN oncogenic transcription factor. MiR-335 is significantly down-regulated in MYCN amplified tumours (Bray et al Plos One 2009; 4:e7850) suggesting a possible tumour suppressive function for this miRNA. This study provides evidence that miR-335 is of functional significance in neuroblastoma tumourigenesis, particularly in the MYCN amplified genetic subtype. We demonstrate that ectopic up-regulation of miR-335 in neuroblastoma cell lines significantly reduces their motility and invasive capacity. Inversely, down-regulation of endogenous miR-335 expression induces a significant increase in the motile and invasive ability of these cells. Microarray mRNA expression analysis, in combination with the TargetScan miRNA target prediction algorithm, identified 43 down-regulated candidate miR-335 target genes following ectopic over-expression of this miRNA in neuroblastoma cell lines. The candidate target genes identified from the array represent a statistically significant enrichment for miR-335 target genes (p=0.0009). Three of these candidate genes were selected for further investigation given their known functional roles in the cellular processes of migration and invasion: *ROCK1*, *MAPK1* and *LRG1*. The down-regulation of these three genes in response to miR-335 over-expression was validated by real-time RT-PCR and at protein level by Western blot in neuroblastoma cell lines. Experimental work using luciferase reporter assays is ongoing to determine if *ROCK1*, *MAPK1* and *LRG1* are direct or indirect targets of miR-335. Understanding the molecular mechanisms by which miR-335 inhibits neuroblastoma cell migration and invasion could ultimately translate to improved outcome for patients.

1. Bray I, Bryan K, Prenter S, Buckley PG, Foley NH, Murphy DM, et al. Widespread dysregulation of MiRNAs by MYCN amplification and chromosomal imbalances in neuroblastoma: association of miRNA expression with survival. PLoS One2009;4(11):e7850.

The role of the chromatin protein HMGB2 in estrogen driven breast cancer

AM Redmond[1], AT Stafford[2], M McIlroy[2], D McCartan[2], K O'Brien[2], ADK Hill[2], JS Carroll[1], LS Young[2]

[1]Nuclear Receptor Transcription, Cancer Research UK Cambridge Research Institute, Cambridge, UK

[2]Endocrine Oncology Research, Dept of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Breast cancer affects one woman in ten in the western world with a mortality rate of approximately 35%. The estrogen receptor (ER) plays a major role in two thirds of these tumours, and drugs targeting this subgroup of patients include tamoxifen and the aromatase inhibitors. However, resistance to these treatments occurs in 30-40% of patients. The ER coactivator protein SRC-1 was found to be a strong predictor of disease recurrence, independent of treatment ($p<0.0001$). Using a mass spectrometry based screen, the high mobility group box protein HMGB2 was identified as an SRC-1-interacting partner. HMGB2 is a chromatin-associated protein, containing two HMG boxes that mediate interactions with the minor groove of the DNA, facilitating binding of a number of different transcriptions factors. Expression of this protein in the ER positive patients on our breast cancer TMA correlated with good prognosis ($p=0.0346$). Chromatin immunoprecipitation with parallel high-throughput sequencing (ChIPseq) of HMGB2 was conducted in the endocrine sensitive MCF-7 cell line and the endocrine resistant LY2 cell line. HMGB2 ChIPseq revealed enrichment at the promoter regions of genes, which would correlate with the reported role for HMGB2 in enhancing binding of RNA polymerase to DNA. When analysed in conjunction with our ChIPseq data for ER & SRC-1, we found that almost half of the HMGB2 binding sites were also bound by these two factors, a strong indication that these three proteins form a complex at the chromatin level. Motif analysis revealed enrichment for the MYC::MAX motif at HMGB2 binding sites with tamoxifen treatment, which warrants further investigation. Microarray experiments are currently underway and will allow cross-analysis with the ChIPseq data to confirm the effects of binding of the HMGB2:ER:SRC-1 complex. This work will shed light on estrogen-driven breast cancer, with the aim to identify new therapeutic targets for breast cancer patients.

Hypoxia in Epithelial Ovarian Cancer: Remodelling the Epigenome and Taxol® response.

S English[1,2], B Li[1], A McGoldrick[1], F Furlong[1], A McCann[1,2]
[1]UCD Conway Institute of Biomolecular and Biomedical Research,University College Dublin,Belfield, Dublin 4, [2]UCD School of Medicine and Medical Science (SMMS), University College Dublin,Belfield,Dublin 4, Ireland.

Purpose: Chemoresistance continually restricts the efficacy of first line Taxol® based regimes in patients with epithelial ovarian cancer (EOC). This is in part due to Taxol's® anticipated pharmacological response in a proliferative normoxic environment despite our knowledge of the characteristic hypoxic and senescent nature of such solid tumours inherently known to be chemoresistant.
Evidence is accumulating that chemoresistance pathways may be epigenetically regulated by the atypical expression of the Chromatin Remodeling Polycomb Group (PcG) proteins BMI1 and EZH2. Specifically, the recent identification of oxygen as a mediator in PcGs function (1) and in light of the emergent understanding of their impedance upon anti-mitotic drug function exemplified by Taxol®, warranted investigation into the potential effects of the hypoxic tumour microenvironment on BMI1 and EZH2 and a combined putative role in chemoresistance to Taxol® in EOC.
Methods: The ovarian cancer cell lines A2780, OVCAR7 and UPN251 were cultured in normoxia (21%) and hypoxia (1% O2) for 24, 48 and 72 hours followed by western blot analyses of BMI1 (35kDa) and EZH2 (90kDa). The effect of hypoxia (1%) on Taxol® (100nm) response was examined by the MTT viability assay. Chemoresponse was also established following siRNA specific targetting of BMI1 and EZH2.
Results: Western blot analysis demonstrated that 72 hours hypoxic (1%) exposure significantly affected the protein expression of BMI1 and EZH2 in A2780, OVCAR7 and UPN251 compared to their normoxic controls. A2780, the cell line most sensitive to Taxol®, over-expressed BMI1 and EZH2 in hypoxia, while OVCAR7 and UPN251 showed decreased expression of both PcG proteins. The MTT assay demonstrated that increased exposure to hypoxia (1%) was coincident with increased resistance to Taxol® (100nm) in the three cell lines. Moreover, siRNA knockdown of both polycomb proteins resulted in increased chemoresistance.
Conclusion: We have previously published (2) that hypoxia, a key feature of the tumour microenvironment alters the global epigenome. This current study further demonstrates that the master chromatin remodellers BMI1 and EZH2 are also altered in hypoxia. Moreover both the hypoxic environment and siRNA selective knockdown of both BMI1 and EZH2 resulted in increased viability and cellular resistance to Taxol®. We suggest that differential PcG levels induced by hypoxia impact on Taxol® responsiveness possibly through global epigenomic changes in the transcriptome.

(1) Crea F, Duhagon Serrat MA, Hurt EM, Thomas SB, Danesi R, Farrar WL. BMI1 silencing enhances docetaxel activity and impairs antioxidant response in prostate cancer. Int J Cancer. 2010 Jun 21. [Epub ahead of print] PubMed PMID: 20568112. (2)Watson JA, Watson CJ, McCrohan AM, Woodfine K, Tosetto M, McDaid J, Gallagher E, Betts D, Baugh J, O'Sullivan J, Murrell A, Watson RW, McCann A. Generation of an epigenetic signature by chronic hypoxia in prostate cells. Hum Mol Genet. 2009 Oct 1;18(19):3594-604. Epub 2009 Jul 7. PubMed PMID: 19584087

BEVACIZUMAB CAUSES INHIBITION OF DENDRITIC CELLS AND IS ASSOCIATED WITH PATIENT SURVIVAL IN COLORECTAL CANCER

A Michielsen[1], S Noonan[1], P Martin[1], J Marry[1], M Tosetto[1], J Hyland[1], K Sheahan[1], D O'Donoghue[1], H Mulcahy[1], D Fenelly[1], E Ryan[1], J O'Sullivan[1,2]
[1]Centre for Colorectal Disease, St. Vincent's University Hospital, Dublin, Ireland
[2]Institute of Molecular Medicine, Trinity Centre for Health Sciences, St. James Hospital, Dublin, Ireland

Introduction: The development of targeted therapies, such as Bevacizumab, has improved survival in colorectal cancer, however many patients develop resistance to this treatment. Currently there are no biomarkers that predict response to Bevacizumab and it is unknown how this treatment influences the immune system in late staged colorectal cancer patients. Dendritic cells (DCs) are important for the induction of a potent anti-tumour immune response; however tumours are able to disable dendritic cells, thus enabling immune evasion.

Aim: To investigate the effect of tumour conditioned media, with the presence or absence of Bevacizumab, on DC maturation, and associate this with survival of colorectal cancer patients.

Method: Tumour explant tissues were cultured for 72 hours in the absence or presence of Bevacizumab, to generate Tumour Conditioned Media (TCM) and Bevacizumab Conditioned Media (BCM) respectively. Monocyte derived DCs were treated with TCM and BCM. Expression of CD80, CD86, CD83, CD54, HLA-DR and CD1d was measured by flow cytometry. IL-10 and IL-12p70 were measured by ELISA. The Cox proportional Hazards model was used to associate inhibition of DCs with survival.

Results: TCM and BCM inhibited DC maturation and IL-12p70 secretion (TCM and BCM $p< 0.0001$), while increasing IL-10 secretion ($p= 0.0033$ and 0.0220 respectively). There was no difference between effects elicited by TCM versus BCM. Inhibition of CD1d ($p=0.021$, hazard ratio=1.096) and CD83 ($p=0.017$, hazard ratio=1.083) expression by TCM was associated with increased risk of death in colorectal cancer patients. Inhibition of CD1d ($p=0.017$, hazard ratio=1.067), CD83 ($p=0.032$, hazard ratio=1.035) and IL-12p70 ($p=0.037$, hazard ratio=1.036) by BCM was also associated with an increased risk of death. Age, stage and gender were not significantly associated with survival.

Conclusion: This is the first study to show that inhibition of DC maturation by TCM and BCM could be used as a prognostic marker for colorectal cancer patients receiving Bevacizumab.

This work was funded by the Irish Research Council for Science, Engineering and Technology (IRCSET)

IDENTIFICATION OF THE TRANSCRIPTIONAL COMPLEX THROUGH WHICH TBX2 DRIVES THE PROLIFERATION OF BREAST CANCER CELLS

NT Crawford, KL Redmond, PB Mullan
[1]CCRCB, Queens University Belfast, Belfast, N.Ireland

The T-box (TBX) family of transcription factors play an important role in developmental gene regulation, binding to target promoters through highly conserved T-box domains. TBX2 is located on chromosome 17q23, a region amplified in a subset of breast cancers and associated with poor prognosis. Few TBX2 target genes have been identified but its role in tumorigenesis has been linked to its ability to repress key growth regulatory genes such as p14ARF, p21WAF1 and Connexin 43.

Gene silencing of TBX2 resulted in a dramatic inhibition of proliferation of TBX2 expressing breast cancer cells. NDRG1 (N-myc DownRegulated Gene 1), a growth control gene, was identified by microarray analysis as a TBX2 target gene. The mechanism through which TBX2 represses genes involved in growth control, apoptosis and senescence is poorly understood. NDRG1 promoter studies resulted in the identification of a novel mechanism by which TBX2 targets this gene, through the association with the Early Growth Response 1 (EGR1) transcription factor. Through Co-IP experiments we have identified two novel co-repressors; KAP1 and HP1, interacting with TBX2. KAP1 is the universal co-repressor for the KRAB domain containing zinc finger superfamily of transcriptional repressors. HP1 is considered the 'gatekeeper' of methyl-K9-H3 mediated silencing. Although neither TBX2 or EGR1 contain KRAB domains (usually a requirement for KAP1 binding), the interaction between KAP1 and HP1 has been well documented. Through site directed mutagenesis we have found two HP1 binding sites on TBX2 essential for its ability to recruit HP1 and KAP1 to target promoters and subsequently repress NDRG1. Downregulation of KAP1 and HP1, similarly to TBX2 and EGR1, results in a decreased ability of breast cancer cells to proliferate.

TBX2 is an oncogene promoting the proliferation and survival of breast cancer cells. We have identified a novel mechanism of transcriptional repression by TBX2 through interaction with the putative tumour suppressor EGR1, allowing TBX2 to repress some of the major growth inhibitory pathways. We have also proposed a mechanism though which TBX2 represses transcription of target genes. Abrogating either this TBX2-EGR1 interaction or TBX2 repression complexes may present opportunities for the development of novel breast cancer treatments.

CD44 AFFILIATION WITH LIPID RAFTS IS ASSOCIATED WITH DECREASED MOTILITY OF BREAST CANCER CELLS.

IS Babina[1], S Donatello[1], AM Hopkins[1]
[1]Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Breast cancer invasion and metastasis are critically regulated by adhesion and migration properties of individual tumour cells. CD44 is an adhesion protein expressed by breast epithelial cells which plays a key role in regulating cell migration. Altered expression of CD44 has been implicated in tumour progression in several carcinomas including breast, but the mechanisms are incompletely understood. CD44 localizes in cholesterol-enriched regions of the cell membrane known as lipid rafts, but the role of rafts in regulating CD44 functions during cell migration remains controversial. Our preliminary data suggested that CD44 must move outside of lipid rafts to drive cancer cell migration. Thus the aim of our study was to determine the role of lipid rafts in regulating the localization of CD44 and its binding partner ezrin during breast cancer cell migration. We examined raft affiliation of CD44 and ezrin in a panel of breast cancer cell lines of varying invasiveness under basal migratory conditions or after induction of CD44-dependent migration using its extracellular matrix ligand hyaluronan. Raft affiliation of CD44 was significantly reduced during migration of highly-invasive cell lines, in parallel with increased recovery of CD44 in non-raft fractions. In contrast, raft-affiliated CD44 was unaltered during migration of non-invasive breast cell lines. Ezrin was recovered only in non-raft fractions in all cell lines. To further investigate the functional control of rafts on CD44 in breast cancer cell migration, we used site-directed mutagenesis to introduce point-mutations into the CD44 palmitoylation sites which control its trafficking to lipid rafts. Results to date support a novel mechanism whereby containment of CD44 within lipid rafts reduces the migratory capabilities of breast cancer cells. As breast tumour cell migration is a precursor to metastasis, targeting the affiliation of CD44 with lipid rafts may offer a new therapeutic target to prevent or reduce cancer cell motility.

microRNA-31 modulates tumour sensitivity to radiation in oesophageal cancer

N Lynam-Lennon[1], JV Reynolds[1], L Marignol[2], OM Sheils[3], GP Pidgeon[1], SG Maher[1]
[1]Department of Surgery, Trinity College Dublin, Dublin, Ireland
[2]Department of Radiation Therapy, Trinity College Dublin, Dublin, Ireland
[3]Department of Histopathology, Trinity College Dublin, Dublin, Ireland

Oesophageal cancer has one of the worst prognoses of all cancers and its incidence is rising rapidly in Ireland. Radiotherapy is fundamental to the treatment of oesophageal cancer. However, radioresistance is a significant clinical problem, with only ~30% of patients responding to neoadjuvant chemoradiotherapy (CRT). We aimed to identify molecular mechanisms and biomarkers underlying this radioresistance, which would be of substantial clinical benefit.

Chronic exposure of OE33 oesophageal adenocarcinoma (OAC) cells to fractionated, clinically-relevant doses of 2 Gy X-ray radiation (cumulative dose, 50 Gy) resulted in a radioresistant subline, OE33R. Extensive characterisation of this model revealed that, relative to the age- and passage-matched parent control (OE33P), the radioresistant cells had a significantly enhanced DNA repair efficiency and a significantly altered miRNA profile, including a downregulation and differential induction of miR-31. Functionally, overexpression of miR-31 re-sensitised OE33R cells to radiation. miR-31 overexpression also altered the mRNA expression levels of 13 DNA repair genes, suggesting miR-31-mediated regulation of DNA repair in this model. In pre-treatment OAC patient tumour biopsies, significantly decreased miR-31 expression was associated with a poor response to neoadjuvant CRT. In parallel, significantly increased expression of the miR-31-regulated DNA repair genes, including SMUG1, MLH1, RAD51L3 and MMS19, was associated with a poor response to neoadjuvant CRT. Increased SMUG1 expression was also significantly associated with the presence of lymph node metastasis.

To our knowledge we have developed the first isogenic model of radioresistance in OAC. Characterisation of this model revealed alterations in DNA repair efficiency and miRNA expression. We also demonstrate for the first time a functional role for miR-31 in modulating the cellular response to radiation. Reduced miR-31 expression and increased expression of its target DNA repair genes in tumour tissue suggests these as potential biomarkers and a mechanism of resistance to neoadjuvant CRT in oesophageal cancer.

The Role of FKBPL and RBCK1 in Oestrogen Receptor Signalling

Donley C, McClelland K, McKeen H, Byrne C, Yakkundi Y, McCarthy HO and Robson T., School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast

Steroid hormone receptor complexes are classified as ligand activated transcription factors that can directly induce transcription of a variety of genes. We have previously demonstrated that the immunophilin-like protein, FKBPL, an Hsp90 co-chaperone protein, is involved in the regulation of glucocorticoid[1], androgen[2] and oestrogen[3] receptor complexes. More specifically for breast cancer, FKBPL has been identified as an oestrogen responsive gene that modulates ER signalling, enhances sensitivity to endocrine therapies and prognosticates for improved patient response[3].
RBCK1 was pulled out of a yeast-two-hybrid screen to identify proteins which interacted with FKBPL. RBCK1 is a RING domain protein which is highly conserved and appears to be bi-functional where it has been shown to act as a transcriptional regulator as well as having E3 ligase activity. The aim of this project was to further characterise the role of FKBPL and its binding partner, RBCK1, in oestrogen receptor (ER) signalling and transcriptional regulation.

Using co-immunoprecipitation, we demonstrate that RBCK1 interacts within an FKBPL/HPS90 complex in the MCF7 breast cancer cell line. MCF7-X8 cells that stably overexpress RBCK1 upregulated the levels of FKBPL and exhibited a similar phenotype to FKBPL overexpressing cells which includes significant perturbation of breast cancer cell growth, an increase in p21 levels and a reduction in ER and cathepsin D levels in comparison to parental cells. Furthermore, ChIP analysis revealed that ER, FKBPL and RBCK1 bind to the FKBPL promoter suggesting that FKBPL transcription can be regulated by itself in association with ER and RBCK1. Finally, we show that FKBPL and RBCK1 also bind to the promoter of several oestrogen responsive genes. In summary, RBCK1 in association with FKBPL, is a potential novel transcriptional regulator of ERα and its target genes with further implications for breast cancer growth and response to endocrine therapy.

1. McKeen HD, McAlpine K, Valentine A, Quinn DJ, McClelland K, Byrne C, O'Rourke M, Young S, Scott CJ, McCarthy HO, Hirst DG and Robson T (2008) A novel FK506-like binding protein (FKBPL) interacts with the glucocorticoid receptor and regulates steroid receptor signalling. *Endocrinology* 149(11):5724-34
2. Sunnotel O, Hiripi L, Lagan K, McDaid J, De León JM, Miyagawa Y, Crowe H, Kaluskar S, Ward M, Scullion C, Campbell A, Downes SC, Hirst DG, Barton D, Mocanu E, Tsujimura A, Cox MB, Robson T and Walsh CP. (2010) Alterations in the steroid hormone receptor co-chaperone FKBPL are associated with male infertility: a case-control study *Reproductive Biology and E*ndocrinology 8;8(1):22.
3. McKeen HD, Byrne C, Jithesh PV, Donley C, Valentine A, Yakkundi A, O'Rourke M, Swanton C, McCarthy HO, Hirst DG, Robson T. (2010) FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. *Cancer Research* 70(3):1090-1100

Dasatinib overcomes acquired temozolomide resistance in metastatic melanoma cell lines.

AJ Eustace[1], P Stafford[1], M Clynes[1], J Crown[2], N O'Donovan[1]
[1]Molecular Therapeutics for Cancer Ireland, National Institute for Cellular Biotechnology, Dublin 9
[2]Molecular Therapeutics for Cancer Ireland, St. Vincents University Hospital, Dublin 4

Background: In Ireland in 2006, 626 cases of metastatic melanoma (MM) werediagnosed and 100 deaths were reported resulting from MM. Despite poor response rates, DNA damaging agents, dacarbazine and temozolomide (TMZ), remain the only registered chemotherapeutics for MM. The aims of this study were to establish *in vitro* models of TMZ resistance and develop strategies to overcome resistance.

Methods: TMZ resistant cell lines, HT144-TMZ and Malme-TMZ, were established by repeated exposure of HT144 and Malme-3M cells to 300 μM TMZ. Using the acid phosphatase assay, we examined the sensitivity of parental/resistant cells to chemotherapy and targeted therapies. We studied the effect of TMZ selection on signalling pathways by western blotting. Finally we examined the combination of TMZ and dasatinib in the parental/TMZ resistant cells.

Results: Repeated TMZ exposure resulted in 1.45 fold TMZ resistance in HT144-TMZ and 1.68 fold TMZ resistance in Malme-TMZ. Interestingly, the TMZ resistant variants displayed increased sensitivity to dasatinib compared to parental cells. Dasatinib (300 nM) inhibited growth of HT144 cells by 23 ± 10 % whilst inhibiting growth of the TMZ resistant cells by 70 ± 10 %. Similar results were observed for Malme TMZ (70 ± 6 % inhibition) versus Malme-3M cells (14 ± 10 % inhibition). The TMZ resistant cells showed elevated levels of phosphorylated SRC, with no significant changes in SRC expression. Combining dasatinib with TMZ in Malme-TMZ inhibited significantly higher levels of proliferation compared to TMZ alone.

Conclusions: Acquired TMZ resistance increased sensitivity to dasatinib which was associated with increased phosphorylation of SRC, a key target of dasatinib. Inhibition of TMZ resistant cells with dasatinib alone or in combination with TMZ increased proliferation inhibition compared to that observed in parental cells. Treatment with SRC inhibitors such as dasatinib may be beneficial in MM that develops resistance to TMZ.

HRG-1, a Heme-Binding Protein with Enhanced Expression in Colorectaladenocarcinoma and Essential for Endosomal Trafficking of Nutrient Transporters

J O'KEEFFE, V AYLLON, F FOGARTY, K O'CALLAGHAN, R O'CONNOR
[1]biochemistry Dept, University College Cork, Cork

The Insulin-like Growth Factor I signalling pathway has a fundamental function in controlling the metabolism of normal cells, and in facilitating the enhanced metabolic demands of cancer cells. There is now considerable evidence to support the idea that targeting essential components of metabolic pathways may be a useful therapeutic strategy. With this in mind we have isolated a panel of novel IGF-I-regulated genes whose expression is enhanced in transformed cells compared with non-transformed cells. Amongst these was a gene encoding an endosomal protein that associated with the Vacuolar (H+)-ATPase, which is essential for controlling the pH of cellular endosomes and thereby essential signalling responses. Interestingly this protein was independently identified in C.elegans as a member of the heme-regulated gene (HRG) family of proteins and called HRG-1. We hypothesized that enhanced expression of HRG-1 in cancer cells may have an important role in cancer cell metabolism.
HRG-1 is very highly expressed in colorectal adenocarcinoma and renal carcinoma with moderate expression in ovarian carcinoma cell lines compared with a wide range of other tumour and normal tissues, which have low expression. Importantly, HRG-1 is detected at the cell surface using the specific mAb, which is also taken into cells. Suppression of HRG-1 with siRNA results in decreased uptake of heme and decreased V-ATPase activity. This is associated with a perturbation of the trafficking of plasma membrane receptors, including the Transferrin Receptor, IGF-I Receptor and the glucose transporter GLUT-1, that results in degradation of these proteins in the lysosome. Conversely, cells over-expressing HRG-1 show less lysosomal activity and increased protein levels of these receptors. This data shows that HRG-1 is essential to controlling the degradation of receptors crucial for cell survival and metabolism. We conclude that HRG-1 has an essential metabolic function as a heme binding protein in regulating endosomal trafficking of cell survival receptors and nutrient transporters. HRG-1 may also be a useful biomarker and/or target in colorectal and renal carcinoma where it is over-expressed.

1. ‘The heme binding protein HRG-1 is induced by IGF-I and associates with the Vacuolar (H+) -ATPase to control endosomal pH and receptor trafficking.' O'Callaghan, K.M., et al., J Biol Chem. 2009 Oct 29 2. ‘Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins.' Rajagopal, A., et al., Nature 2008 Jun 19;453(7198):1127-31

Insulin-like Growth Factor I Receptor Tyrosine Kinase activation is regulated by GSK-3β mediated phosphorylation of serine 1248 in the C terminal tail.

GM Kelly[1], DA Buckley[1], PA Kiely[2], DR Adams[3], R O'Connor[1]
[1]Department of Biochemistry, University College Cork, Cork, Ireland
[2]Department of Life Sciences, University of Limerick, Limerick, Ireland
[3]Department of Chemistry, Heriot-Watt University, Edinburgh, Scotland

The insulin-like growth factor I receptor (IGF-IR) plays important roles in cell transformation, migration and in protection from apoptosis. The C terminal tail of the IGF-IR has been proposed as a regulatory domain and by virtue of divergence in its sequence with that of the insulin receptor (IR) it has also been proposed to potentially mediate differences in IGF-IR and IR signaling. Certain C terminal residues have been shown to be essential for the anti-apoptotic and transforming activities of the receptor. On the contrary, truncation of the receptor by deletion of the C terminus enhances IGF-IR suppression of apoptosis; and a myristoylated C terminal peptide (MyCF) induces apoptosis in cell models and inhibits tumorigenic growth in nude mice. Here we show that mutation of S1248 (S1248A) in the C terminal SFYYS motif enhances IGF-IR autophosphorylation, in vitro kinase activity, basal Akt phosphorylation, and cell proliferation. S1248 phosphorylation occurs preferentially in the absence of IGF-I and is mediated by GSK-3β in a mechanism that involves a priming phosphorylation on S1252. Crystallographic structures of the IGF-IR kinase domain reveal that the SFYYS motif adopts a conformation tightly packed against the C-lobe of the kinase domain when S1248 is in the unphosphorylated state that favours kinase activity. S1248A mutation is predicted to lock the motif in this position. In contrast, phosphorylation of S1248 will drive profound structural transition of the sequence, critically affecting connection of the C terminus as well as exposing potential protein docking sites therein. The SFYYS motif therefore controls the organisation of the C terminus relative to the kinase domain and its phosphorylation by GSK-3β negatively regulates IGF-IR kinase activity and signaling output.

Eukaryotic Elongation Factor 2 In Acquired Lapatinib Resistance In HER2 Positive Breast Cancer Cells

M McDermott[1], BC Browne[1], J Crown[1, 2], N O'Brien[3], DJ Slamon[3], M Henry[1], P Meleady[1], M Clynes[1], P Dowling[1], N O'Donovan[1]
[1]National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9
[2]Dept of Medical Oncology, St. Vincents University Hospital, Elm Park, Dublin 4,
[3]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, USA

Approximately 25% of breast cancers overexpress HER2. Although HER2 targeted therapies, trastuzumab and more recently lapatinib, have improved prognosis for HER2 positive breast cancer patients not all tumours respond to these therapies. We developed an in vitro model of acquired lapatinib resistance by conditioning SKBR3 cells with 250 nM lapatinib for 6 months, after which time the resulting SKBR3-L cells exhibited significantly reduced growth inhibition (27.7 ± 0.1 %) when treated with lapatinib (1 μM) compared to parental SKBR3 (90.7 ± 0.2 %) ($p<0.01$). Using 2D-DIGE analysis of phospho-enriched samples, eukaryotic elongation factor 2 (eEF2) was identified as a phosphoprotein which was significantly lower in the SKBR3-L cells compared to the parental SKBR3 cells. eEF2 plays an essential role in protein synthesis by facilitating the binding of mRNA to the ribosome. Phosphorylation of eEF2 by eEF2 kinase (eEF2k) prevents it from binding to ribosomes and thus inhibits its activity. eEF2k is regulated by p70S6 kinase, which is activated by signalling through mTOR.
We examined mTOR signalling, and its effect on p-eEF2 in SKBR3-par and SKBR3-L cell lines. Although the level of p-eEF2 was lower in SKBR3-L compared to SKBR3-par cells, the levels of p-eEF2k and p-p70S6K were unchanged. There were no changes in the levels of the total proteins. Treatment of SKBR3-L cells with lapatinib, rapamycin or a combination of the two failed to increase the levels of p-eEF2. This suggests that the alterations in p-eEF2, in the lapatinib resistant SKBR3-L cells are mediated by an alternative pathway, other than the mTOR pathway.

DIRECT CONTROL OF CDC7 AND DBF4 BY MIR-29

LR Barkley[1], B Howley[1], C Santocanale[1]
[1]National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Galway, Ireland

Cdc7 is a serine/threonine kinase essential for initiation of DNA replication and maintenance of cell viability during replication stress (1, 2). The binding of a regulatory subunit, Dbf4, to Cdc7 is essential for kinase activity (3,4). The molecular mechanism(s) underlying Cdc7 kinase regulation during the cell cycle and replication stress is largely unknown. Inappropriate regulation of Cdc7 kinase could lead to deregulated cell growth and genetic instability, two hallmarks of cancer. In this study, we are testing the hypothesis that microRNAs (miRNAs) are key regulators of Cdc7 kinase.
In silico analysis identifies miR-29 binding sites in the 3' UTRs of both Cdc7 and Dbf4. We have found that Cdc7 and Dbf4 RNA levels are up-regulated in both lung and breast cancer and this negatively correlates with the expression of miR-29. In addition, miR-29 and CDC7 elicit an inverse relationship in response to the tobacco smoke carcinogen, benzo[a]pyrene (B[a]P). Cdc7 levels increase in response to B[a]P whereas miR-29 levels go down. Interestingly, miR-29 levels negatively correlates with Cdc7 and Dbf4 RNA levels during the cell cycle. miR-29 levels are high during G1 when Cdc7 and Dbf4 RNA levels are low. Therefore these data strongly suggest miR-29 is an important regulator of Cdc7 and Dbf4. Indeed, we find that miR-29 directly targets the 3'UTR of both Cdc7 and Dbf4 via specific miR-29 binding sites. Overexpression of miR-29 substantially decreases Cdc7 and Dbf4 protein levels in normal lung and lung cancer cells resulting in a decrease in cell growth/viability. This data indicates the use of a miRNA-based strategy to inhibit Cdc7 as a potential chemotherapeutic. We have shown that Cdc7 deficiency sensitises cells to chemotherapeutic-induced death (5). Potentially, miRNA therapies that inhibit Cdc7 kinase activity could also sensitise cancer cells to anticancer drug-induced death.
This work was supported by funding from the Irish Cancer Society.

1. Bousset, K. and Diffley, J. F. (1998) Genes Dev. 12, 480-490 2. Tenca, P., Brotherton, D., Montagnoli, A., Rainoldi, S., Albanese, C., and Santocanale, C. (2007) J Biol Chem. 282, 208-215 3. Jiang, W., McDonald, D., Hope, T. J., and Hunter, T. (1999) EMBO J 18, 5703-5713 4. Kumagai, H., Sato, N., Yamada, M., Mahony, D., Seghezzi, W., Lees, E., Arai, K., and Masai, H. (1999) Mol Cell Biol 19, 5083-5095 5. Montagnoli, A., Tenca, P., Sola, F., Carpani, D., Brotherton, D., Albanese, C., and Santocanale, C. (2004) Cancer Res. 64, 7110-7116

GENERATION OF DECOY RECEPTOR INSENSITIVE TRAIL VARIANTS

L O' Leary[1], E Szegezdi[1], A van der Sloot[2], T Sessler[1], R Cool[3,4], C Reis[3], L Serrano[2], W Quax[3], A Samali[1]
[1]Department of Biochemistry, National University of Ireland, Galway, Galway, Ireland
[2]Centro de Regulación Genómica, CRG-EMBL Systems Biology Unit, Barcelona, Spain
[3]Department of Pharmaceutical Biology, University of Groningen, Groningen, The Netherlands
[4]Triskel Therapeutics BV, Antonius Deusinglaan 1, Groningen, The Netherlands

Tumour necrosis factor-related apoptosis inducing ligand (TRAIL) is a cytokine expressed by immune cells which selectively eradicates a wide variety of cancer cells leaving untransformed cells unharmed. TRAIL binds to 4 membrane bound receptors. However, only two of these receptors, death receptor 4 (DR4) and DR5, can launch the apoptotic machinery into action leading to cancer cell death. The other 2 receptors, decoy receptor-1 (DcR1) and DcR2 can also bind TRAIL but are unable to induce apoptosis. These decoy receptors can attenuate TRAIL-induced apoptosis by competing with DR4 and DR5 for TRAIL binding or directly binding and inhibiting the death inducing receptors. While TRAIL shows tremendous promise as an anti-cancer agent, it is this promiscuous receptor binding capacity that limits the utilisation of TRAIL as a therapeutic. Our objective is to engineer a TRAIL variant (TRAIL-45) with the ability to bind to DR4 and DR5 with high affinity, but not to the DcRs. Using the computational design software, FoldX, such TRAIL variants were designed and the recombinant proteins generated. Surface-plasmon-resonance uncovered several variants which displayed reduced binding to DcRs. The death inducing capabilities of these mutants were investigated with 5 variants (TRAIL45-1, TRAIL45-a, TRAIL45-b, TRAIL45-c and TRAIL 45-d) identified as having the desired characteristics. We found the variants were capable of activating both DR4 and DR5 and proved to be potent inducers of cell death with a 2-4 fold reduction in cell viability compared to wild type TRAIL. The efficacy of variants TRAIL45-1, TRAIL45-a, TRAIL45-b and TRAIL45-c could not be increased by neutralising the DcRs indicating that they can successfully evade these receptors. We currently aim to further characterise the higher anti-cancer activity of these TRAIL variants in vitro and examine the importance of the decoy receptors in in vivo systems.

STABILISATION OF MAD2 EXPRESSION THROUGH INHIBITION OF MIR-433; A NOVEL MECHANISM FOR RESTORING CHEMO-RESPONSIVENESS IN OVARIAN TUMOURSF FURLONG, S PHLEAN, A MCGOLDRICK, A MAGUIRE, E GALLAGHER, P MCGETTIGAN, P FITZPATRICK, S O'TOOLE, O SHEILS, L MCEVOY, C MARTIN, A O'GRADY, E KAY, J O'LEARY, A MCCANN
[1]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4
[2]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4
[3]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4
[4]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4
[5]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4,
[6]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4
[7]UCD School of Public Health and Population Science, University College Dublin, Dublin 4
[8]Departments of Histopathology & Obstetrics & Gynaecology, Trinity College Dublin, Dublin 2
[9]Departments of Histopathology & Obstetrics & Gynaecology, Trinity College Dublin, Dublin 2
[10]Departments of Histopathology & Obstetrics & Gynaecology, Trinity College Dublin, Dublin 2
[11]Departments of Histopathology & Obstetrics & Gynaecology, Trinity College Dublin, Dublin 2
[12]Department of Pathology, Royal College of Surgeons in Ireland (RCSI), Beaumont Hospital, Dublin
[13]Department of Pathology, Royal College of Surgeons in Ireland (RCSI), Beaumont Hospital, Dublin
[14]Departments of Histopathology & Obstetrics & Gynaecology, Trinity College Dublin, Dublin 2
[15]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4
[20]

Epithelial ovarian carcinoma (EOC) is characterised by late diagnosis and recurrences, both of which contribute to the high morbidity and mortality of this cancer. Unfortunately, EOC has an innate susceptibility to become chemo-resistant. Specifically, up to 30% of patients may not respond to current standard chemotherapy (paclitaxel and platinum in combination) and of those who have an initial response, some patients relapse within a few months. Therefore, in order to improve patient outcome it is crucial to establish what factors influence a patients' individualised response to chemotherapy. We analysed MAD2 protein expression in a patient cohort of 35 ovarian tumours and a panel of 5 ovarian cancer cell lines. We have demonstrated that low nuclear MAD2 expression intensity was significantly associated with chemo-resistant ovarian tumours (p=0.0136). Moreover, in vitro studies of the 5 ovarian cancer cell lines revealed that reduced MAD2 expression was associated with paclitaxel resistance. In silico analysis identified a putative miR-433 binding domain in the MAD2 3'UTR and expression profiling of miR-433 in the ovarian cancer cell lines showed that low MAD2 protein expression was associated with high miR-433 levels. In vitro over-expression of miR-433 attenuated MAD2 protein expression with a concomitant increase in cellular resistance to paclitaxel. Over-expression of a morpholino oligonucleotide that blocks miR-433 binding to the MAD2 3'UTR stabilised MAD2 protein expression and protects from miR-433 induced degradation. Furthermore, miR-433 expression analysis in 35 ovarian tumour samples revealed that high miR-433 expression was associated with advanced stage presentations (p=0.0236). In conclusion, ovarian tumours that display low nuclear MAD2 intensity are chemo-resistant and stabilising MAD2 expression by antagonising miR-433 activity is a potential mechanism for restoring chemo-responsiveness in these tumours.

Oral Poster Board Listing

(Please note that posters are listed alphabetically by category and then authors are listed alphabetically within these categories)

Surname	Category	Abstract Title	Poster Board No.
Cichon	Cell Biology	THE CELL NON-AUTONOMOUS EFFECTS OF PTEN	**1**
Oram	Cell Biology	BRCA1 is a master regulator of breast cell fate through co-ordinated activation of a Notch signalling program.	**2**
Walsh	Cell Biology	The role of the developmental protein HOXC11 in breast cancer tumour adaptability	**3**
Dasgupta	Cell Signalling	ADAM-17 is a key regulator of ErbB survival response in colorectal cancer.	**4**
Dunne	Cell Signalling	ONCOGENIC KRAS CONTROLS CHEMOTHERAPY-INDUCED GROWTH FACTOR SHEDDING BY INDUCING MEK AND ERK-MEDIATED ACTIVATION OF ADAM17 IN COLORECTAL CANCER	**5**
Cotter	Translational	Poly (ADP-Ribose) Polymerase 1: A New Target for the Treatment of Triple Negative Breast Cancer	**6**
Lysaght	Translational	The human omentum is a rich source of activated inflammatory CD4+ and CD8+ T cells, with a potential pathological role in oesophageal adenocarcinoma development.	**7**
Prencipe	Translational	Mechanisms of docetaxel resistance in prostate cancer: is NFkB involved?	**8**

Oral Poster Abstracts

Poster No. 1

THE CELL NON-AUTONOMOUS EFFECTS OF PTEN

AC CICHON[1], A PICKARD[1], D PATEL[1], D MCCANCE[1]
[1]Center for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland

The tumour the microenvironment is gaining importance in regulating tumourigenesis, invasion and metastasis. The Phosphoinositide-3-Kinase pathway is a pathway well known for its involvement in tumourigenesis in a cell autonomous manner, with many of its constituents being altered in a broad range of cancers. AKT and its negative regulator PTEN have a wide variety of cellular functions and loss of PTEN in stromal fibroblasts results in an increased tumour occurrence in ErbB2 over-expressing tumours. Using an organotypic raft model that structurally resembles human skin we have shown that loss of the PTEN tumour suppressor in stromal fibroblasts increases invasive potential of Human Papilloma Virus Type 16 E6 and E7 expressing keratinocytes. Furthermore we show that PTEN is essential for normal development of the epithelium. We are currently investigating whether altered expression of Keratinocyte Growth factor (KGF) and Interleukin-1 alpha and beta are essential for a cross-talk between the epithelium and stroma and whether they regulate differentiation and invasion. Loss of PTEN leads to an upregulation of AKT activity. There are three isoforms of AKT expressed in human fibroblasts, AKT1, AKT2 and AKT3. By depleting stromal fibroblasts of each individual AKT isoform we have been able to establish that AKT2 plays an important role in mediating the invasiveness of pre-malignant keratinocytes. Hence, the PTEN/AKT pathway presents itself as one mechanism by which the tumour microenvironment is able to regulate tumourigenesis and tumour invasion.

Poster No. 2

BRCA1 is a master regulator of breast cell fate through co-ordinated activation of a Notch signalling program.

C Nic An tSaoir, NE Buckley, LC Oram, NT Crawford, PB Mullan
[1]Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, UK

BRCA1 is known to act as an important regulator of transcription in breast tissue to enforce growth control and differentiation processes. Here we show that BRCA1 activates the Notch pathway in breast cells through its ability to simultaneously upregulate the transcription of Notch ligands and receptors. We show that Notch activation is dependent on both BRCA1 mutation status and expression levels. Mechanistically we demonstrate that BRCA1 is localised to a conserved intronic enhancer region within the Jagged-1 (JAG1) gene, with BRCA1 recruitment dependent on ΔNp63 proteins. Knockdown of Notch1 and JAG1 phenocopied BRCA1 knockdown in luminal MCF-7 cells resulting in loss of ERα and luminal marker expression. We could artificially activate Notch using a mimetic peptide (DSL) resulting in activation of an ERα promoter reporter in a BRCA1-dependent manner. Conversely, inhibition of Notch signalling using a γ-secretase inhibitor reversed this process. BRCA1/ΔNp63-mediated induction of JAG1 may be important for the regulation of breast stem/precursor cells since knockdown of all three proteins resulted in increased mammosphere growth and ALDH1 activity. Knockdown of Notch pathway components also resulted in decreased expression of stem cell (Nanog) and luminal progenitor (CD61, Muc1) markers but upregulation of the proliferation-associated marker FoxM1 and markers of basal-like breast cancer (CXCL1, p-cadherin). Additionally, γ-secretase inhibition also resulted in the upregulation of basal-like breast cancer markers. Together these findings suggest that BRCA1 regulation of Notch signalling is a key event in the mammary cell fate determination and the normal differentiation process in breast tissue.

Poster No. 3

THE ROLE OF THE DEVELOPMENTAL PROTEIN HOXC11 IN BREAST CANCER TUMOUR ADAPTABILITY

C Walsh, M McIlroy, D McCartan, C Byrne, ADK Hill, LS Young
[1]Endocrine Oncology Research Group, Royal College of Surgeons in Ireland, Dublin 2, Ireland

Hox genes are 'master regulators' of normal development and control processes such as proliferation, apoptosis, migration and invasion2. HOXC11 is actively transcribed during embryogenesis where it directs cell fate and identity. In breast cancer, HOXC11 functionally interacts with the steroid receptor coactivator SRC-11. SRC-1 is increasingly active in steroid independent tumour growth and has been associated with resistance to treatment, clinically aggressive tumours and promotion of metastasis3. Nuclear HOXC11 was found to strongly predict poor disease-free survival in breast cancer patients (n = 560; hazard ratios: 5.79; $P < 0.0001$)1. As HOXC11 is a developmental protein, it is hypothesised that in breast cancer; HOXC11 acts to reprogramme the identity of the tumour by increasing its cellular plasticity, in order to evade the effects of therapy. HOXC11 acts to downregulate expression of characteristic luminal cell markers as well as induce retro-differentiation of epithelial tumour cells thus redirecting the tumour cells into an increasingly primitive and aggressive phenotype. Cells which are resistant to both tamoxifen (LY2) and aromatase inhibitors (Let.R) have enhanced HOXC11 expression in comparison to their endocrine sensitive parent cells (MCF-7s). CD24 and CD44 are common cell surface markers used to characterise breast cancer tumourigenicity. In this context, CD24 is strongly associated with luminal-like tumours. In the endocrine resistant LY2 and Let.R cells there is a clear decrease in CD24 expression (23.2% and 29.6% respectively) compared to the endocrine sensitive cells (82.2%). Other well known luminal markers; including E-cadherin, are also downregulated in the LY2 cells. Furthermore, LY2 cells are unable to form functional acini in 3D Matrigel cultures however siRNA against SRC-1 reverses this retro-differentiation process. HOXC11 activity in conjunction with SRC-1 and another homeobox transcription factor; Cux1 have the potential to redefine the transcriptional landscape of a tumour and identify those tumours which are more likely to acquire resistance to endocrine therapy.Text

1. McIlroy M, McCartan D, Early S, Ó Gaora P, Pennington S, Hill ADK, Young LS. Interaction of Developmental Transcription Factor HOXC11 with Steroid Receptor Coactivator SRC-1 Mediates Resistance to Endocrine Therapy in Breast Cancer. Cancer Research (2010) 2. Lewis, MT. Homeobox genes in mammary gland development and neoplasia. Breast Cancer Research (2000) 3. Redmond AM, Bane FT, Stafford AT, et al. Coassociation 1 of ER and p160 proteins predicts resistance to endocrine treatment; SRC-1 is an independent predictor of breast cancer recurrence. Clin Cancer Res (2009)

Poster No. 4

ADAM-17 is a key regulator of ErbB survival response in colorectal cancer.

S Dasgupta[1], S Van Schaeybroeck[1], J Kyula[1], DB Longley[1], PG Johnston[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, N. Ireland

Background

We previously reported that colorectal cancer (CRC) cells respond to chemotherapy by activating an epidermal growth factor receptor (EGFR)-mediated survival response and are thereby sensitized to EGFR inhibitors. In order to elucidate potential biomarkers for response to chemotherapy treatment and potential novel targets, we decided to identify the molecules which mediate chemotherapy-induced EGFR activity in CRC cells.

Method

Apoptosis was measured by Flow Cytometry or PARP cleavage. EGFR phosphorylation and ADAM-17 expression were assessed by Western blotting. ADAM-17 activity was measured using a fluorometric (MCA) assay kit while TGF-a levels were analysed by ELISA. Inhibition of ADAM-17 and TGF-α expression was achieved by siRNA and measured by real time PCR. HCT116-p53wt xenografts mouse models were established from female BALB/c SCID mice by sc. inoculation using Matrigel. Once tumours reached approximately 200mm3, they received no treatment or 75mg/kg 5-FU by IP injection for 24 hours. Mice were sacrificed and whole blood was collected from the axillary vessels and tumours were harvested.

Results

We found that chemotherapy-induced EGFR phosphorylation was mediated by metalloproteases. Further studies using gene silencing and over-expressing techniques indicated that ADAM-17 was the principal ADAM involved in chemotherapy-induced EGFR activation. Moreover, we found that ADAM-17 induced TGF-a, amphiregulin (EGFR ligands) and heregulin (HER3 ligand) shedding following chemotherapy treatment. Inhibition of ADAM-17 sensitized CRC cells to chemotherapy-mediated apoptosis while exogenous EGFR and HER3 ligands protected CRC cells from chemotherapy-mediated apoptosis. This suggests that inhibiting the ADAM17/EGFR ligand autocrine loop may enhance the response of CRC tumours to chemotherapy. In vivo studies also demonstrated enhanced ADAM-17 activity, TGF-a shedding and EGFR phosphorylation following treatment of CRC xenografts with chemotherapy.

Conclusion

Taken together, we have identified a novel mechanism of acute resistance to chemotherapy treatment which is regulated by ADAM-17-mediated shedding of EGFR like ligands, such that targeting ADAM-17 may enhance the response of CRC tumours to chemotherapy. Moreover, serum levels of TGF-α and other EGF-like ligands post-chemotherapy may be a biomarker for the application of such therapeutic approaches.

Poster No. 5

ONCOGENIC KRAS CONTROLS CHEMOTHERAPY-INDUCED GROWTH FACTOR SHEDDING BY INDUCING MEK AND ERK-MEDIATED ACTIVATION OF ADAM17 IN COLORECTAL CANCER

P Dunne[1], S Van Schaeybroeck[1], J Kyula[1], A Fenton [1], C Fenning [1], D Longley[1], P Johnston[1]
[1]CCRCB, Queen's University Belfast, Belfast, Northern Ireland

Background

Oncogenic mutations in Kras occur in 40%-45% of patients with advanced colorectal cancer (CRC). We have previously shown that chemotherapy acutely activates ADAM17 which results in growth factor shedding, growth factor receptor activation and drug resistance in CRC tumours. In this study, we examined the role of mutant Kras in regulating growth factor shedding and ADAM17 activity using isogenic Kras mutant (MT) HCT116 cells and Kras wild type (WT) HKH-2 CRC cells.

Method

In vitro and in vivo KrasMT and KrasWT models of CRC were assessed. ADAM-17 activity was measured using a fluorometric assay. Ligand shedding was assessed by ELISA. Apoptosis was assessed by flow cytometry and Western blotting.

Results

Compared to the KrasWT cell line, significantly higher levels of TGF-α and VEGF were observed in the culture medium of KrasMT HCT116 cell line, both basally and following chemotherapy treatment, and this correlated with increased pErk1/2 levels and ADAM17 activity. Small molecule and/or siRNA-mediated Kras, MEK1/2 or Erk1/2 inhibition resulted in inhibition of TGF-α shedding and ADAM17 activity following chemotherapy treatment. Importantly, we found that these effects were not drug or cell line specific. In addition, MEK1/2 inhibition in KrasMT xenografts resulted in a significant decrease in ADAM17 activity and serum ligand shedding in vivo and this correlated with dramatically attenuated tumour growth. Moreover, we found that MEK1/2 inhibition led to a potent increase in apoptosis both alone and when combined with chemotherapy in KrasMT cells. Importantly, we found that sensitivity to MEK1/2 inhibition was dependent on ADAM17 activity levels in vitro and in vivo.

Conclusion

Taken together, our findings indicate that oncogenic Kras regulates ADAM17 activity and growth factor ligand shedding via a MEK1/2-dependent pathway. Furthermore, KrasMT CRC tumours are vulnerable to MEK1/2 inhibitors, at least in part due to their dependency on ADAM17 activity.

Poster No. 6

Poly (ADP-Ribose) Polymerase 1: A New Target for the Treatment of Triple Negative Breast Cancer

M Cotter[1,2], A Pierce[1,2], PM McGowan[1,2], S Madden[3], L Flanagan[1,4], C Quinn[1,4], D Evoy[1,2], J Crown[1,2], EW McDermott[1,2], MJ Duffy[1,2]
[1]Breast Cancer Research Group, St. Vincent's University Hospital, Dublin 4
[2]UCD School of Medicine & Medical Science, University College Dublin, Dublin 4
[3]National Institute of Cellular Biotechnology, Dublin City University, Dublin 9
[4]Department of Histopathology, St. Vincent's University Hospital, Dublin 4

Introduction:
Triple negative breast cancers (TNBC) are so named as they lack expression of ER, PR and do not exhibit overexpression of HER2. Consequently, targeted therapy is currently unavailable for this subgroup of patients. The aims of this study were to investigate the expression of poly (ADP-ribose) polymerase 1 (PARP1) in TNBC and test the effect of the PARP inhibitor, Olaparib, on the growth of TNBC cell lines in vitro.
Methods:
PARP1 protein was measured in 90 breast cancers using Western blotting. Pooled data from 22 published gene expression databases (N=approx 3000 patients) was used to relate PARP1 mRNA to patient outcome. The effect of Olaparib on the growth of TNBC lines was investigated using the acid phosphatase assay and Calcusyn software.
Results:
Using Western blotting, 4 forms of PARP1 were found in extracts of breast cancer, migrating at 116 kDa (main form), 89 kDa (apoptotic form), 50 kDa (necrotic form) and 27 kDa (apoptotic form). The parental form of PARP, ie, the 116 kDa form was detected in almost all TN (12/12) and non-TN (73/78, 94%) samples. Using Kaplan-Meier analysis, high PARP1 expression was significantly related to adverse patient outcome ($p=0.002$). IC50 values for the inhibitory effect of Olaparib on the growth of the TNBC cell lines, MDA231 and BT20, were 1.12 μM and 3.9 μM respectively.
Conclusions:
Our results suggest that PARP1 is expressed in the majority of TN and non-TN breast cancer and that high levels are associated with adverse outcome. Furthermore, our in vitro findings showing strong growth inhibition by Olaparib in 2 TNBC cell lines suggest that this PARP1 inhibitor may be a new treatment for patients with TNBC.

Acknowledgement: The authors thank SFI (SRC award, 08/SRC/B1410 to MTCI) for funding this work.

Poster No. 7

The human omentum is a rich source of activated inflammatory $CD4^+$ and $CD8^+$ T cells, with a potential pathological role in oesophageal adenocarcinoma development.

J Lysaght[1], E Allott[1], C Donohoe[1], J Howard[2], N Ravi[1], GP Pidgeon[1], JV Reynolds[1]
[1]Surgery, Trinity College Dublin, Dublin, Ireland
[2]Diagnostic Imaging, St. James's Hospital, Dublin, Ireland

Introduction: Visceral adipose tissue is believed to be fuelling a state of chronic inflammation in obese patients, establishing an environment favourable for tumour growth. However, the exact immunological mechanisms remain unclear. Recent animal studies have pointed to an important role for visceral adipose tissue T cells in driving inflammation. This novel study aimed to phenotypically characterise $CD4^+$ and $CD8^+$ T cells in the omentum of oesophageal adenocarcinoma patients (OAC).

Methods: Peripheral blood and omentum were taken from 35 OAC patients and 12 non-cancer patients. T cell activation status and cytokine production were assessed by flow cytometry. IFN-γ concentrations were determined by ELISA.

Results: The omentum is a rich source of T cells which display an activated inflammatory phenotype, expressing significantly higher ($p<0.001$) CD69, CD107a, CD45RO and IFN-γ than T cells in the blood. Omental $CD8^+$ T cells expressed significantly higher ($p<0.001$) IFN-γ than $CD4^+$ T cells, suggesting that $CD8^+$ T cells are an important source of this inflammatory cytokine within visceral fat. Interestingly, significantly higher ($p<0.05$) IFN-γ expression was observed by both omental $CD8^+$ T cells and conditioned media from omental fat fragments, isolated from cancer patients compared with non-cancer controls. The highest circulating levels of IFN-γ were detected in viscerally obese cancer patients compared with non-obese or non-cancer patients.

Conclusions: This novel study demonstrates that omental T cells may be key players in fuelling adipose tissue inflammation, potentially mediated by IFN-γ, and may provide an immunotherapeutic target to attenuate chronic inflammation associated with visceral obesity.

Poster No. 8

Mechanisms of docetaxel resistance in prostate cancer: is NFkB involved?

M Prencipe[1], A O'Neill[1], C Dowling[1], Y Fan[1], R O'Connor[2], A Devery[2], L Mulrane[3], W Gallagher[3], J Fitzpatrick[1], W Watson[1]

[1]UCD School of Medicine and Medical Science, UCD Conway Institute of Biomolecular and Biomedical Research and Mater Misericordiae University Hospital, University College Dublin, Dublin, Ireland

[2]National Institute for Cellular Biotechnology, Dublin City University, Dublin

[3]UCD School of Biomolecular and Biomedical Science, UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin

Docetaxel (Taxotere®) is the most effective chemotherapeutic agent in metastatic castrate resistant prostate cancer. However, one of the major obstacles in the treatment of this stage of disease is the development of docetaxel resistance. Factors including slow growth rates, multi-drug resistance mechanisms and expression of anti-apoptotic proteins, all contribute to such resistance. Investigations into the molecular basis of docetaxel resistance are therefore critical for the identification of new targets for therapeutic manipulation.

We generated two docetaxel-resistant PC-3 prostate cancer sublines. Resistance to docetaxel treatment was confirmed by assessing cell viability (MTT assay), apoptosis (propidium iodide staining and flow cytometry) and cell proliferation (colony forming assay) following docetaxel treatment.

Resistance was assessed at the level of classical drug pump expression, cellular senescence and the expression of apoptotic proteins. We demonstrated no increased expression of p-glycoprotein or cellular senescence as mechanisms of resistance. Low density array showed alterations in the expression of both pro and anti-apoptotic genes and identified NFkB as a candidate transcription factor involved in this resistance. The baseline NFkB activity, assessed by luciferase assay, was decreased in the resistant cell lines compared to the parental PC-3 cells. This result was confirmed by an ELISA based assay which showed a significant decrease in the activity of p52 ($p<0.01$) and RelB ($p<0.05$) NFkB subunits, in the resistant cells compared to the parental PC-3. We are currently assessing NFkB activity following docetaxel treatment.

Due to the heterogeneous nature of prostate cancer and the complex changes associated with the development of drug resistance, manipulating the upstream transcription factors such as NFkB may represent a better therapeutic targeting approach to reverse docetaxel resistance.

Display Poster Board Listing

(Please note that posters are listed alphabetically by category and then authors are listed alphabetically within these categories)

Surname	Category	Abstract Title	Poster Board No
Bowe	Cell Biology	PDLIM2 regulates transcription to sustain an EMT phenotype in DU145 and MDA-MB-231 cells	**9**
Byrne	Cell Biology	Impaired protein processing and secretion by bile acids as a mechanism for oesophageal cancer progression	**10**
Casey	Cell Biology	OBESITY INTERRUPTS THE REGULATION OF SPINDLE ASSEMBLY CHECKPOINTS AND ANAPHASE BRIDGE FORMATION IN BARRETT'S OESOPHAGUS	**11**
Creevey	Cell Biology	ELUCIDATING THE ROLE OF MIR-15A IN THE CHEMO-SENSITIVITY OF NEUROBLASTOMA	**12**
CROWLEY	Cell Biology	AUTOPHAGY INDUCTION BY BCR-ABL EXPRESSING CELLS CAN LIMIT EFFICACY OF IMATINIB TREATMENT;	**13**
ELMASHAD	Cell Biology	Evaluation of markers of apoptosis and autophagy as predictive markers of chemotherapeutic response in oesophageal cancer patients.	**14**
FOLEY	Cell Biology	CELL LINE-SPECIFIC HETERODIMER FORMATION BETWEEN NON-MUSCLE ACTININS	**15**
Hannon	Cell Biology	REGULATION OF TRIB2 GENE EXPRESSION IN NORMAL AND AML CELLS	**16**
Kerr	Cell Biology	HDAC6 inhibition down regulates c-FLIP and induces caspase 8-dependent apoptosis in colorectal cancer cells	**17**
LAIOS	Cell Biology	PRE-TREATMENT OF OVARIAN CANCER CELL LINES WITH AN MMP-9 INHIBITOR PRIOR TO CISPLATIN TREATMENT INCREASES CYTOTOXICITY AS DETERMINED BY HIGH CONTENT SCREENING CELL-BASED ASSAYS	**18**
Lamers	Cell Biology	MICROARRAY BASED EXPRESSION PROFILING OF BRCA1-MUTATED BREAST TUMOURS USING A BREAST SPECIFIC PLATFORM TO IDENTIFY A PROFILE OF BRCA1-DEFICIENCY	**19**
Maher	Cell Biology	The contribution of the RNA-binding protein RNPC1 to p21-mediated cell cycle accumulation and radiation sensitivity in oesophageal cancer	**20**
McEvoy	Cell Biology	HYPOXIA POTENTIATES THE INVERSE RELATIONSHIP BETWEEN RESISTANCE TO CISPLATIN AND PACLITAXEL IN OVARIAN CANCER CELL LINES	**21**
Nyhan	Cell Biology	The BH3 mimetic HA14-1 can improve the cytotoxicity of 5-Fluorouracil by induction of Type II cell death in oesophageal cancer cells	**22**
O'Connor	Cell Biology	TRIB2 LEADS TO CHEMORESISTANCE AND DIFFERENTIATION DEFECTS	**23**
Orr	Cell Biology	THE IDENTIFICATION OF PATHWAYS INVOLVED IN THE PROLIFERATION AND CHEMOTHERAPY RESPONSES OF TRIPLE NEGATIVE BREAST CANCER	**24**
Owens	Cell Biology	Cardiac Glycosides as Potential Anti-Cancer Agents	**25**
Riley	Cell Biology	HDAC INHIBITORS INDUCE FLIP DOWN-REGULATION AND CASPASE-8 DEPENDENT APOPTOSIS IN NON-SMALL CELL LUNG CARCINOMA.	**26**

Display Poster Board Listing

(Please note that posters are listed alphabetically by category and then authors are listed alphabetically within these categories)

Surname	Category	Abstract Title	Poster Board No
STASIK	Cell Biology	HDAC INHIBITOR VORINOSTAT INDUCES c-FLIP DOWNREGULATION AND CASPASE 8-DEPENDENT APOPTOSIS IN MALIGNANT PLEURAL MESOTHELIOMA CELLS	**27**
Stevenson	Cell Biology	IDENTIFICATION OF NOVEL DETERMINANTS OF RESISTANCE TO 5-FU AND OXALIPLATIN IN COLORECTAL CANCER USING A SYSTEMS BIOLOGY APPROACH	**28**
Wilson	Cell Biology	Mitochondrial VDAC2 is transcriptionally regulated by p63 and confers shorter survival in non-small cell lung cancer	**29**
Corcoran	Cell Signalling	FLT3-DRIVEN REDOX SIGNALLING IN ACUTE MYELOID LEUKAEMIA	**30**
Dolan	Cell Signalling	SOCS1: A CRITICAL MEDIATOR OF 5-FU/IRINOTECAN RESPONSE IN COLORECTAL CANCER CELLS	**31**
Eustace	Cell Signalling	2-D DIGE analysis of phospho-enriched fractions from dasatinib treated melanoma cell lines	**32**
Fatehullah	Cell Signalling	aPKC ZETA CONTROLS CELL POLARIZATION AND CELLULAR MORPHOGENESIS	**33**
Fatehullah	Cell Signalling	A PHOSPHATASE-INDEPENDENT ROLE FOR PTEN IN CDC42-MEDIATED EPITHELIAL POLARIZATION AND MORPHOGENESIS	**34**
Holohan	Cell Signalling	RACK1, A NOVEL BINDING PARTNER FOR CFLIP	**35**
Howley	Cell Signalling	A MICRORNA-206/CYCLIN D1 AXIS IS CONSERVED IN MUSCLE DIFFERENTIATION AND BREAST CANCER PROLIFERATION	**36**
Hughes	Cell Signalling	Using novel bio-marker S100β to determine responsiveness of endocrine resistant breast cancer cells to dasatinib	**37**
McBryan	Cell Signalling	SRC-1 IN AROMATASE INHIBITOR RESISTANT BREAST CANCER	**38**
O'Flanagan	Cell Signalling	INVESTIGATION OF THE FUNCTIONAL RELATIONSHIP BETWEEN PTEN-INDUCED KINASE 1 AND PI3-KINASE/AKT SIGNALLING IN CANCER DEVELOPMENT	**39**
van Dijk	Cell Signalling	MECHANISM OF TRAIL RESISTANCE IN NON-TRANSFORMED CELLS	**40**
Vareslija	Cell Signalling	INSIGHTS INTO AROMATASE INHIBITOR RESISTANCE: NEW MECHANISM OF OESTROGEN SIGNALLING	**41**
Woznicki	Cell Signalling	REGULATION OF A PUTATIVE TUMOUR SUPPRESSOR GENE BCL-G BY PRO-INFLAMMATORY CYTOKINES IN INTESTINAL EPITHELIAL CELLS	**42**
Majkut	Drug Design Development	RxDL MOTIFS OF THE c-FLIP DED DOMAINS ARE CRITICAL FOR ITS ANTI-APOPTOTIC FUNCTION.	**43**
TRAN	Drug Design Development	TOWARDS THE ELUCIDATION OF THE AUTOPHAGIC ACTIVITY OF OBATOCLAX	**44**
Baban	Experimental Therapeutics	NON-PATHOGENIC BACTERIA AS CELL THERAPY VECTORS FOR CANCER TREATMENT	**45**

Display Poster Board Listing

(Please note that posters are listed alphabetically by category and then authors are listed alphabetically within these categories)

Surname	Category	Abstract Title	Poster Board No
Collins	Experimental Therapeutics	AAV2-mediated in vivo immune gene therapy of solid tumours	**46**
Fay	Experimental Therapeutics	CD-33 targeted nanoparticles for specific drug delivery to acute myeloid leukaemia (AML) cells	**47**
Halpin-McCormick	Experimental Therapeutics	UNCOUPLING OF DEATH RECEPTOR INDUCED NF-KB ACTIVATION FROM APOPTOSIS	**48**
Jarzabek	Experimental Therapeutics	Pre-clinical development of pro-apoptotic cancer therapeutics for the treatment of glioma: A combined in vitro/ in vivo molecular imaging approach.	**49**
Monaghan	Experimental Therapeutics	KILLING DRUG RESISTANT TRIPLE NEGATIVE BREAST CANCER CELLS WITH THE ANTI-FUNGAL CICLOPIROX OLAMINE	**50**
OOMMEN	Experimental Therapeutics	BENZYLIDENE LACTAM COMPOUND, KNK437, AN INHIBITOR OF STRESS RESPONSE IS A NOVEL RADIOSENSITIZER	**51**
Carson	Genetics and Genomics	COMPARATIVE ANALYSIS OF PYROSEQUENCING AND SEQUENCING FOR THE DETECTION OF KRAS AND BRAF MUTATIONS IN COLORECTAL CANCER	**52**
LANGHE	Genetics and Genomics	MICRORNA PROFILING IN SERUM/ BLOOD OF OVARIAN CANCER	**53**
Favre	Metastasis	The IGF-I inducible Pyrimidine Nucleotide Carrier (PNC1) regulates mitochondrial biogenesis and the invasive phenotype of cancer cells.	**54**
Ahmed	Other	Regulation of PML by NF-κB	**55**
O'Grady	Other	THE PREDICTION OF RADIOTHERAPY RESPONSE "IN VITRO" FOR CERVICAL CANCER PATIENTS.	**56**
Rani	Other	INVESTIGATING THE POTENTIAL OF CIRCULATING EXOSOMES AND THEIR MIRNA CONTENTS AS MINIMALLY-INVASIVE BIOMARKERS FOR TRIPLE NEGATIVE BREAST CANCER	**57**
White	Other	HUMAN PAPILLOMAVIRUS DNA/mRNA DETECTION AND SMOKING AS A RISK FACTORIN PREDICTING PROGRESSION OF LOW GRADE CERVICAL ABNORMALITIES TO HIGH GRADE CERVICAL DISEASE	**58**
Bourke	Translational	Increasing tumour cell permeability to chemotherapeutic agents results in enhanced responses in intractable cutaneous lesions from breast cancer	**59**
Casey	Translational	Pro-Inflammatory and Pro Metastatic Pathways are activated by Obesity in Barrett's Metaplasia	**60**
Doyle	Translational	INSULIN-LIKE GROWTH FACTOR-1 IS ELEVATED IN VISCERALLY OBESE ESOPHAGEAL ADENOCARCINOMA PATIENTS AND EXPRESSION OF ITS RECEPTOR CORRELATES WITH TUMOUR INVASIVENESS	**61**
Fan	Translational	A proteomics and bioinformatics approach towards biomarker panel identification in prostate cancer: from discovery to targeted validation	**62**
Forde	Translational	AN ENHANCED NON VIRAL (EEV) GIVING IMPROVED RESPONSES IN IMMUNOGENE MELANOMA TREATMENT OVER STANDARD VECTORS	**63**

Display Poster Board Listing

(Please note that posters are listed alphabetically by category and then authors are listed alphabetically within these categories)

Surname	Category	Abstract Title	Poster Board No
Forde	Translational	TARGETING THE INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR (IGF-1R) SIGNALLING PATHWAY IN NON-SMALL CELL LUNG CANCER (NSCLC)	**64**
NOONAN	Translational	HIGH LEVELS OF IMMATURE BLOOD VESSELS IN COLORECTAL TUMOURS CORRELATES WITH LONGER SURVIVAL FOLLOWING BEVACIZUMAB TREATMENT	**65**
O'FARRELL	Translational	MITOCHONDRIAL INSTABILITY IS INCREASED DURING DISEASE PROGRESSION IN BARRETTS OESOPHAGUS	**66**
MARTIN	Translational	PROTEOMIC ANALYSIS OF DIFFERENTIALLY EXPRESSED PROTEINS IN PATIENTS WITH METASTATIC COLORECTAL CANCER RESPONDING TO BEVACIZUMAB	**67**
Mulgrew	Translational	Overexpression of FKBPL confers resistance to chemotherapy in breast cancer cells	**68**
Paul	Translational	CISPLATIN INDUCES MITOCHONDRIA-INDEPENDENT APOPTOSIS BY A CASPASE 8 DEPENDENT PATHWAY FUNCTIONALLY DISTINCT FROM TRAIL	**69**
Paul	Translational	PARP INHIBITION MEDIATES BAX/BAK INDEPENDENT SYNTHETIC LETHALITY OF BRCA1-DEFICIENT NON-SMALL CELL LUNG CANCER	**70**
Prencipe	Translational	Identification of transcription factors associated with castrate resistant prostate cancer cells	**71**
Turkington	Translational	THE ROLE OF FGFR4 SIGNALLING IN SENSITISING COLORECTAL CANCER CELLS TO OXALIPLATIN/5-FLUOROURACIL CHEMOTHERAPY	**72**
O'Callaghan	Tumour Immunology	EP1 receptor specific antagonist (ONO-8713) reduces colon tumor cell growth in vivo.	**73**

DISPLAY POSTER ABSTRACTS

Poster No. 9

PDLIM2 regulates transcription to sustain an EMT phenotype in DU145 and MDA-MB-231 cells

R Bowe[1], OT Cox[1], V Ayllon[1], N Healy[1], R Deevi[1], M Huiglsoot[1], R O'Connor[1]
[1]Department of Biochemistry, UCC, Cork, Ireland
[2]BioSciences Institute, UCC, Cork, Ireland
[3]BioSciences Institute, UCC, Cork, Ireland
[4]BioSciences Institute, UCC, Cork, Ireland
[5]BioSciences Institute, UCC, Cork, Ireland
[6]BioSciences Institute, UCC, Cork, Ireland

Over-expression or constitutive activation of the Insulin-like Growth Factor-1 Receptor (IGF-1R) facilitates cellular transformation and tumorigenesis, but the molecular mechanisms that mediate these effects are unknown. PDLIM2 was identified as a potential mediator of IGF-1 signalling in cancer because it is over-expressed in IGF-IR transformed cells. The pdlim2 gene is a PDZ-LIM domain protein located at the actin cytoskeleton and in the nucleus where it promotes degradation of NF-κB and STAT transcription factors. PDLIM2 expression is suppressed in cancer cell lines but is highly expressed in cancer cell lines derived from metastatic tumours.
The objective of this study was to investigate the function of PDLIM2 in the highly invasive prostate and breast cancer cell lines DU145 and MDA-MB-231 by stably suppressing PDLIM2 expression in these cells. These cells displayed increased cell-cell contacts, exhibited loss of directional cell migration and displayed defects in cytoskeleton reorganization following wounding. RNA profiling of these cells revealed global changes in gene expression patterns that control these processes suggesting that PDLIM2 stimulates cell motility by regulating a large number of genes that determine cancer phenotype.
Importantly we found that suppression of PDLIM2 increased the proteasomal degradation of Snail which correlates with up-regulated E-Cadherin expression at cell-cell contacts. All of this is consistent with a reversed Epithelial to Mesenchymal Transition (EMT) phenotype referred to as Mesenchymal to Epithelial reverting Transition (MErT) where cells lose their migratory and invasive properties but re-express E-Cadherin at metastatic sites necessary for secondary tumour formation.
Overall, these data indicate that PDLIM2 regulates migration and the reversible differentiation of DU145 cells by controlling Snail and a transcription programme necessary for EMT and invasion of distant tissue. We also propose that PDLIM2 may prevent colonization and secondary tumour formation by sustaining Snail protein stability, which would prevent E-Cadherin re-expression and subsequently MErT.

Poster No. 10

Impaired protein processing and secretion by bile acids as a mechanism for oesophageal cancer progression

AM Byrne, R Sharma, D Kelleher, A Long, [1]Clinical Medicine, Trinity College Dublin

Introduction

Deoxycholic acid (DCA) has been implicated in gastrointestinal cancer progression. We had previously shown DCA caused complete breakdown of the Golgi structure in a colon cancer model [1]. Patients with gastro-oesophageal-reflux disease are predisposed to developing an inflammatory condition called Barrett's oesophagus (BO). Patients with BO are 30 times more likely to develop oesophageal cancer. DCA is present in reflux therefore we hypothesised it may play a role in oesophageal cancer progression. The Golgi is responsible for protein processing, glycosylation and secretion. Altered protein glycosylation and secretion are hallmarks of cancer and contribute to inflammation, cell to cell communication and metastasis. In this study we investigated the effects of DCA on Golgi structure and downstream consequences with respect to protein secretion, glycosylation and wound healing in normal oesophageal (HET1A) and Barrett's metaplastic (QH/ CP-A) cell lines as a potential mechanism of oeosophageal cancer progression.

Methods

The effects of DCA (0-300 μM) on Golgi structure was assessed by immunofluorescence and quantified by High Content Analysis. Effects of DCA on protein secretion were assessed using a Gaussia luciferase assay. To determine the effect of DCA on the key glycosylation processes, a panel of FITC-conjugated plant lectins were used. WGA and CONA were used to identify N-linked glycoproteins, PNA for O-linked glycoproteins and UEA-1 for fucosylated glycoproteins. Expression and localisation were detected by flow cytometry and fluorescence microscopy. Functional effects of impaired glycosylation on cell migration were investigated by the scratch wound assay.

Results

Exposure of HET1A or QH cells to DCA induced Golgi fragmentation and consequently decreased protein secretion compared to untreated controls ($p<0.05$). DCA disrupted intracellular localisation and decreased cell surface expression of N-linked glycoproteins. Impaired glycosylation resulted in complete inhibition of wound closure by 300μM DCA.

Conclusion

DCA disrupted Golgi structure, decreased protein secretion and impaired N-linked glycosylation in normal and Barrett's epithelial cells. This is a novel phenomenon uniquely associated with bile acids. Disruption of glycoprotein synthesis impaired the wound healing process which has important implications for Barrett's metaplasia and oesophageal cancer progression.

[1]Byrne, A.M, Foran, E., Sharma, R., Mahon ,C., O Sullivan, J., O Donoghue, D., Davies, A., Kelleher, D., Long, A. Bile acids modulate the Golgi membrane fission process via a protein kinase cη and protein kinase D-dependent pathway. Carcinogenesis. 2010 Apr;31(4):737-44.

Poster No. 11

OBESITY INTERRUPTS THE REGULATION OF SPINDLE ASSEMBLY CHECKPOINTS AND ANAPHASE BRIDGE FORMATION IN BARRETT'S OESOPHAGUS

R Casey[1], JV Reynolds[1], N Ravi[1], J O'Sullivan[1], GP Pidgeon[1]
[1]Dept. of Surgery, Trinity College, Dublin, Ireland

Introduction

Barrett's oesophagus is a known clinical precursor of oesophageal adenocarcinoma. Excess adiposity is understood to be a causal factor in Barrett's oesophagus and oesophageal adenocarcinoma, though the exact molecular mechanisms remain elusive. The aim of this study is to determine whether obesity can drive genomic instability events 1) anaphase bridge formation, crucial in bridge breakage fusion events and 2) regulatory proteins involved in spindle assemly checkpoint control.

Materials and Methods

Adipose conditioned media (ACM) was prepared from omental adipose tissue from viscerally obese and non obese male oesophageal adenocarcinoma patients. The HET-1a, QH and GO cell lines which represent the different stages of the metaplastic-dysplastic-adenocarcinoma progression associated with Barrett's oesophagus were grown in the presence of this ACM over a 24hr period and variations in gene expression of the spindle assembly checkpoint genes BUB1B, CDC20, CENPE, ESPL1 and, MAD2L2 were analysed using q-PCR. In parallel, anaphase bridges were scored as lagging chromosomes not fully resolved following anaphase.

Results

On average, there was a 34% increase in anaphase bridges across all 3 cell lines exposed to adipose conditioned media from obese versus non obese patients. The greatest changes in BUB1B, CDC20, CENPE, ESPL1 expression was detected in the GO cells (dysplastic Barrett's cells) exposed to ACM from obese patients (all p values <0.002). In addition, CDC20 and MAD2L2 significantly correlated with anaphase bridging ($p=0.016$, $r=0.7$ and $p=0.04$, $r=0.6$ respectively).

Conclusions

This study demonstrates, for the first time, that genomic instability events are activated due to factors released from visceral adipose tissue and these early genomic instability events could potentially contribute to the progression of Barrett's oesophagus to oesophageal cancer.

Poster No. 12

ELUCIDATING THE ROLE OF MIR-15A IN THE CHEMO-SENSITIVITY OF NEUROBLASTOMA

L Creevey[1,2], J Ryan[1,2], I Bray[1], K Bryan[1], M Meehan[1,2], J Fay[1,2], RL Stallings[1,2]
[1]Cancer Genetics, Royal College of Surgeons, Dublin, Ireland
[2]National Childrens Research Centre, Our Ladys Childrens Hospital Crumlin, Dublin, Ireland

Neuroblastoma is a paediatric cancer that originates from precursor cells of the sympathetic nervous system. MiRNAs regulate gene expression at a post-transcriptional level. The dysregulation of miRNAs is known to contribute to the pathogenesis of neuroblastoma and our lab has identified a miRNA expression profile that is predictive of clinical outcome. Expression of miR-15a was one of the miRNAs identified as being highly significant ($p>0.01$) (Bray et al., 2009). MiR-15a has been well documented as a tumour suppressor in various cancers, most notably chronic lymphocytic leukemia. The aim of this study was to elucidate a functional role of miR-15a in the pathogenesis of neuroblastoma and the effect on response to the front line chemotherapeutic drug cisplatin. Knock-down of endogenous miR-15a in neuroblastoma cell lines results in a significant decrease in cell viability, while ectopic over-expression of this miRNA had no detectable affect. *WEE1*, a key regulator of the G_2/M phase of the cell cycle, is a computationally predicted target of miR-15a. Using western blots and luciferase reporter assays, we confirmed that *WEE1* is a direct target of miR-15a. Given the role that WEE1 plays in the regulation of the G_2/M transition in the cell cycle, we are now testing the hypotheses that 1) the up-regulation of WEE1 following knock-down of endogenous miR-15a accounts for the observed decrease in cell viability; and 2) that miR-15a targeting of WEE1 can modulate resistance to the chemotherapeutic drug cisplatin.

BRAY, I., BRYAN, K., PRENTER, S., BUCKLEY, P. G., FOLEY, N. H., MURPHY, D. M., ALCOCK, L., MESTDAGH, P., VANDESOMPELE, J., SPELEMAN, F., LONDON, W. B., MCGRADY, P. W., HIGGINS, D. G., O'MEARA, A., O'SULLIVAN, M. & STALLINGS, R. L. (2009) Widespread Dysregulation of MiRNAs by MYCN Amplification and Chromosomal Imbalances in Neuroblastoma: Association of miRNA Expression with Survival. PLoS ONE, 4, e7850.

Poster No.13

AUTOPHAGY INDUCTION BY BCR-ABL EXPRESSING CELLS CAN LIMIT EFFICACY OF IMATINIB TREATMENT; STRATEGIES TO IMPROVE TREATMENT REGIMES.

L.C. Crowley[1], B.M. Elzinga[1], G.C. O'Sullivan[1], M.R. Cahill[2], S.L. McKenna[1]
[1]Cork Cancer Research Centre, University College Cork, Cork, Ireland
[2]Cork University Hospital, Wilton, Cork, Ireland

Chronic Myeloid Leukaemia (CML) has benefited hugely from the introduction of the tailored therapeutic, Imatinib (Tyrosine Kinase Inhibitor/TKI). However CML requires lifelong treatment and is rarely cured due to the persistence of cells that can re-generate the disease. Evidence suggests that the failure to eliminate these cells may be related to inadequate engagement of apoptotic cell death and the induction of autophagy.
We have examined the response of human (K562) and mouse (32D Bcr-Abl) Bcr-Abl expressing cells to Imatinib. Imatinib treatment induces both apoptosis and autophagy in all populations. Following withdrawal of drug, autophagy predominates. These populations rapidly recover. siRNA knockdown of key autophagy regulators, Beclin1 and ATG7, caused a significant reduction in the recovery of K562 cells following Imatinib treatment, indicating that autophagy is important for this recovery[1].
Removal of these persistent CML cells through combining autophagy inhibiting agents with selective TKIs, could potentially improve treatment of CML. While there are currently no specific inhibitors of autophagy regulators, there are indirect inhibitors that target cellular components important for the processing of autophagic vesicles. These include; disruption of Golgi regulation and initiation of autophagy (Brefeldin A), disruption of the microtubule network and trafficking of autophagic vesicles (Vincristine) and inhibition of lysosomal fusion (Chloroquine).
We have found that all of these agents reduce the recovery of Imatinib treated cells and disrupt expression of the autophagy markers, LC3 and Beclin1. Therefore these agents have an impressive potential to reduce autophagic recovery of CML cells and thus improve treatment regimes involving TKIs.

1; Crowley, L. C., Elzinga, B. M., O'Sullivan, G. C. and McKenna, S. L. (2011), Autophagy induction by Bcr-Abl-expressing cells facilitates their recovery from a targeted or nontargeted treatment. American Journal of Hematology, 86: 38–47. doi: 10.1002/ajh.21914

Poster No. 14

Evaluation of markers of apoptosis and autophagy as predictive markers of chemotherapeutic response in oesophageal cancer patients.Title

DR ELMASHAD[1], DR O DONOVAN[1], PROF KAY[2], PROF O SULLIVAN[1], DR MC KENNA[1, 1]CCRC, CCRC, CORK, IRELAND
[2]PATHOLOGY, RCSI, DUBLIN, IRELAND

Oesophageal carcinoma is an aggressive disease with a poor outcome despite improvements in early detection, surgical procedure and (neo-) adjuvant therapy. Only 20% of patients with advanced oesophageal cancer benefit from neo-adjuvant therapy. Defining predictive determinants of drug sensitivity on an individual tumour and patient basis would be of major value, particularly in the metastatic and adjuvant setting.

Our studies with oesophageal cancer cell lines indicate that if cells can undergo apoptosis then chemotherapy is likely to be successful. However, if primarily autophagy is induced then cells are more resistant and can recovery following treatment withdrawal. Autophagy is a major intracellular pathway for the recycling of proteins and entire organelles following exposure to stress. In this study we aimed to evaluate markers of autophagy and apoptosis in oesophageal cancer tissue, pre and post (neo)-adjuvant therapy, to establish whether induction of autophagy or apoptosis is predictive of clinical response.
35 pre-treatment and 25 post-treatment paraffin-embedded oesophageal tumours were collected. The corresponding paraffin embedded normal oesophageal mucosa, dysplastic lesion and lymph node metastasis (if available) were also collected. The surgical oesophageal cancers were constructed into tissue microarrays (TMAs).

The following clinical and histopathological data were collected from medical charts and pathology reports: age, gender, (neo)-adjuvant therapy, follow up, tumour stage and differentiation, vascular and neural invasion.

Expression of LC3, P62 (markers of autophagy) and Active Caspase 3 (CA3; marker of apoptosis) were investigated using immuno-histochemistry. In patients who received neo-adjuvant chemotherapy, 83% showed high LC3 expression and almost all had less than 5 year survival. While a smaller percentage of patients (16.7%) had prominent CA3 expression and this expression correlated with a significant response to neo-adjuvant chemotherapy and better prognosis; more than 5 year survival.

Therefore LC3 and CA3 could be used as markers for predicting a patient’s response to neo-adjuvant chemotherapy.

Poster No. 15

CELL LINE-SPECIFIC HETERODIMER FORMATION BETWEEN NON-MUSCLE ACTININS

K Foley, P Young
[1]Biochemistry, University College Cork, Cork, Ireland

α-Actinin is a cytoskeleton protein that crosslinks actin filaments. The non-muscle actinin isoforms 1 and 4 show 87% amino acid identity but are reported to play different roles in cancer cell migration, adhesion, proliferation and cell survival (1, 2). While the non-muscle actinins are implicated in cancer, the mechanisms and extent to which each isoform contributes to disease development and progression are undetermined. Each non-muscle isoform has been reported to exert distinct effects depending on the cancer type studied (3-6). Actinins are dimeric and while heterodimer formation has been reported between the muscle actinins this has not been systematically examined for the non-muscle isoforms (7). To begin to understand the differential functions of the non-muscle actinins we first sought to examine whether they are present in cells purely as homodimers or whether heterodimers also exist. Using a Yeast-Two-Hybrid screen we show that the rod domains of actinin-1 and -4 do indeed have the potential to form heterodimers. To examine whether heterodimers exist *in vivo* we used native gel electrophoresis of cell lysates from a panel of cancer cell lines. Surprisingly, we find that heterodimers do exist in some cell lines but not in others. This finding indicates that actinin-1 and -4 cannot be thought of as strictly distinct entities but rather that in some cells they form heterodimers which might have unique functional characteristics. It also raises the question of how heterodimer formation is regulated and why it occurs in some cells but not in others.

1) Quick Q. and Skalli O. (2010) α-Actinin 1 and α-Actinin 4: Contrasting roles in survival, motility, and RhoA signalling of astrocytoma cells. Exp. Cell. Res. doi: 10.1016/j.yexcr.2010.02.011 2) Sen S., Dong M., Kumar S. (2009) Isoform-specific contributions of alpha-actinin to glioma cell mechanobiology. PLoS ONE 4(2):e8427 3) Craig D.H., Haimovich B., Basson M.D. (2007) α-Actinin-1 phosphorylation modulates pressure-induced colon cancer cell adhesion through regulation of focal adhesion kinase-Src interaction. Am. J. Physiol. Cell. Physiol. 293:1862-1874 4) Glück U. and Ben-Ze'ev (1994) Modulation of α-actinin levels affects cell motility and confers tumorigenicity on 3T3 cells. J. Cell. Sci. 107:1773-1782 5) Honda K., Yamada T., Hayashida Y. (2005) Actinin-4 increases cell motility and promotes lymph node metastasis of colorectal cancer. Gastroenterology 128:51-62 6) Nikolopoulos S.N., Spengler B.A., Kisselbach K., Evans A.E., Biedler J.L., Ross R.A. (2000) The human non-muscle α-actinin protein encoded by the ACNT4 gene suppresses tumorigenicity of human neuroblastoma cells. Oncogene 19:380-386 7) Chan Y., Tong H., Beggs A.H., Kunkel L.M. (1998) Human skeletal muscle-specific alpha-actinin-2 and -3 isoforms form homodimers and heterodimers in vitro and in vivo. Biochem. Biophys. Res. Commun. 248:134-139

Poster No. 16

REGULATION OF TRIB2 GENE EXPRESSION IN NORMAL AND AML CELLS

M. Hannon[1], F. Al-Shahrour[2, 3], B. L. Ebert[3, 4], K. Keeshan[1]
[1]Haematopoiesis and Oncogenes Lab, Department of Biochemistry, University College Cork, Cork, Ireland
[2]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA
[3], Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts, USA
[4], Harvard Medical School, Boston, Massachusetts, USA

The mammalian Trib family consists of Trib1, Trib2 and Trib3. Elevated levels of Trib2 have been linked with murine AML and associates with a specific subset of human AML with Notch1 mutations and dysregulated C/EBPα. However the regulation of Trib expression is poorly understood; understanding this regulation is important both physiologically and pathologically.

Promoter analyses indicated that several possible E2F1 binding sites exist within the Trib2 promoter region. A complex regulatory relationship exists between C/EBPα and E2F1, and proliferation arrest mediated by C/EBPα involves repression of E2F1-target genes. Luciferase assays using a Trib2 promoter construct demonstrated that E2F1 is a positive regulator of Trib2 and that this positive induction was dependent on E2F1's ability to bind directly to DNA. Further analyses revealed that this activation is repressed by wild type C/EBPα. Importantly, C/EBPαp30, an N-terminal truncated dominant negative C/EBPα isoform commonly associated with AML co-operated with E2F1 to induce Trib2 promoter activity. Bioinformatic analysis of human AML datasets revealed a negative correlation between Trib2 and C/EBPα expression, and a positive correlation between Trib2 and Notch1 expression. These results correspond with our previous findings that Trib2 is a Notch1 target gene and functions to degrade and inhibit wild type C/EBPα. Further analyses demonstrated a positive correlation between Trib2 and E2F1 expression in the human AML dataset supporting our findings.

Together these data indicate a positive regulatory pathway for Trib2 expression mediated by E2F1 and C/EBPαp30, and a negative regulatory pathway involving E2F1 and wild type C/EBPα.

Poster No.17

HDAC6 inhibition down regulates c-FLIP and induces caspase 8-dependent apoptosis in colorectal cancer cells

E Kerr[1], K McLaughlin[1], C Holohan[1], J Riley[1], P Johnston[1], D Longley[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, N. Ireland

Background:
c-FLIP is an anti-apoptotic protein that blocks death receptor-mediated apoptosis by inhibiting caspase 8 activation. c-FLIP is frequently over-expressed in colorectal cells, and it has previously been shown that high c-FLIP expression limits the effectiveness of chemotherapy treatment. We have previously shown that reduction of c-FLIP expression using siRNA synergistically enhances the response of CRC cells to chemotherapy or TRAIL. Pan-HDAC inhibitors (HDACi) such as SAHA have been shown to be effective anti-cancer agents. Our aims were to assess the effect of HDACi on c-FLIP expression in colorectal cancer.

Results:
Treatment with HDAC inhibitors was found to induce apoptosis in a panel of colorectal cancer cell lines in a manner that was dependent on caspase 8, TRAIL receptor DR5 and down-regulation of c-FLIP protein expression. Similar results were obtained using in vivo models of colorectal cancer. Further analysis revealed that HDAC inhibitors down-regulate c-FLIP expression post-tranlationally by triggering its ubiquitination and proteasomal degradation. Using more selective HDAC inhibitors, we identified HDAC6 as the key HDAC involved in regulating c-FLIP expression as specific HDAC6 inhibition recapitulated the effects of SAHA on c-FLIP expression and apoptosis induction in colorectal cancer cell models.

Conclusions:
HDAC inhibitors induce apoptosis in colorectal cancer cells by down-regulating c-FLIP and thereby activating DR4/5-dependent, caspase 8-mediated apoptosis. Thus, HDAC inhibitors act as effective post-translational suppressors of c-FLIP expression. Moreover, we have identified HDAC6 as the key HDAC involved in regulating c-FLIP expression in colorectal cancer cells. Use of more specific HDAC inhibitors may significantly reduce the cytotoxic side-effects caused by pan-HDAC inhibitors such as SAHA.

Poster No.18

PRE-TREATMENT OF OVARIAN CANCER CELL LINES WITH AN MMP-9 INHIBITOR PRIOR TO CISPLATIN TREATMENT INCREASES CYTOTOXICITY AS DETERMINED BY HIGH CONTENT SCREENING CELL-BASED ASSAYS

A Laios[1], M Bashir[2], R Flavin[3], L Kelly[1], L McEvoy[3], C Martin[3], O Sheils[3], M Gallagher[3], N Gleeson[1], T D'Arcy[1], E McGuinness[1], B Sheppard[1], J O'Leary[3], S O'Toole[1]
[1]Obstetrics and Gynaecology, Trinity College Dublin, Dublin, Ireland
[2]Clinical Medicine, Trinity College Dublin, Dublin, Ireland
[3]Histopathology, Trinity College Dublin, Dublin, Ireland

Background: Platinum compounds, in the form of cis-and carboplatin, constitute the most active and standard chemotherapy treatment for nearly all women diagnosed with ovarian cancer. Despite its efficacy, platinum resistance is a major cause of treatment failure. We previously described an integrative transcriptomic and miRNA model for recurrence in ovarian cancer and identified MMP-9 as a potential marker of recurrence/chemoresistance. We hypothesized that incorporating a chemical inhibitor of MMP-9 (MMP-9i) in the treatment of ovarian cancer, the chemoresistant phenotype could be overcome.
Design: A2780cis (cisplatin-resistant) and A2780 (cisplatin-sensitive) human ovarian carcinoma cell lines were used. MMP-9/MMP-2 inhibitor, (2R)-2-[(4-Biphenylsulfonyl)amino]-3 phenylpropionic Acid (C21H19NO4S) was used. The cytotoxic effects of MMP-9i and MMP-9i+cisplatin were determined using the multiparameter cytotoxicity 1 Kit (Thermo Fisher). A pre-incubation for 3 hours with MMP-9i prior to treatment with cisplatin was also assessed and cytotoxicity was observed at 3 and 6 hours. Image acquisition analysis was performed using automated microscopy and image analysis (high content analysis). The system allows for live cell imaging and observation of the changes in the following cellular properties: (1) nuclear morphology/size (nuclear condensation and nuclear size), (2) cell membrane permeability, (3) lysosomal mass/pH and (4) cell density (cell count, number of cells per field).Relative fluorescent intensity was used to quantify changes. Statistical analysis was performed by t-test or two-way ANOVA using the PRISM software
Result: Co-incubation of cisplatin and MMP-9i resulted in significantly greater cytotoxicity as compared to cisplatin or MMP-9i treatment alone. In addition preincubating resistant cells with MMP-9i for 3h and then treating with cisplatin further enhanced this cytotoxic effect.
Conclusion: We propose that MMP-9i may be utilised in the treatment of recurrent/chemoresistant ovarian cancers that over express the gene but its role in vivo remains to be evaluated.

Poster No.19

MICROARRAY BASED EXPRESSION PROFILING OF BRCA1-MUTATED BREAST TUMOURS USING A BREAST SPECIFIC PLATFORM TO IDENTIFY A PROFILE OF BRCA1-DEFICIENCY

E Lamers[1], DJ Cochrane[1], GW Irvine[1], KI Savage[1], JJ Gorski[1], FA McDyer[2], JM Mulligan[2], PB Mullan[1], FJ Couch[3], RD Kennedy[2], DP Harkin[1,2], JE Quinn[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University , Belfast, Northern Ireland
[2], Almac Diagnostics Ltd, Craigavon, Northern Ireland
[3], Mayo Clinic College of Medicine, Rochester, USA

Background
The BRCA1 tumour suppressor gene is mutated in a significant proportion of hereditary breast cancer cases. In addition, downregulation of BRCA1 mRNA and protein expression is also reported in approximately one third of sporadic breast cancers. BRCA1 is strongly implicated in the maintenance of genomic stability by its involvement in multiple cellular pathways including DNA damage signalling, DNA repair, cell cycle regulation, apoptosis chromatin remodelling and transcriptional regulation. Gene expression profiling has identified at least five different breast cancer subtypes with BRCA1 mutated breast tumours clustering with basal-like/ triple negative breast cancers. The aim of this study is to identify the key genes and pathways that drive proliferation of BRCA1-deficient breast cancer.
Methods
Extensive gene expression profiling and data analysis was performed on a cohort of 70 FFPE (formalin fixed paraffin embedded) derived BRCA1 mutated breast tumours and matched sporadic controls using the Almac Diagnostics Breast DSA research tool. Functional analysis was performed using Oncomine, DAVID and Metacore. Validation of gene targets was performed by qRT-PCR and western blotting. High throughput siRNA screens were performed using the Qiagen siRNA Flexiplate.
Results
A list of differentially expressed transcripts has been derived from the comparison of 35 BRCA1 mutant breast tumours and 35 matched sporadic controls. Functional analysis of this gene list has identified the main pathways and processes that are deregulated by these transcripts. BRCA1-deficiency was associated with deregulation of pathways involved in: (1) immune response, (2) metastasis and invasion, (3) cell cycle regulation, (4) cell adhesion, (5) apoptosis and survival. Validation of the key genes underlying this BRCA1-deficient breast cancer profile has been performed. High throughout siRNA screening in several BRCA1-deficient breast cancer cell lines has identified a panel of overexpressed transcripts that when inhibited have a negative impact on cellular proliferation.
Conclusions
This approach has identified a novel panel of tumour derived transcripts implicated in the proliferation of BRCA1 mutated breast cancer. These transcripts could potentially be used to identify both hereditary and sporadic breast cancer patients with BRCA1-deficiency. The ability to perform gene expression profiling from FFPE derived breast tissue could also have significant clinical application.

Poster No.20

The contribution of the RNA-binding protein RNPC1 to p21-mediated cell cycle accumulation and radiation sensitivity in oesophageal cancer

GJ Hotte[1], **SG Maher[1]**, JV Reynolds[1]
[1]Surgery, Trinity College Dublin, Dublin, Ireland

Background:
Recently, we identified that the RNPC1 gene was upregulated in the tumours of oesophageal cancer patients who had a poor response to neoadjuvant CRT. RNPC1 encodes an RNA-binding protein that is required for maintaining the stability of p21, which regulates cell cycle transition from G1 to S phase. Overexpression of the RNPC1 protein has been shown to induce cell cycle arrest through G1 phase accumulation. As cells are most resistant to radiation in the G1 phase, we hypothesize that the observed upregulation of RNPC1 contributes to resistance to radiation therapy through a p-21-mediated mechanism.

Methods & Materials:
OE33 adenocarcinoma cells were transiently transfected with a plasmid (pCMV6-AC-GFP) encoding a C-terminal GFP-tagged RNPC1 and overexpression (relative to an empty vector control (expressing GFP only)) was confirmed by qPCR. p21 transcript levels and RNPC1/p21 association were assessed by qPCR and protein/RNA co-immunoprecipitation, respectively. Cell cycle phase distribution was measured by propidium iodide staining and flow cytometry. Radiosensitivity was assessed by clonogenic assay.

Results: In a pre-treatment setting, RNPC1 is overexpressed in the tumours of oesophageal cancer patients who are subsequently unresponsive to neoadjuvant CRT. In OE33 cells, overexpression of RNPC1-GFP mRNA resulted in increased levels of the p21 transcript levels, G0/G1 cell cycle accumulation and enhanced resistance to radiation.

Conclusions: Our patient and in vitro data suggest that RNPC1 contributes to resistance to radiotherapy, which likely occurs through a p21-mediated G0/G1 arrest mechanism. Therefore, RNPC1 may represent a potential therapeutic target for enhancing tumour sensitivity to ionising radiation.

Poster No. 21

HYPOXIA POTENTIATES THE INVERSE RELATIONSHIP BETWEEN RESISTANCE TO CISPLATIN AND PACLITAXEL IN OVARIAN CANCER CELL LINES

LM McEvoy[1], SA O'Toole[1,2], CD Spillane[1,3], CM Martin[1,3], O Sheils[1], JJ O'Leary[1,3]
[1]Histopathology, Trinity College Dublin, Dublin, Ireland
[2]Obstetrics & Gynaecology, Trinity College Dublin, Dublin, Ireland
[3]Molecular Pathology, Coombe Women and Infants University Hospital, Dublin, Ireland

Long term survival for ovarian cancer is poor (<30%). Patients undergo debulking surgery followed by platinum-taxane based chemotherapy. Most patients respond well initially, however relapse with chemoresistance is frequent. Tumour hypoxia is a factor in chemoresistance via HIF-1 (hypoxia inducible factor-1) pathway activation. Recent reports indicate an inverse relationship between resistance to cisplatin and paclitaxel across a variety of tumour cell lines. This study aimed to examine whether hypoxia could play a role in this inverse relationship in two ovarian cancer cell lines, A2780 and its cisplatin-resistant daughter line, A2780cis.
Cell lines were grown in RPMI 1640 media (10% FBS, 1mM glutamax and 1% penicillin/streptomycin) at 37°C, 5% CO_2. Hypoxic exposure was performed in an Invivo 2000 hypoxic chamber at 0.5% O_2. Drug resistance following hypoxic exposure was determined using an MTT assay. Results were analysed via Student's t-test and Graph Pad Software. All experiments were carried out in triplicate, with significance of $p<0.05$. A2780cis cells were 5-fold more resistant to cisplatin than A2780 cells (IC_{50} 10μM vs. 2μM, $p<0.05$). Conversely A2780cis were more than 10-fold more sensitive to paclitaxel than A2780s (IC_{50} 250pM vs. 3.4nM, $p<0.05$). Exposure to acute hypoxia (4 hours) prior to drug treatment significantly increased resistance to cisplatin in both cell lines. However, acute hypoxia before paclitaxel treatment increased sensitivity to the drug in A2780s, while increasing resistance in A2780cis cells. Chronic hypoxia (5 days) before treatment increased sensitivity to both cisplatin and paclitaxel in A2780s (Table 1). A2780 and A2780cis have an inverse relationship between resistance to cisplatin and paclitaxel. This relationship can be further potentiated by exposing the cells to hypoxia prior to drug treatment. Genes involved in this process may include HIF-1, p53 and BRCA; known to play a role in cisplatin and paclitaxel resistance whilst also regulated by hypoxia.

Cell Line	Acute Hypoxia Before Drug Treatment	Chronic Hypoxia Before Drug Treamtent	Cisplatin IC_{50} (fold change)	Paclitaxel IC_{50} (fold change)
A2780	+	-	2.9μM (1.6)*	1nM (0.3)*
A2780	-	+	1.2 μM (0.6)*	765nM (0.2)**
A2780cis	+	-	14 μM (1.35)**	1.9nM (7.6)**
A2780cis	-	+	10.9 μM (1.04)	600nM (2.4)

Poster No. 22

The BH3 mimetic HA14-1 can improve the cytotoxicity of 5-Fluorouracil by induction of Type II cell death in oesophageal cancer cells

M Nyhan, T O'Donovan, B Elzinga, L Crowley, GC O'Sullivan, SL McKenna
[1]Cork Cancer Research Centre, University College Cork, Cork, Ireland

Resistance to DNA damaging drugs has been associated with a failure of cancer cells to induce apoptotic cell death (Type I cell death), due in part to an imbalance between negative and positive regulators of the Bcl-2 family. Strategies to restore apoptosis have led to the development of BH3 mimetic compounds that mimic the pro-apoptotic activity of BH3 only proteins, or small molecule inhibitors of the anti-apoptotic Bcl-2 family members.

We examined the sensitivity of three oesophageal cancer cell lines to 5-fluorouracil (5-FU) and the cell death mechanisms induced. OE21 cells induce apoptosis and are the most drug sensitive. KYSE450 and KYSE70 cells induce autophagy which is accompanied by Type II cell death. This induction of autophagy results in a substantial number of surviving cells that will re-populate, thus rendering treatment ineffective. In this study, we evaluated the potential of the BH3 mimetic HA14-1 to reduce this survival and assessed the death mechanisms involved.
Western blot analysis confirmed that all cell lines express the anti-apoptotic proteins MCL-1, Bcl-xL and Bcl-2 and the pro-apoptotic protein Bax. Differential expression of the BH3 only protein NOXA was identified by array analysis and confirmed by Real-Time RT-PCR. OE21 cells have 15 fold higher expression of NOXA compared to KYSE450 or KYSE70 cells.

Drug sensitivity of OE21 (Type I death) and KYSE450 cells (Type II death) cells was improved by combining 5-FU and HA14-1 as determined by clonogenic assay. In the apoptosis resistant cell line KYSE450, less than 5% of cells were apoptotic in the presence of HA14-1. The enhanced cytotoxicity was a consequence of Type II cell death. In contrast, HA14-1 did not enhance Type I or Type II death in KYSE70 cells. In conclusion, the efficacy of the BH3 mimetic, HA14-1 is cell line dependent and is not reliant on apoptosis induction.

Poster No. 23

TRIB2 LEADS TO CHEMORESISTANCE AND DIFFERENTIATION DEFECTS

L Liang[1], **C O'Connor[1]**, K Keeshan[1]
[1]Haematopoiesis and Oncogenes Lab, Department of Biochemistry,, BioSciences Institute, University College Cork, Cork, Ireland

The current ‘‘standard of care’’ for AML patients involves an initial phase of intense chemotherapy followed by post-remission treatment[1]. We have identified Trib2, one of three homologs, as a potent leukemogen that clusters in a specific subset of human AML[2]. However, the prognostic implications of this subset are unknown. We aim to mimic clinical AML induction therapy to check the chemotherapeutic response of leukemic cells expressing high Trib2.

Our results showed that Trib2 AML cells are more resistant to chemotherapy compared with controls. Trib2-mediated degradation of the cell cycle activator cdc25 is one of the key mechanisms for drug resistance. In addition, Trib2 maintains but does not increase p53 and p21 expression after chemotherapy, which also contributes to drug resistance. Our previous data showed Trib2 directly inhibits the function of the transcription factor C/EBPalpha[2], which is a target of ATRA (all trans retinoic acid) to induce APL differentiation[3]. We assessed the ATRA response in AML cells and APL cells expressing high level of Trib2, and demonstrated that Trib2 blocks ATRA induced differentiation by degrading C/EBP alpha. Taken together, Trib2 leads to chemoresistance and differentiation defects.

[1] Döhner H et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010 Jan 21;115(3):453-474. [2] Keeshan K et al. Tribbles homolog 2 inactivates C/EBPalpha and causes acute myelogenous leukemia. Cancer Cell. 2006 Nov;10(5):401-411. [3] Lee YJ et al. CCAAT/enhancer binding proteins alpha and epsilon cooperate with all-trans retinoic acid in therapy but differ in their antileukemic activities. Blood. 2006 Oct 1;108(7):2416-2419.

Poster No. 24

THE IDENTIFICATION OF PATHWAYS INVOLVED IN THE PROLIFERATION AND CHEMOTHERAPY RESPONSES OF TRIPLE NEGATIVE BREAST CANCER

KS Orr, NE Buckley, NT Crawford, PB Mullan
[1]CCRCB, Queens University Belfast, Belfast, Northern Ireland

Triple negative breast cancer (TNBC), which accounts for approximately 15% of all breast cancers, is characterised by absence of the oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2).1 Although TNBC is initially chemo-sensitive, aggressive relapse resistant to chemotherapy is common and results in poor overall survival and a disproportionate amount of breast cancer deaths from this disease.2,3 To date, there are no targeted therapies available for the treatment of TNBC since current treatments focus on inhibiting ER, PR and HER2 which TNBC already lacks.4,5 Our aim is to identify the specific molecular pathways involved in driving proliferation and drug resistance in TNBC to predict which tumours will not respond to chemotherapy and ultimately to discover a potential therapeutic target to improve clinical outcome.
We have performed microarray profiling of 16 triple negative breast tumours (8 good and 8 poor FEC response tumours). Unsupervised hierarchical clustering of gene expression data showed that the tumours cluster into distinct poor response and good response tumour groups. Functional analysis of genes up-regulated in poor or good responders was carried out in TNBC cell lines by siRNA screens. Following siRNA knockdown of the genes, cell viability was assessed by MTT assay to identify genes of interest. The ongoing siRNA screens have revealed several potential targets to date in which the knockdown of gene expression resulted in a marked reduction in cell viability. siRNA screens combined with chemotherapy treatment are also underway in cell line models to detect genes which may be involved in drug resistance.
By identifying genes which drive growth and chemo-resistance of TNBC we hope to shed some light on the ill-defined molecular pathways involved, with the ultimate goal of finding a possible target for treatment to improve the poor outcome typical of this aggressive cancer.

1. Kumar Pal S, Mortimer J. Triple-negative breast cancer: Novel therapies and new directions. Maturitas 2009; 63: 269-74 2. Podo F, Buydens LMC, Degani H, Hilhorst R, Klipp E et al. Triple-negative breast cancer: Present challenges and new perspectives. Molecular Oncology 2010; 4: 209-29 3. Carey L. Directed therapy of subtypes of triple-negative breast cancer. The Oncologist 2010; 15: 49-56 4. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncology 2007; 8: 235-44 5. Bosch A, Eroles P, Zaragoza R, Viña JR, Lluch A. Triple-negative breast cancer: Molecular features, pathogenesis, treatment and current lines of research. Cancer Treatment Reviews 2010; 36: 206-15

Poster No. 25

CARDIAC GLYCOSIDES AS POTENTIAL ANTI-CANCER AGENTS

MB Owens[1], ADK Hill[1], AM Hopkins[1]
[1]Dept of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Breast cancer causes 17.5% of cancer deaths in Ireland . Cardiac glycosides, a class of naturally-occurring treatments for heart failure, have recently shown promise as potential anti-cancer agents. Specifically, breast cancer patients taking the cardiac glycoside digoxin reportedly had significantly reduced rates of relapse and metastasis . The cellular target of cardiac glycosides is the Na-K-ATPase ion channel. A pool of Na-K-ATPase is thought to be ion-independent and to activate several signalling cascades, however little is known about the contribution of such signalling to breast cancer progression. The ion-independent Na-K-ATPase pool reportedly localizes to caveolae, specialized cholesterol-enriched regions of the cell membrane. We therefore hypothesized that Na-K-ATPase partitioning into caveolae regulates tumourogenic behaviour in breast cancer cells; and that pharmacological inhibition of the Na-K-ATPase exerts anti-tumorigenic effects by modulating its interactions with caveolae. We have demonstrated by confocal immunofluorescence microscopy that Na-K-ATPase co-localises with the caveolar structural protein caveolin-1 in cell membranes of the breast cell lines MCF7 (weakly-invasive) and MDA-MB231 (highly-invasive). In parallel we have shown by isopycnic sucrose density gradient fractionation that Na-K-ATPase is enriched in lipid raft fractions of MCF7 cells. Finally we have evidence that proliferation and migration of MCF7 and MDA-MB-231 cells is significantly reduced by treatment with the cardiac glycosides digoxin, ouabain and oleandrin. Future work will concentrate on investigating alterations between Na-K-ATPase/caveolin interactions following treatment with sub-lethal doses of cardiac glycosides; and translation of these results into primary cell cultures and clinical breast cancer specimens.

Cancer in Ireland 1994-2007. Annual report of the National Cancer Registry Stenkvist B, Bengtsson E, Eriksson O, Holmquist J, Nordin B, Westman-Naeser S. Cardiac glycosides and breast cancer. Lancet. 1979 Mar 10;1(8115):563. Liang M, Tian J, Liu L, Pierre S, Liu J, Shapiro J, Xie ZJ. Identification of a pool of non-pumping Na/K-ATPase. Biol Chem. 2007 Apr 6;282(14):10585-93.

Poster No. 26

HDAC INHIBITORS INDUCE FLIP DOWN-REGULATION AND CASPASE-8 DEPENDENT APOPTOSIS IN NON-SMALL CELL LUNG CARCINOMA.

JS Riley[1], I Stasik[1], DA Fennell[1], PG Johnston[1], DB Longley[1]
[1]Centre for Cancer Research & Cell Biology, Queen's University Belfast, Belfast, United Kingdom

Introduction: Non-small cell lung carcinoma accounts for 17% of cancer related deaths in the UK. A major barrier to effective treatment is drug resistance. Over-expression of c-FLIP, an anti-apoptotic protein which blocks caspase 8 activation and death receptor-mediated apoptosis, has been identified in various cancers and is a possible mechanism of drug resistance. In several models vorinostat (SAHA) has been shown to suppress c-FLIP expression. HDAC inhibitors, such as vorinostat, are an emerging class of compounds, some of which are already approved for the treatment of cancer.

Aim: The aim of this research was to characterise NSCLC cells' responses to clinically relevant HDACi and elucidate possible mechanisms of their action.

Methods: H460 and A549 NSCLC cell lines and CCD-34Lu normal lung fibroblast cells were utilised. Protein expression was determined by Western blotting. Apoptosis was assessed by flow cytometry with PI staining and PARP cleavage detection. Caspase activation was established by caspase 3/7 and -8 activity assays. Caspase 8 was silenced by caspase 8 siRNA transfection.

Results: Treatment with HDACi caused an early down-regulation of c-FLIP protein in a time- and dose-dependent manner. PARP cleavage, indicative of apoptosis, and activation of caspases 8 and -3/7 were observed. FACS analysis by propidium iodide staining confirmed that HDACi treatment induced apoptosis in NSCLC cells. Normal lung fibroblasts CCD-34Lu did not undergo apoptosis following treatment with HDACi at clinically relevant doses. Silencing of caspase 8 suggested that HDACi-induced apoptosis was partly mediated by caspase 8.

Conclusions: We have previously shown that c-FLIP down-regulation in other cancers sensitises cells to chemotherapy and death-receptor ligands such as TRAIL and Fas. Therefore targeting c-FLIP with HDACi in NSCLC has promising therapeutic relevance.

Poster No.27

HDAC INHIBITOR VORINOSTAT INDUCES c-FLIP DOWNREGULATION AND CASPASE 8-DEPENDENT APOPTOSIS IN MALIGNANT PLEURAL MESOTHELIOMA CELLS

I Stasik, JL Hurwitz, K McLaughlin, D Fennell, DB Longley
[1]Centre for Cancer Research and Cell Biology, Queen's University, Belfast, Northern

Malignant pleural mesothelioma (MPM) is a rare and highly aggressive neoplasm, arising from the mesothelial lining of the lungs, for which no optimal treatment is known so far. Recent findings suggest that histone deacetylase inhibitors, such as vorinostat/SAHA, could be a promising novel therapeutic strategy in MPM.

We studied the effect of SAHA on a MPM cell line viability. In REN cells SAHA induced apoptosis in a dose- and time-dependent manner. Cell death was preceded by downregulation of c-FLIP, a major inhibitor of the extrinsic apoptotic pathway. Silencing of either caspase 8 or FADD expression resulted in attenuation of SAHA-induced apoptosis.

We also tested the potential use of vorinostat in combined therapy. Co-treatment with SAHA and TRAIL or cisplatin resulted in synergistic enhancement of apoptosis. c-FLIP over-expression resulted not only in reduced sensitivity to SAHA alone, but also abolished its ability to overcome the resistance to TRAIL and cisplatin.
These results show that HDAC inhibition in MPM leads to apoptosis induction via c-FLIP downregulation with following caspase 8 activation. This approach could be an attractive therapeutic option for treating MPM.

Poster No.28

IDENTIFICATION OF NOVEL DETERMINANTS OF RESISTANCE TO 5-FU AND OXALIPLATIN IN COLORECTAL CANCER USING A SYSTEMS BIOLOGY APPROACH

L Stevenson[1], W Allen[1], R Turkington[1], P Jithesh[1], I Proutski[1], G Stewart[1], HJ Lenz[2], D Longley[1], P Johnston[1]

[1]for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, NI

[2]Division of Medical Oncology, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, USA

A major factor limiting the effectiveness of chemotherapy against colorectal cancer (CRC) is drug resistance, either inherent or acquired. Therefore, the identification of key mediators of resistance is a priority. We report the results from an RNAi screen of genes identified in a microarray profiling experiment that was carried out to identify mediators of resistance to 5-FU and oxaliplatin.

Microarray profiling of pre-treatment metastatic CRC liver biopsies, HCT116 parental CRC cell line and sub-lines resistant to 5-Fluorouracil or Oxaliplatin was performed using a colorectal cancer disease specific array. Pathway analysis using KEGG and Gene Set Enrichment Analysis (GSEA) of the microarray data identified MAPK signalling, Focal Adhesion and Insulin signalling as key pathways involved in regulating response to 5-FU and Oxaliplatin. Fifty-seven candidate genes that were common to the in vitro and clinical genelists were selected from the identified pathways. RNAi was used to examine the effects of these candidates on the chemosensitivity of HCT116 CRC cells to 5-FU and Oxaliplatin using cell viability and cytotoxicity assays as end-points. The primary RNAi screen identified 25 genes, the knockdown of which led to a statistically significant increase in sensitivity to 5-FU and/or Oxaliplatin. A secondary RNAi screen confirmed on-target effects of multiple siRNAs for 8 genes and a tertiary screen identified 6 genes with a chemosensitising affect in ≥ 3 out of 7 independent CRC cell lines: HCT116, HT29, H630, LoVo, LS-174T, RKO and SW620.

Thus, this study demonstrates the utility of DNA microarray profiling followed by pathway analysis/GSEA and RNAi screening to identify genes from microarray expression data that are involved in 5-FU/ Oxaliplatin resistance in CRC and provides the foundation for further studies examining the clinical and therapeutic relevance of these genes.

Poster No. 29

Mitochondrial VDAC2 is transcriptionally regulated by p63 and confers shorter survival in non-small cell lung cancer

BD Wilson[1], C Grills[2], S McDade[1], D McCance[1], A Ashworth[3], C Lord[3], DA Fennell[1, 2]
[1]Centre for Cancer Research and Cell Biology, Queen's University , Belfast, UK
[2]Center for Cancer Bioinformatics, Queen's University, Belfast, UK
[3]Institute of Cancer Research, London, UK

Introduction:
Resistance to apoptosis is a hallmark of cancer and a major problem limiting the effectiveness of treatment in non-small cell lung cancer (NSCLC). The mitochondrial dependant anion channel isoform 2 (VDAC2) gene is amplified in cancers, and inhibits apoptosis by binding to, and preventing activation of BAK(1). VDAC2 is required for cancer cell cytotoxicity of a new class of drugs called erastin(2) however the variability of VDAC2 expression in NSCLC is unknown. Here we show that VDAC2 is prognostic in NSCLC, and using gene expression meta-analysis, identify p63 as a critical transcriptional regulator and potential biomarker for classifying erastin sensitive NSCLC.

Results:
We conducted a gene expression meta-analysis of 601 tumours from 7 public gene expression datasets in NCBI GEO to generate a meta-signature corresponding VDAC2 overexpression in NSCLC. We identified VDAC2 as an independent prognostic factor in both univariate and multivariate analyses (versus histology, gender, age, and stage). A conserved 6 gene-metasignature was identified comprising ANKRD27, BICD2, IL6ST, MEF2C, PCYT1A, TP63. we identified a potential p63 binding site in VDAC2 implicating potential transcriptional regulation. This was verified by chromatin immunoprecipitation sequencing (ChIPseq). This interaction was verified by quantitative real-time polymerase chain reaction. Furthermore, in H157 NSCLC cells expressing high levels of both p63 and VDAC2, siRNA silencing of p63 was associated with commensurate reduction in VDAC2 expression. Importantly, ANKRD27, BICD2, MEF2C, PCYT1A were also found to be transcriptional targets.

Conclusions:
In summary, using meta-signature analysis of large scale gene expression data, we have validated a relevant biological connection between p63 and VDAC2 transcription. Since p63 expression is found selectively in the squamous subtype of NSCLCs, we propose that this group of cancers might be selectively targeted by erastins, thus enabling personalized therapy with this agent.

1. E. H. Cheng, T. V. Sheiko, J. K. Fisher, W. J. Craigen, S. J. Korsmeyer, Science 301, 513 (Jul 25, 2003) 2. N. Yagoda et al., Nature 447, 864 (Jun 14, 2007)

Poster No.30

FLT3-DRIVEN REDOX SIGNALLING IN ACUTE MYELOID LEUKAEMIA

A Corcoran, R Naughton, JF Woolley, TG Cotter
[1]Biochemistry Department, University College Cork, Cork, Ireland

FLT3 is a gene that is mutated in 35% of acute myeloid leukaemia patients and recent evidence suggests that as part of its signalling pathway it stimulates the production of reactive oxygen species. These in turn can lead to increased genomic instability and also specifically enhance cell survival signalling. The NADPH oxidase (Nox) family of enzymes produce reactive oxygen species in response to signalling from growth factors and oncogenic receptor tyrosine kinases, thus implicating the Nox family as possible candidates for oxidant generation in response to FLT3 signalling. In this project we are seeking to investigate the relationship between FLT3 and Nox as well as the downstream targets of reactive oxygen species generated in acute myeloid leukaemia.

Results: Using flow cytometry and oxygen sensitive fluorescent probes, we demonstrate that FLT3 induces the production of reactive oxygen species and that this induction can be blocked by inhibition of Nox. We also show that inhibition of FLT3 signalling in cell lines expressing the FLT3-ITD mutation leads to a decrease in the levels of reactive oxygen species. This decrease coincides with a reduction in the p22phox subunit of the Nox complex. In addition to this we use phosphotyrosine immunoblotting and two-dimesional gel electrophoresis to identify downstream targets of Nox-generated reactive oxygen species. Research is currently ongoing to determine the mechanism by which Nox regulates these targets and whether this regulation is in response to FLT3 signalling.

Conclusion: Overall these data suggest a significant role for Nox-derived reactive oxygen species in FLT3 signalling and this implicates redox signalling as a possible therapeutic target in the treatment of acute myeloid leukaemia.

Poster No. 31

SOCS1: A CRITICAL MEDIATOR OF 5-FU/IRINOTECAN RESPONSE IN COLORECTAL CANCER CELLS

SC Dolan[1], I Proutski[1], M Crudden[1], WL Allen[1], L Stevenson[1], DB Longley[1], M Gordon[2], HJ Lenz[2], PG Johnston[1]

[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland

[2]Division of Medical Oncology, University of Southern California/Norris Comprehensive Cancer Center, Keck School of Medicine, Los Angeles, California 90033, USA

Advanced colorectal cancer patients largely have poor response rates to current treatments demonstrating the need for identification of novel drug targets and improved treatment strategies.

Methods

Microarray profiling of pre-treatment metastatic colorectal cancer biopsies, HCT116 parental and chemotherapy-resistant cell lines was carried out using a colorectal cancer disease-specific array. Bioinformatic pathway analysis was carried out to identify pathways and genes involved in chemo-sensitization. Functional analysis was carried out on the identified genes using custom-designed siRNA screens, initially in the HCT116 cell line followed by further investigation of positive hits in a panel of CRC cell lines.

Results

The bioinformatic pathway analysis identified a number of genes that could be functionally tested using a custom-designed siRNA screen. Primary, secondary and tertiary siRNA screens were carried out leading to the identification of 6 genes involved in chemo-sensitisation from either the cell cycle, insulin or interferon pathways. SOCS1, a gene involved in the insulin pathway, was selected for further analysis in a panel of chemotherapy sensitive and resistant cell lines. The results demonstrated that SOCS1 silencing increased the sensitivity of most cell lines to 5-FU and SN38 treatment, however it did not re-sensitise the 5-FU resistant and SN38 resistant HCT116 cells to 5-FU or SN38 respectively. SOCS1 silencing alone and in combination with either 5-FU or SN38 resulted in decreased activation of the JAK/STAT signalling pathway and apoptosis induction. Further work is being carried out to elucidate role of SOCS1 in chemotherapy resistance.

Poster No.32

2-D DIGE analysis of phospho-enriched fractions from dasatinib treated melanoma cell lines

AJ Eustace[1], P Dowling[1], M Henry[1], P Doolan[1], P Meleady[1], M Clynes[1], J Crown[2], N O'Donovan[1]
[1]National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland
[2]St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland

Current therapeutic regimes for metastatic melanoma have failed to provide robust clinical responses. Dasatinib has shown anti-proliferative and anti-invasive effects in vitro, however, not all melanoma cells tested were sensitive to dasatinib.

We used 2D-DIGE analysis of phospho-enriched fractions to identify phosphoproteins involved in regulating response to dasatinib in an isogenic pair of melanoma cell lines, one sensitive to dasatinib (WM-115) and the other resistant (WM-266-4).
In WM-115 cells treated with dasatinib, 18 unique protein species with altered phosphorylation levels were detected. Dasatinib treatment of WM-266-4 cells resulted in phosphoprotein alterations to four unique protein species. Four phosphorylated forms of Annexin-A2 (ANXA2) were increased in WM-115 cells treated with dasatinib, whilst dasatinib treatment did not alter ANXA2 phosphoprotein levels in WM-266-4 cells. Immunoblotting confirmed that phosphorylation of ANXA2, on tyrosine residues, was increased in WM-115 cells treated with dasatinib. Subsequent knockdown of ANXA2 by siRNA significantly inhibited proliferation of WM-115 cells but did not significantly reduce proliferation of WM266-4 cells.

Therefore, ANXA2 plays a role in regulating proliferation in dasatinib sensitive WM-115 cells and could potentially play a role in sensitivity to dasatinib in melanoma cells.

Poster No.33

aPKC ZETA CONTROLS CELL POLARIZATION AND CELLULAR MORPHOGENESIS

A Fatehullah[1], RK Deevi[1], V Bingham[1], I Jagan[1], FC Campbell[1], FC Campbell[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland

Cell polarization is essential for the growth and maintenance of epithelial tissue morphology. Derugluation of this cellular polarization is one of the hallmarks of cancer. PTEN is a tumour suppressor and a controller of cell polarization, the antagonist PI3Kinase is the upstream regulator for aPKC zeta. aPKCζ controls cell polarization and morphogenesis via regulation of tight junction formation and regulation of polarity complexes. The small Rho GTPase Cdc42 is a master regulator of cell polarization. Using a Caco2 colorectal cells, we have shown that aPKC zeta is upstream of Cdc42. aPKCζ is essential for cdc42-mediated polarization.
LKB1 is a known downstream target of aPKCζ. Co-transfection of LKB1 with aPKCζ enhances cdc42 activation. STRADα isrequired for cytoplasmic localisation for LKB1. We show that STRADα enhances LKB1 activation of cdc42.
In a three dimensional Caco2 culture model, wild type cells form regular gland-like structures around a single central lumen. Suppression of aPKC leads to misalignment of the apical membrane and inhibition of lumen formation.
Taken together, these data show that the cdc42- aPKCζ signalling axis is enhanced by LKB1 and STRAD and is a key mediator of 3D morphogenesis.

Poster No. 34

A PHOSPHATASE-INDEPENDENT ROLE FOR PTEN IN CDC42-MEDIATED EPITHELIAL POLARIZATION AND MORPHOGENESIS

I Jagan[1], RK Deevi[1], **A Fatehullah[1]**, V Bingham[1], M Nagaraju[1], FC Campbell[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast BT9 7BL, Northern Ireland

PTEN loss promotes high grade dysmorphic cancers by unclear phosphatase-dependent and -independent mechanisms. Here we show that PTEN deficiency is associated with blunting of cdc42-dependent morphogenic growth of colorectal cancer (CRC) cells. PTEN null HCT116 cells showed impaired cdc42 signaling and polarized growth vs wild type (wt) PTEN+/+ HCT116 cells, in monolayer assays. ShRNA knockdown of PTEN in Caco-2 cells (Caco-2 ShPTEN) cells impaired cdc42 activation and attenuated morphogenesis in three-dimensional cultures, leading to formation of abnormal intra- and intercellular vacuoles and multiple abnormal lumens, within cyst-like structures. Modulation of phosphatidinylinositol 3-kinase (PI3K) signaling by EGF or wortmannin treatment influenced cell size within cysts but did not affect morphogenesis. Transfection of Caco-2 ShPTEN cells with cdc42 or the catalytically-inactive PTEN C2 domain but not by a PTEN C2 domain mutated at the CBR3 membrane targeting loop rescued cyst morphogenesis. Taken together, our data show PTEN phosphatase-dependent or -independent effects on key elements of CRC morphogenesis including cell size, vacuolation and luminogenesis, in a model system.

Poster No. 35

RACK1, A NOVEL BINDING PARTNER FOR CFLIP

C Holohan[1], J Majkut[1], PA Kiely[2], R O'Connor[2], PG Johnston[1], DB Longley[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland
[2]BioSciences Institute, University College Cork, Cork, Ireland

c-FLIP is an inhibitor of apoptosis mediated by the death receptors Fas, DR4, and DR5 and is expressed both as long (c-FLIPL) and short (c-FLIPS) splice forms. Previous studies by this group have shown that c-FLIP is an important regulator of colorectal cancer cell death and that silencing of c-FLIPL can overcome resistance to chemotherapy in a variety of disease models. In order to learn more about the biology of c-FLIP a yeast two hybrid screen was carried out to identify novel binding partners of c-FLIPL.

Receptor for activated protein kinase C1 (RACK1) was identified as an interacting partner of c-FLIPL. RACK1 is a seven-WD-domain-containing protein with numerous downstream effectors regulating various cellular functions such as growth and migration. The RACK1-FLIPL interaction was verified in mammalian cells by co-immunoprecipitation. Co-immunoprecipitation studies also revealed an interaction between FLIPS and RACK1. In addition, the silencing of RACK1 by RNA interference enhanced apoptosis induced by the death ligand TRAIL. TRAIL treatment was also shown to attenuate the binding of FLIPL and RACK1. These results suggest that RACK1 may act as a key regulator of death receptor signalling. Peptide array studies further verified the RACK1-c-FLIPL interaction and localised the c-FLIPL binding interface to specific sites on the RACK1 propeller. Site-directed mutagenesis has been carried out on these residues and current studies are ongoing using the RACK1 mutants to determine the functional significance of this novel partnership.

Poster No. 36

A MICRORNA-206/CYCLIN D1 AXIS IS CONSERVED IN MUSCLE DIFFERENTIATION AND BREAST CANCER PROLIFERATION

B Howley[1], HO Fearnhead[1], C Santocanale[1], SJ Elliman[1], LR Barkley[1]
[1]National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Galway, Ireland

To better understand the regulation of microRNAs (miRNAs) in the development of skeletal muscle, we generated miRNA expression profiles of differentiating murine C2C12 myocytes. MicroRNA-206 (miR-206) is significantly upregulated during differentiation. We investigated the potential role of miR-206 in promoting skeletal myogenesis. Ectopic miR-206 reduced cell number and increased the proportion of cells in the G1 phase of the cell cycle. Transient over-expression of miR-206 prompted an increase in activity of myogenic transcription factors, MEF2 and myogenin. Bioinformatic analysis of Affymetrix Gene Expression Microarray (GEM) data revealed that ectopic miR-206 expression in C2C12 myoblasts initiated a transcriptional profile consistent with early myogenesis and identified Cyclin D1 as a putative miR-206 target. We demonstrate that miR-206 targets Cyclin D1 protein for repression via its 3'UTR, likely contributing to the observed G1 cell cycle arrest. In contrast to differentiated muscle cells, miR-206 levels are low in proliferating breast cancer cells, concomitant with high levels of Cyclin D1 protein. We show that ectopic miR-206 reduces Cyclin D1 protein levels in human breast cancer cell lines. Interestingly, miR-206 induces a G1 cell cycle arrest followed by cell death in Estrogen Receptor- α positive (ERα+) cell lines. This data suggests a tumour suppressor function for miR-206 through appropriate regulation of Cyclin D1 in breast cancer. We are currently investigating if ERα -signaling represents a common mechanism that regulates this miR-206/Cyclin D1 axis in differentiating murine myoblasts and proliferating human breast cancer cells.

This work was supported by funding from the Irish Cancer Society.

Poster No.37

Using novel bio-marker S100β to determine responsiveness of endocrine resistant breast cancer cells to dasatinib

E Hughes[1], C Byrne[1], ADK Hill[1], LS Young[1]
[1]Dept of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Dasatinib is a new drug that inhibits Src/Abl family tyrosine kinase activity. In preclinical breast cancer studies it has shown to inhibit metastasis, cause cell cycle arrest, block proliferation, induce apoptosis (Finn 2008). However, the clinical efficacy of dasatinib as a single agent therapy for both triple negative and ER/PR-positive breast cancer has shown to be limited. We suggest that selection of ER positive patients with high levels of a biomarker could dramatically improve dasatinib sensitivity.
Our group has previously identified the novel serum biomarker S100β which is associated with decreased disease free survival in patients treated with endocrine therapies. This protein was found to be transcriptionally activated by interaction between SRC-1 (Steroid Receptor Coactivator 1/NCoA1) and HOXC11 (McIlroy M et al 2010). SRC-1 is activated via src tyrosine kinase phosphorylation therefore drugs such as Dasatinib offer a potential strategy to reduce SRC-1's interactions and its role in mediating resistance to endocrine therapy. Since S100β is produced by SRC-1's interaction with HOXC11, it is an ideal candidate as a predictive biomarker of patients who would benefit from dasatinib treatment.
In this study we observed higher levels of tyrosine kinase activity in two endocrine resistant cell lines and that dasatinib reduced this activity. Further, dasatinib decreased the interaction of SRC-1:HOXC11 leading to diminished expression of S100β. Dasatinib alone did not affect proliferation but when used in combination with tamoxifen a reduction in cell number was observed. Dasatinib also reduced their migration but had no effect on their parental (endocrine sensitive) cell line MCF-7. In Xenograft studies we have observed reduced tumour volume in endocrine resistant tumours treated with dasatinib in combination with tamoxifen, compared to tumours treated with tamoxifen alone. These results indicate that the efficacy of dasatinib is cell specific and that pre-selection of patients using a biomarker such as S100β offers a potentially exciting way of overcoming resistance to endocrine therapies.

Marie McIlroy, Damian McCartan, Sarah Early, Peadar Ó Gaora, Stephen Pennington,Arnold D.K. Hill, and Leonie S. Young (2010);Interaction of Developmental Transcription Factor HOXC11 with Steroid Receptor Coactivator SRC-1 Mediates Resistance to Endocrine Therapy in Breast Cancer; Cancer research(4)1585-1594 Finn RS, Dering J, Ginther C, Wilson CA, Glaspy P, Tchekmedyian N, Slamon DJ.(2007); Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing in vitro; Breast Cancer Research and Treatment; 105(3):319-26

Poster No. 38

SRC-1 IN AROMATASE INHIBITOR RESISTANT BREAST CANCER

J McBryan[1], S Theissen[1], C Byrne[1], S Cocchiglia[1], E Hughes[1], J O'Hara[1], S Sande[1], A Hill[1], L Young[1]
[1]Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Aromatase inhibitors (AIs) are an effective and frequently used method for treating many hormone receptor positive breast tumours. However, as with tamoxifen, endocrine resistance can develop over time leading to tumour recurrence. In this study, a cell model of AI resistance has been developed to investigate the molecular mechanisms involved. The AI resistant cells are more migratory and less capable of forming organised 3D structures than their parental sensitive cells. The resistant cells have higher levels of the transcriptional co-activator SRC-1, the transcription factor Ets2 and the SRC-1/Ets2 target gene Myc. Chromatin immunoprecipitation studies have revealed binding of both SRC-1 and Ets2 to the promoter region of the Myc gene suggesting a mechanism of direct regulation of the Myc oncogene by SRC-1 in the resistant cells. Knockdown of SRC-1 with targeted siRNA reduced the migratory capacity of resistant cells and increased their ability to form organised 3D structures. These results suggest an important functional role for SRC-1 in mediating AI resistance and highlight SRC-1 signalling pathways as potential targets for new therapies in endocrine resistance.

Poster No.39

INVESTIGATION OF THE FUNCTIONAL RELATIONSHIP BETWEEN PTEN-INDUCED KINASE 1 AND PI3-KINASE/AKT SIGNALLING IN CANCER DEVELOPMENT

CH O'Flanagan[1], C O'Neill[1]
[1]Department of Biochemistry, University College Cork, Cork, Ireland
[2]Department of Biochemistry, University College Cork, Cork, Ireland

Deregulated PI3-kinase (PI3K)/Akt signalling is a significant driver of tumourigenesis, and mutations or deletions of the main inhibitor of this pathway, PTEN, are a major cause of many different cancers. *PTEN-induced kinase 1* (*PINK1*) was first identified in cancer cells as a gene up-regulated by overexpression of PTEN. Subsequently, loss-of-function mutations in PINK1 were discovered to cause autosomal recessive Parkinson's disease. Since this discovery, research has revealed that PINK1 function is central to mitochondrial health, mitophagy and in protection from oxidative/mitochondrial stress with emphasis on brain function. Emerging evidence indicates PINK1 function is important in cancer development, though results are conflicting showing both tumour promoting and tumour suppressing properties.
This aims of this study were to investigate the function of PINK1 in the development of cancer, with emphasis on the relationship between PINK1 and PTEN/PI3K/Akt signalling. Firstly, meta-analysis using the Oncomine database showed PINK1 mRNA expression was significantly decreased in a number of different cancers, suggesting PINK1 could function as a tumour suppressor. Screening the same cancers for PTEN expression showed a significant decrease in PTEN expression in only a subset of these cancers. While PTEN was not found to be essential for PINK1 protein expression in cancer cell lines, deletion of PTEN, with concomitant activation of Akt, caused a 70% decrease in PINK1 mRNA levels in MCF-7 cells. In concordance, IGF-1 induced activation of Akt caused a time-dependent decrease in PINK1 mRNA expression that was blocked by PI3K inhibition using LY2949002. Together, these results indicate that reduction of PINK1 expression may be mechanistically important in cancer cell survival induced by PI3K/Akt signalling. Current research is focused on examining the effects of modulation of PINK1 expression and function on cancer development, with emphasis on PI3K/Akt signalling.

Unoki, M. and Nakamura, Y. (2001) 'Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signalling pathway' Oncogene 20 4457-4465 Valente EM, Salvi S, Ialongo T, Marongiu R, Elia AE, Caputo V, Romito L, Albanese A, Dallapiccola B, Bentivoglio AR. (2004) ' PINK1 mutations are associated with sporadic early onset parkinsonism' Ann. Neurol. 56 336-41 Gegg ME, Cooper JM, Schapira AH, Taanman JW. (2009) 'Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells.' PLoS One. 4(3) e4756. Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. (2010) 'PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1.' Nat Cell Biol. 12(2):119-31. Berthier A, Navarro S, Jiménez-Sáinz J, Roglá I, Ripoll F, Cervera J, Pulido R. (2011) 'PINK1 displays tissue-specific subcellular location and regulates apoptosis and cell growth in breast cancer cells.'Hum Pathol. 42(1):75-87

Poster No. 40

MECHANISM OF TRAIL RESISTANCE IN NON-TRANSFORMED CELLS

M van Dijk[1], A Samali[1], E Szegezdi[1]
[1]Department of Biochemistry, National University of Ireland, Galway, Galway, Ireland

Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of death ligands. TRAIL can bind to two death-inducing receptors, DR4 and DR5, and to three decoy receptors, DcR1, DcR2 and osteoprotegerin. TRAIL can induce apoptosis selectively in tumour cells but not in non-transformed cells. Approximately 50-60% of tumours are also resistant to TRAIL. Currently arrays of drug combinations are being evaluated for their ability to sensitise tumours to TRAIL. The potential effect of such drug combinations on normal cells is currently unpredictable due to the lack of information on the mechanisms protecting non-transformed cells from the cytotoxic effect of TRAIL.

The aim of the current project is to identify the mechanism of TRAIL resistance in non-transformed cells using primary fibroblasts and smooth muscle cells as model cell types. We found that receptor expression cannot explain TRAIL resistance in these cells. We show that the anti-apoptotic proteins cFLIP and XIAP inhibit the TRAIL-induced apoptotic signalling pathway. Inhibition of protein synthesis, using cycloheximide (CHX), could sensitise the cells to TRAIL-induced apoptosis by activating the mitochondrial apoptotic pathway. CHX reduced the expression of the non-specific, anti-apoptotic Bcl-2 proteins Bcl-XL and Mcl-1, and also cFLIP, which is a specific inhibitor of death receptor-induced apoptosis. Expression of these proteins was found to be regulated by cyclin dependent kinase 1 (cdk1; also known as cdc2) and to a lesser extent also by glycogen synthase kinase 3 (GSK3) and inhibition of either kinase resulted in sensitisation to TRAIL-induced apoptosis.

In summary we show that in non-transformed cells cFLIP inhibits the TRAIL-induced apoptotic pathway at the level of the receptors and anti-apoptotic Bcl-2 family members inhibit the pathway at the level of the mitochondria and that expression of these proteins is regulated by cdk1 and GSK3.

Poster No.41

INSIGHTS INTO AROMATASE INHIBITOR RESISTANCE: NEW MECHANISM OF OESTROGEN SIGNALLING

D Vareslija[1], J O'Hara[1], AD Hill[1], LS Young[1]
[1]Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Aromatase Inhibitors (AIs) as adjuvant endocrine therapy have shown a clear supremacy over other anti-oestrogens through their clinical success [1-4]. However, as is the case with selective oestrogen receptor modulators (SERMs), prolonged exposure to AIs eventually leads to resistance [5-7]. In this study an AI (Letrozole) resistant model cell line was developed (Let-R). Let-R cells have higher levels of the oestrogen receptor (ERα) and the steroid receptor coactivator AIB1 compared to the AI sensitive cells. Gene expression of classically regulated target gene, pS2, and non-classically regulated cyclinD1 was investigated and evidence of differential gene expression was observed. In the resistant cells recruitment of ERα and AIB1 to the pS2 promoter and subsequent gene expression was steroid independent. Moreover, transcription factor recruitment to the cyclin D1 promoter and expression remained sensitive to treatment with steroids which was successfully inhibited by AI treatment. ER mediates cyclinD1 expression through binding to the AP transcription factors c-jun and c-fos. As cyclinD1 expression remains oestrogen regulated in AI resistant cells, it is thought that cyclinD1 might not be solely regulating through ER but through oestrogen signalling to c-jun N-terminal kinase (JNK). Here, recruitment of c-jun and c-fos onto AP1 sites on the cyclinD1 promoter was mediated by oestrogen signalling through JNK. To relate these findings to the clinic, a tissue microarray was constructed from 447 patients in which 63% showed AIB1 expression which co-associated with ERα (p=0.009). AIB1 significantly associated with disease recurrence in AI-treated population (p=0.047). Here we describe a differential oestrogen regulation of its target genes in AI resistance and describe a new signalling pathway for oestrogen, independent of ER through JNK mediated c-jun and c-fos. Moreover, AIB1 is required for aberrant ER signalling in AI resistance and as such has the potential to be a significant biomarker for this powerful therapy.

1.Coombes, R.C., et al., A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med, 2004. 350(11): p. 1081-92. 2.Dowsett, M., et al., Mechanisms of resistance to aromatase inhibitors. J Steroid Biochem Mol Biol, 2005. 95(1-5): p. 167-72. 3.Goss, P.E., et al., Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. J Natl Cancer Inst, 2005. 97(17): p. 1262-71. 4. Coates, A.S., et al., Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. J Clin Oncol, 2007. 25(5): p. 486-92. 5.Chen, S., et al., What do we know about the mechanisms of aromatase inhibitor resistance? J Steroid Biochem Mol Biol, 2006. 102(1-5): p. 232-40. 6.Hiscox, S., E.L. Davies, and P. Barrett-Lee, Aromatase inhibitors in breast cancer. Maturitas, 2009. 63(4): p. 275-9. 7.Long, B.J., et al., The effect of second-line antiestrogen therapy on breast tumor growth after first-line treatment with the aromatase inhibitor letrozole: long-term studies using the intratumoral aromatase postmenopausal breast cancer model. Clin Cancer Res, 2002. 8(7): p. 2378-88.

Poster No. 42

Regulation of a putative tumour suppressor gene BCL-G by pro-inflammatory cytokines in intestinal epithelial cells

Jerzy Woznicki[1], Fergus Shanahan[1], Silvia Melgar[1,2], Kenneth Nally[1]

[1] Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland

[2] Immuno-Inflammation, CEDD, GlaxoSmithKline, Stevenage, UK

IFN-□ and TNF-□ are potent pro-inflammatory cytokines with opposing roles in tumourigenesis: IFN-□ being primarily a tumour suppressor whereas TNF-□ a context-dependent tumour promoter. Our recent microarray analysis indentified a putative tumour suppressor gene BCL-G, a pro-apoptotic member of the Bcl-2 family, as being significantly up-regulated by IFN-□ plus TNF-□ in intestinal epithelial cells (IECs). The human BCL-G gene through alternative splicing gives rise to two transcripts and protein isoforms, BCL-G short (BCL-G_S) and long (BCL-G_L), both of which display a pro-apoptotic activity. The aim of the present study was to examine regulation of BCL-$G_{S/L}$ expression in response to selected inflammatory cues.

We found that in colon cancer cells BCL-$G_{S/L}$ mRNAs are significantly up-regulated by IFN-□ plus TNF-□ as compared to the individual cytokines and this paralleled induction of apoptosis. Expression of BCL-$G_{S/L}$ was markedly repressed by IKK2 and JAK inhibitors as well as RNAi-mediated knockdown of the SWI/SNF chromatin remodelling complex. Interestingly, apoptosis was also reduced by RNAi to SWI/SNF. Tissue-specific profiling indentified that both splice variants show highest expression levels in the gastrointestinal tract (small intestine and colon). Under pathological conditions, BCL-G_S was significantly down-regulated in Crohn's disease, colitis and colon cancer whereas BCL-G_L only in colon cancer.

We propose a novel regulatory mechanism of BCL-G induction by IFN-□ plus TNF-□ that is most likely NF-□B-dependent and requires IFN-□-mediated chromatin remodelling. Reduced expression of BCL-$G_{S/L}$ in IBD and colon cancer may reflect its involvement in altered cell death pathways in these disease states. Future studies will also investigate the contribution of the elevated BCL-G expression to the immune stress-mediated apoptosis of IECs.

This work was supported by Science Foundation Ireland.

Poster No. 43

RxDL MOTIFS OF THE c-FLIP DED DOMAINS ARE CRITICAL FOR ITS ANTI-APOPTOTIC FUNCTION.

J Majkut[1], A Logan[1], M Huang[1], C Holohan[1], J Johnston[1], D Longley[1]
[1]Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, United Kingdom

Introduction:

cFLIP is a major anti-apoptotic protein that blocks the apoptotic pathway mediated by death receptors. cFLIP is overexpressed in many cancers resulting in chemoresistance and limiting the effectiveness of commonly used anticancer therapies. Both the long (FLIPL) and the short (FLIPS) splice forms compete with procaspase 8 for binding with the adaptor protein FADD at the death inducing signaling complex (DISC), which is formed after stimulation of death receptors by their ligands. Developing a strategy to prevent recruitment of cFLIP to the DISC would be of potential therapeutic value. The aim of this project was to identify the critical regions in cFLIP which are required for binding to FADD and to examine whether disruption of this binding can promote death receptor mediated apoptosis.

Results:
As a member of the death effector domain (DED) protein family, cFLIP contains tandem DED domains, each with a characteristic RxDL conserved binding motif, which were shown previously to be important for interaction and function of viral FLIP1. The crystallographic structure of cFLIPS remains unresolved, so computer modelling using an homology model of cFLIPS based on viral FLIP was carried out. This suggested that the two conserved RxDL motifs in each DED were important sites of interaction with FADD. To validate the model's predictions, site-directed mutagenesis was performed to generate mutations in the RxDL motif in each individual DED or in both. We have demonstrated that the ability of RxDL cFLIPS mutants to interact with FADD and be recruited to the DISC is significantly impaired compared to wild-type cFLIPS. Moreover overexpression of cFLIP RxDL mutants no longer protects cells from death ligand-induced apoptosis.

Conclusion:
This study demonstrates that the RxDL motifs in the DEDs of FLIP are important for cFLIPS function.

1. Yang JK, Wang L, Zheng L, Wan F, Ahmed M, Lenardo MJ et al. Crystal structure of MC159 reveals molecular mechanism of DISC assembly and FLIP inhibition (2005). Mol Cell; 20: 939–949

Poster No.44

TOWARDS THE ELUCIDATION OF THE AUTOPHAGIC ACTIVITY OF OBATOCLAX

F Tran[1], F McCoy[2], D Fennell[2], D Haigh[1]
[1]Drug Discovery Research Division, Centre for Cancer Research and Cell Biology, Queen's University Belfast , Belfast, UK
[2]Thoracic Oncology Research Group, Centre for Cancer Research and Cell Biology, Queen's University Belfast , Belfast, UK

Obatoclax (Figure 1a) is a small molecule currently in clinical development, which has been shown to induce apoptosis by inhibiting the interaction between pro- and anti-apoptotic Bcl-2 family proteins. Furthermore, such mediation of apoptosis has been reported to be dependent on BAX/BAK expression. However recent evidence has suggested that the drug may be promoting cell death via a non-apoptotic pathway, possibly via the induction of autophagy. The molecular mechanism of this alternative cell death pathway has not yet been identified.

To investigate, these effects of Obatoclax, we envisaged that an affinity probe would aid the elucidation of the key target protein(s). Suitable affinity probes can be designed by attaching a biotin-linker to the drug and utilizing the well-documented biotin-streptavidin affinity protocol1 (Figure 1b). Following affinity capture, it is envisioned that the identification and characterisation of the protein(s) responsible for this mechanism of action of Obatoclax will be determined.

This poster will illustrate the strategy and progress towards the identification of suitable sites of attachment of biotin to Obatoclax. Limited structure-activity data have identified at least 3 possible positions, which will be described.

Elia, G. Proteomics, 2008, 8, 4012 and 2004, 4, 2296

Poster No. 45

NON-PATHOGENIC BACTERIA AS CELL THERAPY VECTORS FOR CANCER TREATMENT

C Baban[1], M Cronin[1], S Collins[1], D O'Hanlon[2], G C O' Sullivan[1], M Tangney[1]
[1]Cork Cancer Research Centre, University College Cork, Cork, Ireland
[2]Department of Surgery, South Infirmary Victoria University Hospital , Cork, Ireland

The ideal cancer treatment would eradicate tumour cells selectively with minimum side effects on normal tissue. Bacteria have emerged as gene vectors with natural tumour specificity, capable of homing to tumours and replicating locally to high levels when systemically administered. Pathogenic, invasive species have been utilised to deliver plasmid intracellularly to tumour cells, and some genera possess oncolytic capability, such as Clostridium and Salmonella. However, their inherent toxicity has outweighed therapeutic responses in patients, despite efforts to reduce toxicity through genetic modification.

A promising alternative exploits non-pathogenic bacteria. Non-invasive, apathogenic species expressing heterologous genes can secrete therapeutic proteins locally within the tumour. We are investigating a range of commensal bacteria (natural inhabitants of the human GIT, and often employed as probiotics). Our group has developed imaging systems using luminescent reporter gene (lux) tagging of various bacteria permitting real-time visualization of vector, in subcutaneous and orthotopic murine models.
We have also demonstrated that certain species (Bifidobacterium breve) are capable of trafficking to systemic tumours following oral administration, with equal efficiency to intravenous injection. Our studies indicate a low level, delayed anti-vector immune response in tumours, with no bacterial clearance from tumours for up to 1 month post administration. Through engineering of secreting constructs, these replication competent bacteria can mediate high-level production of soluble agents within tumour masses, presenting a powerful and safe approach to specific gene/cell therapy of primary tumours and secondary metastases.

Poster No.46

AAV2-mediated in vivo immune gene therapy of solid tumours

SA Collins[1,2], A Buhles[1], MF Scallan[2], PT Harrison[3], DM O'Hanlon[4], GC O'Sullivan[1], M Tangney[1]
[1]Cork Cancer Research Centre, University College Cork, Cork, Ireland
[2]Department of Microbiology, University College Cork, Cork, Ireland
[3]Department of Physiology and Biosciences Institute, University College Cork, Cork, Ireland
[4]Department of Surgery, South Infirmary Victoria University Hospital, Cork, Ireland

Background
Many strategies have been adopted to unleash the potential of gene therapy for cancer, involving a wide range of therapeutic genes delivered by various methods. Immune therapy has become one of the major strategies adopted for cancer gene therapy and seeks to stimulate the immune system to target tumour antigens. In this study, the feasibility of AAV2 mediated immunotherapy of growing tumours was examined, in isolation and combined with anti-angiogenic therapy.

Methods
Immune-competent Balb/C or C57 mice bearing subcutaneous JBS fibrosarcoma or Lewis Lung Carcinoma (LLC) tumour xenografts respectively were treated by intra-tumoural administration of AAV2 vector encoding the immune up-regulating cytokine granulocyte macrophage-colony stimulating factor (GM-CSF) and the co-stimulatory molecule B7-1 to subcutaneous tumours, either alone or in combination with intra-muscular (IM) delivery of AAV2 vector encoding Nk4 14 days prior to tumour induction. Tumour growth and survival was monitored for all animals. Cured animals were re-challenged with tumourigenic doses of the original tumour type. In vivo cytotoxicity assays were used to investigate establishment of cell-mediated responses in treated animals.

Results
AAV2-mediated GM-CSF, B7-1 treatment resulted in a significant reduction in tumour growth and an increase in survival in both tumour models. Cured animals were resistant to re-challenge, and induction of T cell mediated anti-tumour responses were demonstrated. Adoptive transfer of splenocytes to naïve animals prevented tumour establishment. Systemic production of Nk4 induced by intra-muscular (IM) delivery of Nk4 significantly reduced subcutaneous tumour growth. However, combination of Nk4 treatment with GM-CSF, B7-1 therapy reduced the efficacy of the immune therapy.

Conclusions
Overall, this study demonstrates the potential for in vivo AAV2 mediated immune gene therapy, and provides data on the inter-relationship between tumour vasculature and immune cell recruitment.

Poster No. 47

CD-33 targeted nanoparticles for specific drug delivery to acute myeloid leukaemia (AML) cells

F Fay[1], S Spence[1], S Abdelghany[2], JA Johnston[2], CJ Scott[1]
[1]Molecular Therapeutics Cluster, School of Pharmacy, Queen's University Belfast, Belfast, UK
[2]Centre for Infection and Immunity, Queen's University Belfast, Belfast, UK

The application of encapsulated drug in nanoparticulate carriers has attracted much interest for the development of formulations with improved bioavailability. Moreover, these nanoparticles can be surface modified to incorporate targeting moieties to enable specific drug delivery to diseased cells, whilst minimising off-target side effects 1, 2. Acute Myeloid Leukaemia (AML) and lymphoma cells have been shown to express elevated levels of sialic acid binding Ig-like lectins (Siglec receptors) such as Siglec 3 (CD33) on their cell surface 3, 4. This has been exploited therapeutically to target chemotherapy to diseased cells using drug-conjugated anti-CD33 monoclonal antibodies (Gemtuzumab Ozogamicin/Mylotarg®; Wyeth Ayerst).
Currently, we are examining the application of a novel poly(lactic-co-glycolic acid) (PLGA) nanoparticle formulation to target Siglec receptors for delivery of encapsulated chemotherapeutic agents to acute monocytic leukaemia cells. We have developed a formulation methodology to produce nanoparticles (dia. 150 nm), entrapping a model chemotherapy camptothecin (3 µg ± 0.5 per mg PLGA). Using a carbodiimide conjugation approach, a sialic acid, di(α,2 → 8) N-Acetylneuraminic acid, was conjugated by esterification onto the surface of the PLGA nanoparticles (15µg ± 8 per mg PLGA).

These targeted nanoparticles were then incubated with human THP1 and mouse RAW 264.7 cells and demonstrated preferential adherence and uptake to cells over control nanoparticle formulations in a Siglec specific manner. Upon internalisation, the encapsulated drug is then released resulting in improved efficacy over non-targeted nanoparticles. We are currently developing this formulation for application in vivo. This approach has the potential to be applied to specifically target diseased cells whilst minimising exposure of normal tissues to the chemotherapeutic drug cargo, thus improving both the therapeutic effect and minimising off-target side-effects.

1 Scott CJ, Marouf WM, Quinn DJ et al.: Immunocolloidal targeting of the endocytotic siglec-7 receptor using peripheral attachment of siglec-7 antibodies to poly(lactide-co-glycolide) nanoparticles. Pharm. Res. 25, 135-146 (2008). 2 McCarron PA, Marouf WM, Quinn DJ et al.: Antibody targeting of camptothecin-loaded PLGA nanoparticles to tumor cells. Bioconjug. Chem. 19, 1561-1569 (2008). 3 OReilly MK, Paulson JC: Siglecs as targets for therapy in immune-cell-mediated disease. Trends Pharmacol. Sci. 30, 240-248 (2009). 4 Nguyen DH, Ball ED, Varki A: Myeloid precursors and acute myeloid leukemia cells express multiple CD33-related Siglecs. Exp. Hematol. 34, 728-735 (2006).

Poster No.48

UNCOUPLING OF DEATH RECEPTOR INDUCED NF-KB ACTIVATION FROM APOPTOSIS

A Halpin-McCormick[1], A van der Sloot[3], L Serrano[2], A Samali[1], E Szegezdi[1]
[1]Department of Biochemistry, National University of Ireland Galway, Galway, Ireland
[2]Centro de Regulación Genómica, CRG-EMBL Systems Biology Unit, Barcelona, Spain

Traditional approaches targeting cancerous cells such as chemo- and radiation therapies lack specificity and often cause severe toxicity. This has fuelled much research into alternative therapies targeting the apoptotic pathway in tumour cells. Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL), a member of the TNF superfamily, is considered to be a promising anti-cancer agent as it induces apoptosis in only cancerous cells, but not in normal host cells.

When TRAIL binds to its two apoptosis-inducing receptors, Death Receptor 4 (DR4) and 5 (DR5), it can in parallel induce apoptotic signalling and an inflammatory, often pro-survival signalling mediated by Nuclear-Factor-kappa-B (NF-κB). It has been shown that activation of NF-κB by DR4/DR5 contributes to TRAIL resistance, and that prevention of NF-κB activation can sensitize cells to TRAIL. Activation of NF-κB by DR4/DR5 is believed to be mediated by the recruitment of the adaptor protein TNF Receptor Associated Factor (TRAF2) to receptor associated TNF Receptor Associated Death Domain (TRADD) to the TRAIL receptor signal transduction complex. Our experiments have also shown that inhibition of NF-κB upon TRAIL treatment has a synergistic cytotoxic effect. Furthermore, here we show that knockdown of TRAF2 can sensitise tumour cells to TRAIL-mediated apoptosis.

We propose that through the design of novel molecular blockers that prevent the recruitment of TRAF2 to TRADD, we can uncouple DR4/DR5 mediated NF-κB activation, and thus can restore or increase the cell's sensitivity to TRAIL-induced apoptosis.

Here we present the computational design of TRADD-TRAF2 interaction inhibitory peptides. The programme FoldX was utilised to identify the TRADD-TRAF2 interaction surface and design a peptide that by binding to TRADD masks this surface and thus blocks recruitment of TRAF2. Our current studies aim to examine the ability of these peptides to penetrate into tumour cells and assess their efficacy in blocking TRAIL-mediated NF-κB activation *in-vitro*.

Aydin, C., Sanlioglu, A.D., Bisgin, A., Yoldas, B., Dertsiz, L., Karacay, B., Griffith, T.S., and Sanlioglu, S. (2010). NF-kappaB targeting by way of IKK inhibition sensitizes lung cancer cells to adenovirus delivery of TRAIL. BMC Cancer 10, 584. Thomas, R.P., Farrow, B.J., Kim, S., May, M.J., Hellmich, M.R., and Evers, B.M. (2002). Selective targeting of the nuclear factor-kappaB pathway enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated pancreatic cancer cell death. Surgery 132, 127-134.

Poster No.49

Pre-clinical development of pro-apoptotic cancer therapeutics for the treatment of glioma: A combined *in vitro*/ *in vivo* molecular imaging approach.

MA Jarzabek[1], V Amberger-Murphy[2], A Zagozdzon[3], L Shiels[1], C Gao[4], WM Gallagher[3], JHM Prehn[1], AT Byrne[1]
[1]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland
[2]Molecular Therapeutics for Cancer Ireland, Dublin City University, Dublin, Ireland
[3]Conway Institute, University College Dublin, Dublin, Ireland
[4]Department of Microbiology and Immunology, McGill University, Montreal, Canada

Identification of novel therapeutics is critical towards improving patient survival rates and quality of life for glioma patients (1). Anti-apoptotic Bcl-2 family members contribute to cancer progression and confer resistance to apoptosis induced by standard anti-cancer therapies. One promising approach may be to inhibit tumour cell survival using pro-apoptotic agents (2).

We have assessed the efficacy of gossypol treatment alone or in combination with current standard treatment, temozolomide, in cell-based models. MTT cell viability data reveals that gossypol delivered in combination with temozolomide can improve therapeutic efficacy compared with drug treatment alone. Furthermore, we have employed an in vitro 3D glioma cell invasion assay, which gives an early indication of gossypol benefit in affecting tumour cell migration/invasion inhibitory response. We also assessed the benefit of gossypol alone or in combination with temozolomide in endothelial cell tubule formation assays. We have recently used an optically active xenogenic model of glioma to assess in vivo benefit of gossypol alone and in combination with temozolomide. We have seen reduction in SQ U87MG-luc2 tumour volume after two weeks, for animals treated daily with gossypol alone (30 mg/kg p. o.) or in combination with temozolomide (7.5 mg/kg i.p.).
We used a combined MRI/bioluminescent imaging approach towards the development of a novel imageable, locally invasive orthotopic mouse model of glioma. This model will be further used to assess the benefit of a BH3 mimetic treatment regime in combination with current standard treatment.

1. de Vries, N.A. et al., High-grade glioma mouse models and their applicability for preclinical testing. Cancer Treat Rev, 2009. 2. Hetschko, H., et al., Pharmacological inhibition of Bcl-2 family members reactivates TRAIL-induced apoptosisin malignant glioma. J Neurooncol, 2008

Poster No.50

KILLING DRUG RESISTANT TRIPLE NEGATIVE BREAST CANCER CELLS WITH THE ANTI-FUNGAL CICLOPIROX OLAMINE

D Monaghan[1], H Fearnhead[1], E O'Connell[2]
[1]Pharmacology and Therapeutics, NUI Galway, Galway, Ireland
[2]NCBES, NUI Galway, Galway, Ireland

Triple negative breast cancers (lacking Estrogen, Progesterone and HER2 receptor expression) constitute ~15% of breast cancers. Unlike Estrogen receptor positive and HER2 positive cancers, there are currently no targeted therapies for triple negative breast cancers, which are treated cytotoxic drugs. Identifying new therapies for triple negative cancers that lack the adverse effects of cytotoxic therapy is an important goal.

In addition, multi-drug resistance is a major factor limiting the effect of cytotoxic chemotherapeutics and drives the search for novel anti-cancer drugs that can kill multidrug resistant tumors.

However, conventional drug discovery is laborious, time-consuming and inefficient. An alternative approach is “repurposing” existing drugs for new uses. Repurposing is an attractive alternative as a wealth of preclinical and clinical data is already available, greatly reducing the time and resources required to bring a candidate drug to clinical trial.

We screened the John Hopkins Clinical Compound Library, using a multi-drug resistant breast cancer cell line, MDA16, which lacks functional p53, over-expresses MDR1 and is derived from the triple negative breast cancer cell line MDA-MB-468. Screening at ~10μM identified >30 “hit” compounds. One hit, an antifungal drug Ciclopirox induced apoptotic cell death in the drug-resistant cancer cells and also in MDA-MB-231, another triple negative breast cancer cell line. It did not induce apoptosis in non-triple negative breast cancer cell lines (BT474, SkBr3, MCF-7), nor in colorectal cancer cell lines (RKO, HT29, HCT8, SW480) or in a prostate cancer cell line (DU145).

These data suggest that Ciclopirox may potentially be a “selective” therapy for triple negative breast cancers even those that have acquired multiple different mutations that cause multidrug resistance to current chemotherapeutics.

Poster No.51

BENZYLIDENE LACTAM COMPOUND, KNK437, AN INHIBITOR OF STRESS RESPONSE IS A NOVEL RADIOSENSITIZER

D OOMMEN[1], **K PRISE[1]**
[1]CENTRE FOR CANCER RESEARCH & CELL BIOLOGY, QUEEN'S UNIVERSITY, BELFAST, UK

Heat shock proteins (Hsps) have been implicated in contributing resistance to ionizing radiation (IR). Hsp inhibitors such as 17- AAG (Hsp90 inhibitor in phase II clinical trials) have been investigated as potential radiosensitizers. However, 17-AAG was found to induce Hsp70 which in turn contributes to tumour resistance and undermines their potential as a radiosensitizer. Hence identification and characterization of compounds that are radiosensitizers as well as inhibitors of Hsp synthesis could increase the effectiveness of radiotherapy. KNK437 is a benzelydene lactam compound known to inhibit stress induced synthesis of Hsps. Interestingly, no significant changes in the expression of Hsps (Hsp70 and Hsp27) or the activation of heat shock factor 1 (HSF1) were observed in human glioblastoma T98G and breast cancer cell line MDAMB231 after ionizing radiation (IR). However, pre-treatment of KNK437 sensitises both cell lines to IR. Decreased cell survival of KNK437 treated cells was correlated with increased and persistent DNA damage and transient accumulation of cells in G2 phase of the cell cycle. KNK437 induced reactive oxygen species (ROS) which was further enhanced by IR. Moreover, depletion of reduced glutathione by butathionine sulfoxamine enhances the effect of KNK437 and suggested a prominent role for ROS. Interestingly, KNK437 found to down regulate hypoxia inducible factor 1 alpha (HIF 1α), a pro-survival transcriptional factor activated in hypoxic tumour environment. Taken together, these data suggest that KNK437 is an effective radiosensitizer with the potential to override the acquired tumour resistance in a hypoxic environment.

Poster No.52

COMPARATIVE ANALYSIS OF PYROSEQUENCING AND SEQUENCING FOR THE DETECTION OF KRAS AND BRAF MUTATIONS IN COLORECTAL CANCER

RJ Carson[1], S Van Schaeybroeck[1], MA Catherwood[1,2], PG Johnston[1]
[1]Oncology, Centre for Cancer Research and Cell Biology, Belfast, Northern Ireland
[2]Haemato-Oncology, Belfast City Hospital, Belfast, Northern Ireland

Background: Mutations in the Kras proto-oncogene are found in 40-45% of patients with colorectal cancer (CRC) and occur mainly in codon 12 and 13 and to a lesser extent in codon 61 and codon 146. The high prevalence of Kras mutations in CRC and its strong negative predictive value for EGFR monoclonal antibody therapies, has led to its rapid acceptance as a valuable biomarker. The Braf V600E mutation is mutually exclusive with mutations in Kras and occurs in around 8-15% of KrasWT CRC patients. As well as acting as a poor prognostic factor, activating mutations in Braf also predict for non-response to EGFR inhibitors.
Aims: The aim of this study was to identify a reliable method for detection of mutations in Kras and Braf that we could use routinely in clinical practice.
Methods: Genomic DNA was extracted using the Qiagen DNeasy spin column kit. PCR was performed to amplify the regions for mutational analysis. We used the Qiagen PyroMark Q24 MDx system and ABI BigDye Terminator Sequence v3.1 for pyrosequencing and dideoxy sequencing respectively.
Results: DNA was successfully extracted from 38/40 samples. 12 samples (32%) had either a mutation in Kras or Braf. We found 3 Kras codon 12 mutations (GGT > GAT), 1 Kras codon 13 mutation (GGC > GAC), 1 Kras codon 61 mutation (CAA > CTA), 2 Kras codon 146 mutations (GCA > ACA) and 5 Braf (V600E) mutations. All mutations were confirmed by dideoxy sequencing and this method did not detect additional Kras/Braf mutations in the remaining samples.
Conclusions: Our results indicate that pyrosequencing is a sensitive method for the detection of Kras and Braf in CRC samples. We are now performing a blind validating of the test in a larger cohort of CRC samples with known Kras and Braf mutational status.

Tu D, D P, Tebbutt NC, et al. K-ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer. New England Journal of Medicine. 2008:1757-1765. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nature reviews. Cancer. 2007;7(4):295-308. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17384584. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27(5):663-71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19114683. Neumann J, Zeindl-Eberhart E, Kirchner T, Jung A. Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer. Pathology, research and practice. 2009;205(12):858-62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19679400 Wang HL, Lopategui J, Amin MB, Patterson SD. KRAS Mutation Testing in Human Cancers : The Pathologist ' s Role in the Era of Personalized Medicine. Advances in Anatomic Pathology. 2010;17(1):23-32. Oliveira C, Westra JL, Arango D, et al. Distinct patterns of KRAS mutations in colorectal carcinomas according to germline mismatch repair defects and hMLH1 methylation status. Human Molecular Genetics. 2004;13(19):2303-2311. Dufort S, Richard M-J, Fraipont F de. Pyrosequencing method to detect KRAS mutation in formalin-fixed and paraffin-embedded tumor tissues. Analytical biochemistry. 2009;391(2):166-8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19464247

Poster No.53

MICRORNA PROFILING IN SERUM/ BLOOD OF OVARIAN CANCER

R I Langhe[1], S A O' Toole[1], L M McEvoy[1], L A Norris[1], NC Gleeson[2], T D'Arcy[2], D O' Donnell[3], O Sheils[4], JJ O'Leary[4]
[1]Obstetrics&ampGynecology, Trinity centre for health sciences in St James Hospital, Dublin, Ireland
[2]Obstetrics&ampGynecology, St James Hospital, Dublin, Ireland
[3]Medical oncology, St James Hospital, Dublin, Ireland
[4]Histopathology, St James Hospital, Dublin, Ireland

Ovarian cancer is the fourth leading cause of death in women. Approximately 190,000 cases occur worldwide annually with over 350 new cases in Ireland. The vast majority present in advanced stages and this is due to lack of reliable screening test and the absence of symptoms. Despite an initial response rate of 65%–80% to first-line chemotherapy, most ovarian carcinomas relapse. Acquired resistance to further chemotherapy is generally responsible for treatment failure.
Our aim is to profile MicroRNAs in serum /blood from ovarian cancer patients in order to improve the detection and treatment of this disease.
Blood and serum is being collected from all patients undergoing surgery for ovarian cancer and benign ovarian disease. Additional samples are being taken from patients undergoing chemotherapy for ovarian cancer. Ethical approval has been received for this study. RNA extraction from serum/ blood was optimised using modified TRI Reagent ® RT-Blood protocol. Profiling is being carried out using the TaqMan® array MicroRNA cards. Currently, we are profiling 10 cases serous papillary ovarian carcinoma and benign serous cystadenoma as a training set. Validation will be carried out on a larger sample population.

So far 5 malignant and 6 benign cases have been profiled.microRNAs which were expressed in malignant samples but not the benign ones include miR 18a,19b,21,29a,c,30a-5p,30d,93,103,106a,b,126,140,145,144,151-3p, 185,197,223,320,324-3p,331,338,342-3p.378, 409-3p, 520c-3p, 625,720,766, 942, 1274a, b, 1305 were expressed in malignant samples only. Around 180 and 160 out of 384 microRNAs were expressed from both cards.
144,151-3p, 342-3p, 409-3p, 520c.3p, 720, 942, 1274a, b and 1305 have not been previously described in ovarian cancer and warrant further validation
MicroRNAs signatures from ovarian cancer serum/ blood may improve detection and treatment of ovarian cancer.

Poster No.54

The IGF-I inducible Pyrimidine Nucleotide Carrier (PNC1) regulates mitochondrial biogenesis and the invasive phenotype of cancer cells.

C Favre, A Zhdanov, M Leahy, D Papkovsky, R O'Connor
[1]Biochemistry, University College Cork, Cork, Ireland

The Insulin-like Growth Factor-I (IGF-IR) is a transmembrane receptor tyrosine kinase which has important functions in regulating metabolism, survival, and carcinogenesis. Although activation of the PI3-kinase/Akt/mTOR signalling pathway may largely mediate the effects of this receptor on cell proliferation and survival, it is not clear that this pathway mediates all of the effects of IGF-IR signalling on cancer progression, in particular cell migration and invasion. We recently identified a new mitochondrial carrier whose expression is induced by IGF-I. This carrier was designated Pyrimidine Nucleotide Carrier 1 (PNC1) based on its ability to transport pyrimidine nucleotides (UTP) into mitochondria (1).

In order to identify the function of PNC1 in mammalian cells we have investigated the effects of stable and transient suppression of PNC1 on mitochondrial function, cellular signalling pathways, and cell phenotype.
We have shown that PNC1 is essential for mitochondrial function by controlling mtDNA replication and transcription and by maintaining the ratio of mitochondrial to nuclear-encoded component of the respiratory chain. This imbalance in components of the respiratory chain reduces mitochondrial ATP production and promotes leakage of mitochondrial ROS. This leaks of ROS leads to the activation of a retrograde pathway involving ROS-CAMKK2-AMPK-PGC1α and to the increased transcription of nuclear-encoded mitochondrial genes accentuating further more the imbalance of the respiratory chain.

This release of mitochondrial ROS observed under PNC1 suppression also causes dramatic morphological and phenotypic changes in HeLa and MCF-7 cells with acquisition of motile and invasive characteristics associated with activation of a ROS-dependent epithelial mesenchymal transition (EMT).

Taken together our data indicate that PNC1 has an essential function in mtDNA replication and transcription and it can regulate the growth and differentiation of transformed cells. These findings reveal a new way in which the IGF-I/mTOR signalling pathway controls cellular growth by controlling mitochondrial functions and ATP production (2).

Grant: Health Research Board Posdoctoral fellowship PD/2008/22. 1. The insulin-like growth factor-I-mTOR signaling pathway induces the mitochondrial pyrimidine nucleotide carrier to promote cell growth. Floyd S, et al., Mol Biol Cell. 2007 Sep;18(9):3545-55. 2. Mitochondrial pyrimidine nucleotide carrier (PNC1) regulates mitochondrial biogenesis and the invasive phenotype of cancer cells. Favre C, et al., Oncogene. 2010 Jul 8;29(27):3964-76.

Poster No.55

Regulation of PML by NF-κB

A Ahmed, R Carmody
[1]Department of Biochemistry, University College Cork, Cork, Ireland

Nuclear factor-kappa B (NF-κB) plays a critical role in various physiological and cellular processes such as cell proliferation, inflammatory response and cancer and is one most widely studied signalling pathways. Promyelocytic leukaemia protein bodies (PML NBs) are sub-nuclear structures that have been implicated in multiple processes such as apoptosis, senescence and anti-viral responses. Here we demonstrate that PML regulates NF-κB activity by blocking ubiquitination and subsequent proteosomal degradation of NF- κB. However, the precise mechanism behind PML-mediated regulation NF-κB is unclear. Using a mutagenesis approach we have mapped the region of PML essential for interaction and stabilization of NF-kB. In addition we present data on the role of SUMOylation and the PML SUMO interacting motif (SIM) on NF- κB stabilization and transcriptional activity. This data contributes to our understanding of PML mediated control of NF-kB with implications for the role of NF-kB in cancer.

Bernardi, Pandolfi (2008) Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. Nat Rev Mol Cell Biol. Dec;8(12):1006-16. Lallemand-Breitenbach V, de Thé H (2010) PML nuclear bodies. Cold Spring Harb Perspect Biol. May 1;2(5):a000661. Apr 21. Jensen K, Shiels C, Freemont PS (2001) PML protein isoforms and the RBCC/TRIM motif. Oncogene. Oct 29;20(49):7223-33. Brand P, Lenser T, Hemmerich P (2010) Assembly dynamics of PML nuclear bodies in living cells. PMC Biophys. Mar 5;3(1):3.

Poster No.56

THE PREDICTION OF RADIOTHERAPY RESPONSE "IN VITRO" FOR CERVICAL CANCER PATIENTS.

S. O'Grady[1,2], C Gillham[3], F Lyng[2], O Howe[1,2]
[1]Biological Sciences, Dublin Institute of Technology, Kevin Street , Dublin 8, Ireland
[2]Radiation and Environmental Science Center, The Focas Research Institute, DIT, Dublin 8, Ireland
[3]Radiation Oncology, St. Lukes Hospital, Rathgar, Dublin 6, Ireland

Introduction: Tumour response rate and duration of cervical carcinoma treated by radiotherapy varies between individuals. Individual prediction of tumour response to treatment could allow individualised treatment by choosing the best treatment option and /or treatment adaptation. p16INK4A overexpression has been demonstrated in cervical cancers as a result of functional inactivation of RB by the HPV E7 protein thus making this CDK inhibitor an ideal candidate as a biomarker for the disease. There has also been evidence to show a significant association between the overexpression of the tumour suppressor protein p73 and cellular radiosensitivity in cervical cancers after radiotherapy thus underlining it as a possible marker of radiosensitivity.
The aim of this project is to attempt a correlation of the radiosensitivity scores of each patient sample to the expression of these genes to validate their use as respective biomarkers together with the in vitro radiosensitivity assay as potential tools of response in cervical cancer patients to radiotherapy.
Materials and Methods: Whole blood lymphocyte cultures were irradiated in vitro with low doses of gamma radiation. The cultures were used for the G2 chromosomal radiosensitivity assay to assign each patient sample with an intrinsic radiosensitivity score. Radiosensitivity data will be created and analysed for each patient sample. Furthermore, Real-time PCR will be conducted on cDNA synthesised from RNA extracted from the blood cultures to identify potential biomarkers of cervical carcinoma response to radiotherapy.
Results: Preliminary results show that each cervical cancer patient, based on their radiosensitivity score, shows an individual response to radiation regardless of the grade of malignancy. This data will be presented, along with the in vitro culturing and processing of patient samples for the parallel gene expression studies.
Conclusion: It is anticipated this study will demonstrate the potential of testing individual patients for radiation response prior to therapy, thus resulting in more tailored treatment plans with higher efficacy.

Poster No.57

INVESTIGATING THE POTENTIAL OF CIRCULATING EXOSOMES AND THEIR MIRNA CONTENTS AS MINIMALLY-INVASIVE BIOMARKERS FOR TRIPLE NEGATIVE BREAST CANCER

S Rani[1], K O'Brien[1], A Friel[1], FC Kelleher[2], J Crown[3], L O'Driscoll[1]
[1]School of Pharmacy & Pharmaceutical Sciences and MTCI, Trinity College Dublin, Dublin, Ireland
[2]Department of Medical Oncology, St. Vincent's University Hospital, Dublin, Ireland
[3]Molecular Therapeutics for Cancer Ireland , NICB, Dublin City University, Dublin, Ireland

Introduction: Triple-negative breast cancer (TNBC) represents approximately 15-20% of invasive breast cancers, generally occurs in younger women, and is responsible for a disproportionate number of breast cancer deaths. There is thus an urgent need to identify minimally-invasive biomarkers to enable its early diagnosis. Recent studies, by ourselves and others, analysing circulating miRNAs on a one-at-a-time basis, suggest that such miRNAs may have potential as biomarkers. Our aim here was to apply global profiling approaches to explore if miRNAs exist in circulating exosomes from TNBC patients and, if so, do they have potential as diagnostic biomarkers. Methods: This proof-of-principle study involved exosomes isolation from serum procured from TNBC patients and age- and gender-matched controls (n=10 each), using combined filtration and ultracentrifugation techniques. Nucleic acids were isolated from the exosomes and ~670 miRNAs were co-analysed. Individual miRNAs were selected for qPCR validation.
Results: Successful isolation of exosomes was confirmed by Western blotting, detecting TSG101. While the quantities of exosomes in TNBC and control sera did not differ significantly, 226/667 miRNAs were detected in exosomes from TNBC compared to 209/667 in control sera. A panel of seven miRNAs were at >1.2 fold higher levels in TNBC compared to control exosomes. The trend observed using subsequent qPCR analysis reflected our global screening ($p<0.05$). Furthermore, a positive correlation was noted between miRNA expression and tumour stage, although the numbers of specimens included was too limited to derive a meaningful outcome on this. Conclusion:

This, we believe, is the first report of exosomal miRNA profiling from TNBC serum specimens. Differences in miRNA profile identified here suggest that circulating exosomal miRNAs may have potential as diagnostic biomarkers for TNBC. More extensive studies including larger cohorts of serum/exosome specimens from TNBC, as well as other breast cancer subtypes and controls, are warranted to further explore this initial discovery.

Acknowledgements: We wish to thank the Marie Keating Foundation in the form of its PhD Scholarships at Trinity College Dublin (KOB), Trinity Foundation, and Science Foundation Ireland for the Strategic Research Cluster award to Molecular Therapeutics for Cancer Ireland (MTCI; 08/SRC/B1410).

Poster No.58

HUMAN PAPILLOMAVIRUS DNA/mRNA DETECTION AND SMOKING AS A RISK FACTORIN PREDICTING PROGRESSION OF LOW GRADE CERVICAL ABNORMALITIES TO HIGH GRADE CERVICAL DISEASE

C White[1, 2], C Ruttle[2], L Pilkington[2], J McInerney[1, 2], H Keegan[1, 2], S O' Tool[1], C Spillane[1, 2], L Sharp[3], R O' Kelly[2], M Fitzpatrick[4], P Lenehan[4], G Flannelly[4], J O' Leary[1, 2], C Martin[1, 2]

[1]Histopathology, Trinity College Dublin, Dublin, Ireland
[2]Molecular Pathology, Coombe Women's and Infants Universtiy Hopsital, Dublin, Ireland
[3], National Cancer Registry, Cork, Ireland
[4]Obstetrics & Gynaecology, National Maternity Hospital, Dublin, Ireland

Background: High Risk Human Papillomavirus (HR-HPV) infection is identified in up to 80% of women with low grade cervical abnormalities, most of which regress spontaneously. Thus co-factors are believed to be involved in the transition from transient to transforming HPV infections. This study evaluates the significance of smoking, through urinary cotinine analysis, and HPV infection in development of high grade disease. The study is performed under CERVIVA funded by the Health Research Board Ireland and the Irish Cancer Society.
Methods: A urine sample for cotinine analysis and a smear specimen for HPV testing were collected from 618 women presenting with LSIL (low-grade squamous intraepithelial lesion) and ASCUS (atypical cells of undetermined signifance) at their first visit to colposcopy at the National Maternity Hospital, Dublin. HPV DNA was detected by Hybrid Capture II (Qiagen, UK), HPV mRNA by the PreTect™ HPV Proofer (NorChip AS, Norway) and cotinine analysis by the Immulite Nicotine Metabolite assay (Siemens, UK).
Results: Histology confirmation shows 21% (127/618) were CIN1 (cervical intraepithelial neoplasia 1), 24% (147/618) were CIN2+ and 52% (321/618) were normal. The overall prevalence of HR-HPV DNA was 62% (383/618) compared with 38% (232/618) E6/E7 mRNA positive cases. The likelihood of being mRNA positive decreased with age (30-39 vs <30: OR=0.61, 95%CI 0.39-0.95; 40+ vs <30: OR=0.47, 95%CI 0.26-0.86). Of the 618 women 160 were treated by LLETZ. HPV prevalence rates in this cohort are 75% and 49% for HPV DNA and mRNA respectively. For detection of CIN 2+ lesions the sensitivity and specificity was 85% and 52% for HPV DNA and 58% and 80% for HPV mRNA. HPV mRNA was detected in 35% (113/318) of non-smokers (cotinine <50ng/ml) compared with 43% (95/223) of smokers (cotinine >50ng/ml).
Conclusion: HPV testing is a useful tool in the management of women presenting with low grade abnormalities. In addition it suggests that women who smoke are at increased risk of persistent HPV infection, associated with progression to high grade cervical lesions.

Poster No.59

INCREASING TUMOUR CELL PERMEABILITY TO CHEMOTHERAPEUTIC AGENTS RESULTS IN ENHANCED RESPONSES IN INTRACTABLE CUTANEOUS LESIONS FROM BREAST CANCER

MG Bourke[1], S Salwa[1], P Forde[1], JO Larkin[1], M Sadadcharam[1], MC Whelan[1], JP Cashman[1], CG Collins[1], DM Soden[1], GC O'Sullivan[1]
[1]Cork Cancer Research Centre, Biosciences Institute, University College Cork, Cork, Ireland

Introduction
Electrochemotherapy (ECT) is the application of electric pulses to tumour tissue to render the cell membranes permeable to impermeant anticancer drugs and thereby facilitate a potent cytotoxic effect[1]. Cutaneous manifestations of breast cancer affect over 20% of patients and typically occur in the setting of disseminated, intractable disease. They are a significant clinical problem because of evident disease progression, pain, bleeding and ulceration. Current treatment modalities are associated with limited responses[2]. ECT offers the potential for an improved response rate in metastatic cutaneous lesions.

Methods
We performed a retrospective audit of our experience treating intractable cutaneous manifestations of breast cancer with ECT. In combination with systemic and/or intratumoural bleomycin or cisplatin, optimised electric pulses were delivered to locally recurrent cutaneous breast cancer lesions. Patients were assessed at regular intervals to assess responses.

Results
18 patients were treated, all of whom were female. The average age at first treatment was 62.2 years (47-86). All patients had had prior multimodal therapy including mastectomy. In total 139 lesions were treated, including 5 (3.6%) lesions of greater than 5cm2. A response to treatment was seen in 110 lesions (79.1%), 83 complete responses and 27 partial responses. 24 lesions (17.3%) did not respond. Five lesions were not assessed following treatment as the patient was lost to follow-up. Of the 5 lesions greater than 5cm2, two had a partial response to treatment while 3 did not respond. In total the patient cohort received 56 treatments, though 15 patients (83%) received less than 4 treatments. 13/18 patients responded with worthwhile palliation.

Conclusion
Electrochemotherapy is an effective treatment for cutaneous breast cancer lesions that have proven refractory to conventional therapies.

1. Larkin JO, Collins CG, Aarons S, Tangney M, Whelan M, O'Reily S, et al. Electrochemotherapy: aspects of preclinical development and early clinical experience. Ann Surg 2007, 245:469-79. 2. De Giorgi V, Grazzini M, Alfaioli B, Savarese I, Corciova SA, Guerriero G, et al. Cutaneous manifestations of breast carcinoma. Dermatol Ther 2010, 23:581-9.

Poster No.60

PRO-INFLAMMATORY AND PRO METASTATIC PATHWAYS ARE ACTIVATED BY OBESITY IN BARRETT'S METAPLASIA

R Casey[1], N Ravi[1], JV Reynolds[1], GP Pidgeon[1]
[1]Dept. Surgery, Trinity College, Dublin, Ireland

Introduction

Barrett's oesophagus, a squamous to columnar cell metaplastic condition in the oesophagus, is the only known clinical precursor of oesophageal adenocarcinoma. Excess adiposity is understood to be a causal factor in Barrett's oesophagus and oesophageal adenocarcinoma, though the exact molecular mechanisms remain elusive.

Methods

Adipose conditioned media (ACM) was prepared from omental adipose tissue from viscerally obese male oesophageal adenocarcinoma patients. Non-neoplastic Barrett's epithelial cells, were cultured in the presence of this ACM over a 24hr period and variations in gene expression was analysed using Human cancer pathway profilers (SuperArray). Genes that showed significant up/down regulation were validated via q-PCR. Gene targets identified in vitro were subsequently analysed in Barrett's metaplasia biopsies from both obese and non obese patients.

Results

After culture in ACM, non-neoplastic Barrett's epithelial cells showed altered expression of a range of genes associated with tumorigenesis. MMP1, IL-8 and VEGFA mRNA levels, increased after ACM treatment while TIMP-3, and MCAM, mRNA levels decreased. Expression of these targets were then analysed in Barrett's metaplasia biopsies from obese and non obese patients. Both MMP-1 ($p= 0.0413$) and IL-8 ($p=0.0361$) expression were significantly up-regulated, in Barrett's metaplasia from obese patients compared to non obese patients.

Conclusion

Growth of non-neoplastic Barrett's epithelial cells in ACM, altered the expression of several well established oncogenes. In Barrett's metaplasia from obese patients, MMP-1 and IL8 mRNA expression were significantly up-regulated compared to non obese patients. This adipose tissue derived IL-8 may contribute to the inflammatory microenvironment arising from excess adiposity, potentially contributing to the carcinogenenic progression of Barrett's oesophagus.

Poster No.61

INSULIN-LIKE GROWTH FACTOR-1 IS ELEVATED IN VISCERALLY OBESE ESOPHAGEAL ADENOCARCINOMA PATIENTS AND EXPRESSION OF ITS RECEPTOR CORRELATES WITH TUMOUR INVASIVENESS

SL Doyle[1], CL Donohoe[1], FE Lithander[2], JV Reynolds[1], GP Pidgeon[1], J Lysaght[1]
[1]Dept. Surgery, Trinity College, Dublin, Ireland
[2]Dept. Clinical Medicine, Trinity College, Dublin, Ireland

The insulin-like growth factor (IGF) pathway and visceral obesity have been independently linked with esophageal cancer development. This study aimed to delineate the differential and interlinked role of visceral obesity and the IGF-1 system in esophageal adenocarcinoma and squamous cell carcinoma (SCC).
IGF-1 receptor (IGF-1R) mRNA and protein were examined in esophageal SCC (KYSE-410, OE21) and adenocarcinoma (OE19, OE33) cell lines and tumor sections by Western Blotting and immunohistochemistry. Tumor cell proliferation in response to IGF-1 was assessed by BrdU incorporation assay. Circulating levels of IGF-1 were measured in 99 serum samples by ELISA. Results were related to tumor type, gender and obesity status.
Higher IGF-1R mRNA and protein expression were observed in SCC cells, however only adenocarcinoma cell lines showed significantly increased cell proliferation in response to IGF-1 ($p<0.01$). Significantly higher circulating levels of IGF-1 were detected in patients who had adenocarcinoma ($p<0.05$) and who were viscerally obese ($p<0.05$). In resected esophageal adenocarcinoma, IGF-1R expression significantly increased as the tissue progressed along the malignant sequence, with the highest expression observed at the invasive leading edge of the tumor. Uniform IGF-1R expression was observed in SCC tumors.

This novel study is the first of its kind to examine both the differential role of the IGF system in esophageal adenocarcinoma and SCC, and also its association with visceral obesity. These data indicate that in esophageal adenocarcinoma, the IGF-1 axis plays a key role in malignant progression and represents a plausible mechanism by which visceral obesity increases esophageal adenocarcinoma risk.

Poster No.62

A PROTEOMICS AND BIOINFORMATICS APPROACH TOWARDS BIOMARKER PANEL IDENTIFICATION IN PROSTATE CANCER: FROM DISCOVERY TO TARGETED VALIDATION

Y Fan[1], TB Brendan[2], JC Byrne[1], R Inzitari[1], C Rooney[1], B Collins[1], H Klocker[3], JM Fitzpatrick[4], S Pennington[1], RWG Watson[1]

[1]UCD School of Medicine and Medical Science, University College Dublin, Dublin, Ireland

[2]UCD School of Mathematical Sciences, University College Dublin, Dublin, Ireland

[3]Innsbruck Medical University, Innsbruck, Austria

[4]Mater Misericordiae University Hospital, Dublin, Ireland

Prostate cancer (PCa) remains the most common form of male cancer in Ireland with reported incidence of 2720 in 2007. With early diagnosis, most men affected by PCa can be treated effectively through surgery or radiation therapy to prevent the tumour from further growth and metastasis. The accurate determination of the presence of PCa and organ confined or locally advanced disease is essential in the clinical decision making for the patient management strategy. There is an urgent need for the identification and validation of clinically relevant biomarker panels which will improve the diagnosis and management of PCa.

Preclinical serum samples were collected from men with different grades and stages of PCa, benign prostate hyperplasia and age matched health controls as part of the Irish prostate cancer research consortium bioresource and Innsbruck Prostate cancer bioresource. After removing 6 most abundant serum proteins, proteomics profiling of these serum samples was carried out using 2D-DIGE and label-free LC-MS/MS experiments. Biomarker panels were identified and assessed by feature selection and classification using Random Forests method. The prediction performance of the selected biomarker panel was assessed using 10 fold cross validation and AUC values were calculated. The BPH and PCa panel gives an AUC value of 0.926, and the panel for predicting disease stages achieved AUC value of 0.742, which provide improved prediction accuracies than current standard clinical tools. The validation of two proteins Haptoglobin and Glutathione Peroxidise 3 using western blotting on independent samples (n= 80) confirmed the 2D-DIGE results.

Multiple Reaction Monitoring (MRM) experiment was designed and optimized as a multiplexing assay for the validation of 64 proteins which include the identified biomarker panels, potential biomarkers from label-free LC-MS/MS experiment and literature. The MRM methods were applied to both crude and depleted serum samples. We have demonstrated that the MRM method is sensitive enough to detect peptides in crude serum. We are currently validating the developed MRM methods on independent serum sample from Ireland and our international collaborators. With careful validation, these panels in combination with current clinical tools could improve diagnosis and thus patient outcome through the selection of appropriate treatment options.

Poster No.63

AN ENHANCED NON VIRAL (EEV) GIVING IMPROVED RESPONSES IN IMMUNOGENE MELANOMA TREATMENT OVER STANDARD VECTORS

PF Forde[1], LJ Hall, M Sadadcharam, M de Kruijf, GC O' Sullivan, D Soden
[1]Cork Cancer Research Centre, UCC, Cork, Ireland

We compared the efficacy of an enhanced expression vector (EEV) with CMV standard plasmid vector for non viral immunogenetherapy of a mouse melanoma model. The EEV contains its own RNA replicase and transcribes mRNA cytoplasmically. The transgenes LacZ and GM-CSF/B71 were under the control of the CMV promoter. Comparison of the EEV with the standard vector in normal and growing tumours used quantitative RT-PCR, histological tools and in vivo murine tumour growth and survival curves. In all normal tissues (liver, spleen, and colon and oesophageal epithelium) there were significant increased RNA/DNA ratios for the EEV over the standard plasmid. The EEV gives superior results than standard vector in non viral immunogene therapy of solid growing melanoma tumours. The EEV was curative and has potential for clinical development. Antitumor responses of the EEV vector were both from immune cell recruitment to the tumour environment and the unrestrained RNA production in the cytoplasm.

Poster No.64

TARGETING THE INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR (IGF-1R) SIGNALLING PATHWAY IN NON-SMALL CELL LUNG CANCER (NSCLC)

L Forde[1], K Gately[1], S Gray[1], K O'Byrne[1]
[1]Thoracic Oncology research group, Department of Clinical Medicine, Institute of Molecular Medicine, TCD, Dublin, Ireland

Introduction:

Lung cancer is the commonest cause of cancer related deaths worldwide and has a very poor prognosis. The Insulin-like growth factor receptor (IGF-1R) has emerged as an attractive target for anti-cancer drug development. IGF-1R overexpression has been identified in several tumour types and protects cells from apoptosis-inducing agents, including hypoxia and anti-cancer drugs.

Methods:

IGF-1R and EGFR expression was evaluated on a cohort of 200 patients which had undergone lung tumour resection surgery using immunohistochemistry analysis. The same cohort of patients were also screened for mutations in the tyrosine kinase domain of the IGF-1R. Western blot analysis was carried out on 40 patients for IGF-1R and EGFR expression on matched tumour and normal tissue.

Results:

Results showed that patients that had high expression of both IGF-1R and EGFR had poorer survival compared to those which only had high expression of either receptor alone. The percentage of patient tissue samples with IGF-1R and EGFR expression was found to be 76.4% and 87.5% respectively. High coexpression of EGFR and IGF-1R was identified in 35% of the cohort of NSCLC patients and was associated with a poorer overall survival on univariate and multivariate analysis ($p=0.048$; $p=0.05$ respectively). Results from clinical trials have indicated that combination drug therapy may overcome the problem of drug resistance. The sensitivity of NSCLC cell lines to erlotinib, (an EGFR inhibitor) and/or R1507, (an IGF-1R inhibitor) were compared. The IGF-1R gene was then knocked down using siRNA to examine if sensitivity to these drugs increased. Cell proliferation was reduced in A549 and HCC827 cells treated with IGF-1R siRNA and/or erlotinib and R1507 compared to cells treated with either drug alone.The expression of downstream signaling molecules, pAkt and pERK, were also examined following IGF-1R gene silencing.

Conclusion:

Further investigation into the interaction and regulation of the IGF-1R and EGFR signaling pathways is warranted to elucidate the mechanisms involved in drug resistance.

Poster No.65

HIGH LEVELS OF IMMATURE BLOOD VESSELS IN COLORECTAL TUMOURS CORRELATES WITH LONGER SURVIVAL FOLLOWING BEVACIZUMAB TREATMENT

S Noonan[1], P Martin [1], A Maguire[1], M Biniecka[1], M Tosetto[1], J Hyland[1], K Sheahan[1], D O'Donoghue[1], H Mulcahy[1], D Fennelly[1], J O'Sullivan[1]
[1]Centre for Colorectal Disease, St Vincent's University Hospital, Dublin, Ireland

Introduction: Bevacizumab improves overall survival in metastatic colorectal cancer patients. Currently, bevacizumab is a first line treatment in all metastatic colorectal cancer patients, however only 38-44% respond. Currently, there is no good marker to depict treatment response. Tumour microvascular density has been shown be a prognostic factor in late stage colorectal cancer patients, however, it does not predict response. Blood vessels mature by the recruitment of pericytes. We hypothesise those blood vessels that lack pericytes will be more susceptible to regression to Bevacizumab.

Methods: 80 patients had a primary colorectal cancer resection and were subsequently treated with bevacizumab in our tertiary referral centre of excellence. Tumours were stained using dual immunofluorescence staining for factor VIII (an endothelial marker) and α-smooth muscle actin (a pericyte marker). Levels of immature and mature blood vessels were screened using fluorescent microscopy, scoring multiple fields of view. The median levels of immature and mature blood vessels were scored and correlated with overall and progression free survival using Spearman correlations and multivariate analyses.

Results: 37 patients were metastatic at diagnosis and 43 were initially Duke's A, B or C at diagnosis (early stage) and subsequently developed metastases. Mean duration on bevacizumab was 10.6 months. Mean overall survival was 38.6 months. Interestingly, there was no difference between levels of immature and mature vessels in tumours of early stage patients and metastatic patients. Patients with higher levels of immature blood vessels had longer survival following treatment (p value = 0.026). This remained significant following multivariate analyses correcting for gender, stage at diagnosis, whether patients received chemotherapy before or after treatment with bevacizumab and whether or not bevacizumab was first line or not.

Conclusion: We have shown for the first time that the maturity levels of blood vessels in tumours significantly correlates to overall survival on Bevacizumab.

Poster No.66

MITOCHONDRIAL INSTABILITY IS INCREASED DURING DISEASE PROGRESSION IN BARRETTS OESOPHAGUS

NJ O'Farrell[1], M Biniecka[1], EJ Fox[2], SL Picardo[1], N Ravi[1], D O'Toole[3], JV Reynolds[1], JN O'Sullivan[1]

[1]Department of Surgery, Institute of Molecular Medicine, Trinity Centre, St. James's Hospital, Dublin 8, Ireland

[2]Department of Pathology, University of Washington, Seattle, WA, USA

[3]Department of Gastroenterology, Institute of Molecular Medicine, Trinity Centre, St. James's Hospital, Dublin 8, Ireland

Background:

Barrett's Oesophagus is a premalignant lesion which is associated with a 30-40 fold increased risk of developing oesophageal cancer. Understanding the early cellular instability events may allow us to better stratify those in the high risk group. The mitochondria is highly susceptible to mutations due to high levels of reactive oxygen species generated in this organelle, coupled with a low level of DNA repair and random polymorphisms. Defects in mitochondrial function have long been suspected to contribute to the development and progression of cancer, however their timing and functional relevance during disease progression in Barrett's oesophagus is unknown.

Aim:

Using a novel random capture mutation assay we determined the frequency of random mitochondrial mutations in histologically confirmed Barrett's Oesophagus biopsies and in matched normal biopsies from the same patients. Mutation frequency was also determined in resected oesophageal tumour tissue and matching normal tissue.

Results:

A 13.7% increase in the frequency of mitochondrial point mutations was detected in Barrett's biopsies compared to matching normal biopsies from the same patients. A statistically significant increase in the frequency of point mutations was detected in tumour tissue compared to Barrett's biopsies ($p=0.02$). Interestingly, analysis of the tumour and matched normal tissue biopsies revealed a significant increase in the frequency of mutations in surrounding normal tissue from cancer patients ($p=0.035$).

Conclusions:

Our data demonstrates that mitochondrial instability is an early event in Barrett's Oesophagus and significantly increases in oesophageal tumours. The lower levels of mutations in oesophageal tumours compared to matched adjacent normal tissue could be due to the Warburg effect where anerobic metabolism is activated in cancer cells. Tumour cells may protect themselves from oxidative damage by switching to glycolysis instead of oxidative phosphorylation as a means of generating ATP, resulting in lower levels of mitochondrial mutagenesis.

Poster No.67

PROTEOMIC ANALYSIS OF DIFFERENTIALLY EXPRESSED PROTEINS IN PATIENTS WITH METASTATIC COLORECTAL CANCER RESPONDING TO BEVACIZUMAB

P Martin[1], S Noonan[1], B Nolan[1], C Scaife[2], G Elia[2], M Tosetto[1], J Hyland[1], K Sheahan[1], D O'Donoghue[1], H Mulcahy[1], D Fennelly[1], J O'Sullivan[1]
[1]Centre for Colorectal Disease, St Vincent's University Hospital, Dublin, Ireland
[2]Protein Separation Lab, UCD, Dublin, Ireland

Objective/ Background - Treatment of patients with metastatic colorectal cancer includes chemotherapy and a monoclonal antibody (Cetuximab or Bevacizumab). Patients who have k-ras mutated tumours are given Bevacizumab. However, no biomarker exists to determine those patients who will respond to this targeted treatment. The objective of this study was to investigate the differential protein expression between patients who do and do not respond to bevacizumab and compare this with normal controls.

Methods- Serum from 24 patients diagnosed with metastatic colorectal cancer and 11 normal controls were collected pre-treatment and stored. All patients received Bevacizumab along with chemotherapy. Progression free and overall survival data was collected on all patients. Serum was depleted of high abundant proteins and protein expression analysed using fluorescence two-dimensional differential in-gel electrophoresis (2 D-DIGE). Gels were scanned using a Typhoon 9410 Variable Mode Imager (GE Healthcare). The images were cropped (ImageQuant 5.2, GE Healthcare) and exported into Progenesis SameSpots v3.3 (Nonlinear Dynamics, UK) for quantitative analysis.

Results- 80 spots were differentially expressed between responders and non-responders of Bevacizumab, and of these, 10 spots had significant power (80%) to be carried forward for subsequent mass spectrometry analysis. 214 spots were differentially expressed between cancer patients and normal controls ($p<.05$) and 99 of these had power > 0.8.

Conclusion- There is a significant difference in protein expression patterns between responders and non responders to Bevacizumab. Mass spectrometry is currently identifying these proteins which could used as potential biomarkers of response to Bevacizumab and help us understand resistance to this targeted therapy.

Poster No. 68

OVEREXPRESSION OF FKBPL CONFERS RESISTANCE TO CHEMOTHERAPY IN BREAST CANCER CELLS

Nuala Mulgrew, Hayley McKeen, Christopher Donley, Anita Yakkundi, Helen O McCarthy and Tracy Robson
School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL

FKBPL is a divergent member of the FKBP family with increasingly important roles as regulators of cancer progression and treatment response[1]. FKBPL is a gene which has been demonstrated to be down-regulated when exposed to low dose ionising radiation and is postulated to have roles in DNA repair and cell cycle regulation. A known component of the oestrogen receptor chaperone complex, FKBPL stabilises newly synthesised p21 and correlates with increased sensitivity to tamoxifen and fulvestrant treatment[2]. The role of FKBPL in response to other types of chemotherapy has not yet been established. However, data suggest that the FKBP protein family can affect sensitivity to a variety of agents[1]. Here we demonstrate that FKBPL overexpression corresponds with increased resistance to a range of DNA damaging agents and microtubule hyper-stabilising agents.

Clonogenic assays, over a wide dose range, demonstrated that treatment with cisplatin, cyclophosphamide, doxorubicin, 5-fluorouracil, docetaxel and paclitaxel resulted in increased resistance in FKBPL stably overexpressing cell lines, 3.1D2 and 3.1D9, as compared to parental MCF-7 cells. In order to elucidate the pathways involved in this increased resistance we evaluated pAKT levels. Western blot analysis demonstrated increased pAKT levels in the FKBPL stably-overexpressing cell lines suggesting that the PI3K/AKT pathway may be involved in resistance to DNA damaging drugs. To further investigate the role of this pathway we used the PI3K inhibitor, GDC-0941, to treat cells prior to treatment with cisplatin and found that the FKBPL overexpressing 3.1D2 and 3.1D9 cells were resistant to this inhibitor compared to parental cells probably due to enhanced activation of the pathway. This implies that FKBPL overexpression results in a cellular phenotype which is not susceptible to PI3K pathway inhibition. In summary, patients with breast tumours expressing high FKBPL should be targeted with endocrine therapies whilst treatment with some of the standard breast chemotherapies should be avoided.

1. McKeen HD, Brennan DJ, Hegarty S, Lanigan F, Jirstrom K, Byrne C, Yakkundi A, McCarthy HO, Gallagher WM and Robson T (2011) The emerging role of FK506-binding proteins as cancer biomarkers: a focus on FKBPL. *Biochem Soc Trans* (in press)
2. McKeen
   McKeen HD, Byrne C, Jithesh PV, Donley C, Valentine A, Yakkundi A, O'Rourke M, Swanton C, McCarthy HO, Hirst DG, Robson T. (2010) FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. *Cancer Research* 70(3):1090-1100

Poster No.69

CISPLATIN INDUCES MITOCHONDRIA-INDEPENDENT APOPTOSIS BY A CASPASE 8 DEPENDENT PATHWAY FUNCTIONALLY DISTINCT FROM TRAIL

I Paul[1], A Chacko[1], N Crawford[1], D Longley[1], DA Fennell[1]
[1]Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, UK

Background: The BCL-2 proapoptotic proteins BAX and BAK are genetically redundant mediators of mitochondrial apoptosis. Loss of BAX/BAK has been shown to mediate profound chemoresistance. Cisplatin is the most important drug used to treat NSCLC however the requirement for BAX and BAK in mediating apoptosis has not been clearly delineated. We examined the proapoptotic pharmacodynamics in NSCLC cells lacking BAX and BAK expression

Results: BAX and BAK were simultaneously targeted for stable knockdown in H460 & H1299 cells using short hairpin RNAs (H460shBAXBAK). To confirm mitochondrial apoptosis block, lack of caspase 9 was confirmed in response to staurosporine, and cisplatin in both cell lines. Despite this block, marked caspase 3 activation, PARP cleavage and hypodiploidy still occurred post cisplatin in H460shBAX/BAK cells but not H1299shBAX/BAK cells. The extrinsic pathway initiator caspase 8 was markedly activated by cisplatin in H460shBAX/BAK. Silencing of caspase 8, results in significant rescue of cisplatin treated H460shBAX/BAK cells but not in H460shNTNT control cells. This shows redundancy between the extrinsic and intrinsic apoptosis pathways in cisplatin induced apoptosis. Cisplatin induced caspase 8 activation is mediated by components of the Death Inducing Signalling Complex(DISC) and the enzyme responsible for ceramide generation, acid sphingomyelinase. A panel of cisplatin resistant cells, which exhibit cell cycle arrest, H2AX phosphorylation and p53 stabilisation in response to cisplatin, fail to exhibit caspase 3 and 8 activation following cisplatin exposure. However, tumour necrosis factor related apoptosis inducing ligand (TRAIL) induced caspase 8 and 3 in these cells.

Conclusions: Simultaneous loss of both BAX and BAK, is insufficient to cause cisplatin resistance. Extrinsic apoptosis pathway activation by cisplatin bypasses mitochondria. In the cisplatin resistant phenotype, extrinsic apoptosis pathway signalling is blocked, however this can be bypassed by TRAIL, implicating a functional dissociation of the extrinsic pathway activation by death ligand versus chemotherapy. This difference will be exploited therapeutically in the platinum resistant setting.

Poster No.70

PARP INHIBITION MEDIATES BAX/BAK INDEPENDENT SYNTHETIC LETHALITY OF BRCA1-DEFICIENT NON-SMALL CELL LUNG CANCER

I Paul[1], KI Savage[1], K Gately[3], J Blainey[2], K Kerr[5], M Sheaff[4], E Lamers[1], P Hamilton[2], J James[1], KJ O'Byrne[3], DP Harkin[1], JE Quinn[1], DA Fennell[1,2]
[1]Centre for Cancer Research and Cell Biology, Queens University Belfast , Belfast, UK
[2]Centre for Biomedical Informatics, Queens University Belfast, Belfast, UK
[3]Thoracic Oncology Research Group, St James' Hospital , Dublin, Ireland
[4]Department of Pathology, Barts and the London NHS Trust, London, UK
[5]Department of Pathology, University of Aberdeen, Aberdeen, UK

Evasion of apoptosis contributes to both tumorigenesis and drug resistance. The proapoptotic BCL-2 family proteins BAX and BAK are critical regulators of mitochondrial apoptosis. In contrast, prosurvival BCL-2 family proteins eg. MCL-1 and BCLX are among the most frequently amplified in human cancers, consistent with negative regulation of this pathway. New strategies for targeting cancer in a mitochondria-independent manner should bypass this common mechanism of apoptosis block. Mutation of BRCA1 confers a defect in homologous recombination mediated DNA repair that can be exploited by synthetic lethality through inhibition of PARP. It is not known whether BAX and BAK play an essential role in mediating synthetic lethality. We addressed this question by developing cells where both BAX and BAK expression was silenced, these cells are defective for mitochondrial apoptosis. Silencing BRCA1 expression by RNA interference sensitizes non-small cell lung cancer cells to PARP inhibition. Importantly, this sensitivity was not attenuated by loss of BAX/BAK. Furthermore, we demonstrate that BRCA1 inhibition cannot restore platinum sensitivity in platinum resistant cells but can still sensitize to PARP inhibition. Analysis of 302 primary NSCLCs identified BRCA1 immunonegativity in 14-17%, with validation in an independent cohort. In summary, targeting BRCA1 negative NSCLC by PARP inhibitors results in bypass of mitochondrial apoptosis block and can override platinum resistance. The existence of BRCA1-immunodeficient NSCLC suggests that this molecular subgroup could be effectively targeted by PARP inhibitors in the clinic.

Poster No. 71

IDENTIFICATION OF TRANSCRIPTION FACTORS ASSOCIATED WITH CASTRATE RESISTANT PROSTATE CANCER CELLS

M Prencipe[1], S Madden[2], A O'Neill[1], A Culhane[3], D O'Connor[1], H Klocker[4], W Gallagher[1], W Watson[1]
[1]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland
[2]National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland
[3]Department of Biostatistics, Dana-Farber Cancer Institute, Boston, Massachusetts, US
[4]Department of Urology, Innsbruck Medical University, Innsbruck, Austria

Locally advanced and metastatic prostate cancer are treated by hormone ablation therapy. However, despite an initial response, the majority of men relapse within 2-3 years to develop a castrate resistant disease for which there are no effective treatments; therefore defining the mechanisms of resistance represents a significant question. Androgen-independent (AI) sublines were derived from the androgen-dependent (AD) LNCaP cells. Previous studies in our laboratory have shown a complex interplay between changes in pro and anti-apoptotic proteins in the development of this resistance. Manipulating individual genes has only minor alterations in the resistant phenotype so we hypothesise that targeting the central signalling pathways and transcription factors (TFs) would represent a better therapeutic targeting approach.
We have undertaken a transciptomic analysis of the LNCaP parental (AD), LNCaP Abl (AI) and Hof (AI) cell lines, using the Affymetrix HG U133 Plus 2.0 GeneChip. Using a combination of correspondence analysis, between group analysis and co-inertia analysis, we have generated a transriptomic profile showing 900 genes associated with androgen resistance. Most importantly, we have predicted a list of TFs including HSF1, CDP, VDR-RXR, SRF and PBX1, which may be responsible for the differential gene expression observed in the AI compared to the AD cells. Taqman validation of 32 genes, including 6 TFs, has confirmed our gene chip results. Further validation of VDR-RXR and SRF activity was performed using luciferase assays. We are currently focusing on SRF, which has recently been associated with prostate cancer development (1). We have confirmed its up-regulation in the AI cell lines by WB.
Manipulating the upstream TFs may represent a better therapeutic targeting strategy to reverse androgen resistance.

1. Gorlov et al. Prioritizing genes associated with prostate cancer development. BMC Cancer 2010, 10:599

Poster No.72

THE ROLE OF FGFR4 SIGNALLING IN SENSITISING COLORECTAL CANCER CELLS TO OXALIPLATIN/5-FLUOROURACIL CHEMOTHERAPY

RC Turkington[1], WL Allen[1], L Stevenson[1], VM Coyle[2], PV Jithesh[1], I Proutski[1], C Fenning[1], G Stewart[1], DB Longley[1], PG Johnston[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast,
[2]Department of Medical Oncology, Northern Ireland Cancer Centre, Belfast, Northern

Background.
The development of drug resistance limits the effectiveness of current chemotherapeutic agents used to treat colorectal cancer. The discovery of the underlying mechanisms of resistance and the development of novel agents to target these pathways is a priority. Fibroblast Growth Factor Receptor 4 (FGFR4) is one of a family receptor tyrosine kinases which can bind 22 structurally conserved FGF's and has been implicated in the growth and differentiation of colon cancer.[1]

Materials and Methods.
Transcriptional profiling of pre-treatment metastatic colorectal cancer liver biopsies and HCT116 parental, oxaliplatin and 5-Fluorouracil resistant cell lines was performed using the Affymetrix HGU133 Plus 2.0 array. Profiling of the in vitro and clinical samples was also carried out using the Almac Diagnostics Colorectal Cancer Disease Specific Array™ (DSA™). Analysis of the microarray data was performed using Metacore and Gene Set Enrichment Analysis (GSEA).

Results.
Data analysis identified panels of in vitro and clinical genes whose expression is acutely altered in the parental setting following drug treatment and also basally deregulated in the resistant cells. The significant pathways involved in these panels of genes were compared with the results of the GSEA to produce a final ranked gene list of pathways. This list included groups of Cell Cycle, Focal Adhesion, Insulin and MAPK signalling genes. A candidate gene approach was used to select individual genes from these pathways for incorporation into siRNA screens. A number of target genes were identified of which FGFR4 was selected for further study and validation. Knockdown of FGFR4 using siRNA transfection causes an increase in apoptosis across a panel of colorectal cancer cell lines as well as changes in a variety of cell survival and death-related pathways.

Conclusions.
This study demonstrates the utility of microarray expression data analyzed by pathway and Gene Set Enrichment Analysis to identify pathways of Oxaliplatin/5-Fluorouracil resistance in colorectal cancer. FGFR4 and its specific ligand FGF19 represent possible targets for therapeutic intervention in colorectal cancer.

1. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer;10(2):116-29.

Poster No.73

EP1 receptor specific antagonist (ONO-8713) reduces colon tumor cell growth *in vivo*.

G O'Callaghan[1], P Neary[2], C O'Mahony[3], F Shanahan[1, 3], A Houston[1,3]
[1]Medicine, UCC, Cork, Ireland
[2]Surgery, UCC, Cork, Ireland
[3]APC, UCC, Cork, Ireland

EP1 receptor specific antagonist (ONO-8713) reduces colon tumour cell growth *in vivo*.

O'Callaghan G[1], Neary P[2], O'Mahony C[3], Shanahan F[1,3] and Houston A[1,3].
Departments of Medicine[1], Surgery[2], and Alimentary Pharmabiotic Centre[3], National University of Ireland Cork, Ireland

Background: The role of cyclooxygenase-2 in promoting colon carcinogenesis via its main metabolite prostaglandin E2 (PGE2) is well established. Four different receptors signal via PGE_2 –EP1-EP4. Recently we have shown that signalling via PGE2/EP1 induces Fas ligand (FasL/CD95) expression in vitro. Tumour expressed FasL has been identified as a major inhibitor of the anti-tumour immune response, however regulatory mechanisms remain elusive. Targeting FasL expression via ONO-8713 EP1 specific receptor antagonist drug *in vivo* may represent a new therapeutic target in colon cancer.
Aim: To investigate if blocking PGE_2/EP1-induced FasL expression alters tumour development *in vivo*.
Methods: Female BALB/c mice were injected subcutaneously with CT26 colon tumour cells. Once tumors reached 0.5cm^3 volume, ONO-8713 (ONO Pharmaceutical, Japan), was administered either orally in their feed (1000ppm) or by intra-tumoral injection, 30mg/kg thrice weekly. To access FasL downregulation, another group was injected with CT26 cells, stably transfected with short-hairpin RNA (shRNA) suppressing FasL expression. After 51 days, tumours were analysed for FasL expression. Tumour-infiltrating lymphocytes (TIL) were phenotyped by flow cytometry. Splenocytes were tested for CTL activity after 5 days. Cell proliferation was assessed by resazurin reduction.
Results: shRNA suppression of tumour expressed FasL reduced tumour volume by (57%+/-3.4%). Targeting tumour volume via ONO-8713 also decreased tumour volume resulting in an 46%+/-18% reduction following oral administration and an 74%+/-6.7% reduction following intra-tumoral injection. This was associated with downregulation of FasL expression. In contrast to results *in vivo*, ONO-8713 did not affect tumour cell proliferation *in vitro*. This indirect mechanism for efficacy was supported by TIL analysis revealing reduced $CD4^+CD25^+FOXP3^+$ regulatory T cells within tumours of treated mice. Further, splenocytes derived from ONO-8713 treated mice demonstrated increased cytotoxicity against CT26 tumour cells, relative to untreated mice.

SILVER SPONSORS

ACTIVE MOTIF®
Tools to Analyze Nuclear Function

BIO-SCIENCES

Caliper LifeSciences

Enzo® Life Sciences
ALEXIS assay designs BIOMOL Stressgen
www.enzolifesciences.com

IDT
INTEGRATED DNA TECHNOLOGIES

LGC Standards
Excellence through measurement

MSC

MERCK MILLIPORE

NOVARTIS ONCOLOGY

PerkinElmer®
For the Better

Roche

SIGMA-ALDRICH

Other Contributors

abcam

PLATINUM SPONSORS

irish cancer society

Merck Serono
Living science, transforming lives

MERCK

GOLD SPONSOR

LABPLAN
Science Support Solutions