PATHOLOGICAL SOCIETY
OF GREAT BRITAIN AND IRELAND

Winter Meeting Programme

6–7 January 2005

The 187th Meeting of the
Pathological Society of Great Britain and Ireland
will be held at The Robin Brook Centre, St. Bartholomew's Hospital,
West Smithfield, London, and hosted by The Department of
Histopathology, Barts & The London Hospitals NHS Trust.

Barts and The London
Queen Mary's School of Medicine and Dentistry

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PROGRAMME ACKNOWLEDGEMENTS

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FEATURED TOPICS

THURSDAY 6 JANUARY
Slide Seminar: *Skin*
Mini-Symposium: *New Developments in Skin Pathology*
Plenary Lecture
Poster Presentations
Plenary Oral Session
1st Goudie Lecture

FRIDAY 7 JANUARY
Oral communications
Mini-Symposium: *Cancer Cell Biology*
Plenary Lecture

KEY FACTS

ORAL COMMUNICATIONS *(Morris and Paterson Ross Lecture Theatres)*
Oral communication sessions will be held as follows:
Friday 7 January: 09.00 - 12.30 hrs

Speakers are reminded that no communication may exceed the time allocated on the programme without the consent of the meeting, obtained through the Chairman.

PLENARY ORAL SESSION *(Morris Lecture Theatre)*
The plenary oral session, in which the four highest-ranked submitted oral abstracts will be presented, will be held:
Thursday 6 January: 15.30 – 16.30 hrs

A prize for the best presentation, donated by the *Journal of Pathology*, will be presented at the Society Dinner.

POSTERS/VIEWING *(Boyle and Bainbridge Rooms)*
Posters will be displayed on **Thursday 6 January and Friday 7 January**.
The dedicated poster viewing session will be on:
Thursday 6 January: 13.30 – 15.00 hrs

Ideally, posters should be in place by **09.00 hrs** on **Thursday 6 January** and removed by **16.00 hrs** on **Friday 7 January**. At least one of the contributors must be in attendance during the viewing period, as indicated in the programme synopsis. The Sir Alastair Currie Prize and second and third poster prizes will be presented at the Society Dinner.

MINI-SYMPOSIA *(Morris Lecture Theatre)*
Two mini-symposia will be held:
Thursday 6 January: 10.30 – 12.30 hrs
Friday 9 July: 14.00 – 16.00 hrs
Details of topics are listed in the programme along with chairmen (correct as at time of going to press).

SLIDE SEMINAR COMPETITION*: "**Skin Pathology**"* *(Seminar Room 5)*
There will be a slide competition using digital slide images, which will be available for viewing on:
Thursday 6 January: 09.00 – 16.00 hrs

SOCIETY LECTURE *(Morris Lecture Theatre)*
Tuesday 6 July: 16.45 – 17.30 hrs
The Pathological Society of Great Britain and Ireland's 1st Goudie Lecture entitled:
"*Modelling multi-step human tumorigenesis in vitro: the importance of cellular context*" will be given by Professor D Wynford-Thomas, University of Wales, College of Medicine.

TRADE EXHIBITION *(The Foyer and Seminar Room 10)*
Delegates are encouraged to visit the Trade Exhibition and to support the companies represented there.

CONTINUING PROFESSIONAL DEVELOPMENT (CPD)
This Meeting has been approved by the **Royal College of Pathologists** for the purposes of Continuing Professional Development. Credits can be accrued as follows:
For each full day: 7 points
For each half day: 3 points.

Delegates who are eligible for CPD points should collect their certificates at the Registration Desk before leaving the Meeting.

KEY FACTS *continued*

General Arrangements

REGISTRATION

Registration is **only** available via the internet at: **http://pathsoc.conference-services.net/directory.asp**

FEES *including all refreshments and lunch*

SOCIETY MEMBERS

Up to and including 1 December 2004
£130 for the whole meeting, or £80 per day (or part day)

After 1 December 2004
£180 for the whole meeting, or £105 per day (or part day)

NON-MEMBERS

Up to and including 1 December 2004
£180 for the whole meeting, or £105 per day (or part day)

After 1 December 2004
£230 for the whole meeting, or £130 per day (or part day)

OTHER FEE CATEGORIES

PhD Students, Junior Technicians, Residents and Trainees
£65 for the whole meeting, or £40 per day (or part day)
To qualify for this reduced fee, delegates must submit an identification document signed by the Head of Training, including National Training Numbers where applicable. Please send your identification document by post or via email to: julie@pathsoc.org.uk

Undergraduates, Honorary and Senior Members of the Society
£20 per day (or part day)

SOCIETY DINNER
Tickets are available at £50 per person

DELEGATE ENROLMENT *(Foyer of the Robin Brook Centre)*

Enrolment will take place from **09.00 hrs** on **Thursday 6 January**.

ENQUIRIES

Enquiries before the Meeting regarding administration should be directed to:

Mrs R A Pitts
Pathological Society of Great Britain & Ireland
2 Carlton House Terrace, London, SW1Y 5AF
Tel: +44 (0)20 7976 1260
Fax: +44 (0)20 7976 1267
Email: admin@pathsoc.org.uk

PRESENTATION CHECKING AND PREVIEW
This will be available in ***Seminar Room 6***.

ORAL PRESENTATIONS/LECTURES
Presenters are requested to upload their presentation at their nominated lecture theatre **at least 30 minutes before the start of the session**.

SLIDE SEMINAR
PCs for Slide Seminar Viewing will be located in ***Seminar Room 5***.

MESSAGES
During the Meeting, messages for delegates may be left at the Department of Histopathology on the following telephone number: +44 (0)20 7601 8533. There will also be a message board located beside the Enrolment Desk.

REFRESHMENTS
Coffee and tea will be served in ***The Foyer*** and lunch will be served in the ***Wykeham Balme Room***.

BADGES
Delegates are requested to wear their badges at **all** times.

TRAVEL
By Train and Underground
The nearest underground stations are: Barbican, Farringdon, St Paul's and Blackfriars *(allow for a 15–20 minute walk)*. The nearest mainline stations are: Blackfriars, City Thameslink and Farringdon.

By Bus
Buses numbers that stop outside, or close to, the hospital are: 4, 8, 25, 56, 172 and 242.

Car Parking
There are no dedicated parking facilities for patients or visitors. There are a small number of public parking spaces for people with disabilities outside the main hospital gate (Henry VIII Gate) in West Smithfield. There is an NCP car park in West Smithfield.

By Air
There are trains, tube and bus links from all London airports.

ACCOMMODATION: LOCAL HOTELS

☆☆ **Travelodge London City** (Liverpool Street)
Tel: +44 (0)870 191 1689 Fax: +44 (0)20 7626 1105
Website: www.travelodge.co.uk

☆☆☆ **Holiday Inn Express City** (Old Street)
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Website: www.ichotelsgroup.com/h/d/ex/1/en/home

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Tel: +44 (0)20 7618 5000 Fax: +44 (0)20 7618 5001
Website: www.great-eastern-hotel.co.uk

Or contact **The Hotel Directory**
Tel: +44 (0)870 770 8181
Website: www.thehoteldirectory.co.uk/london/

SMOKING
Smoking is prohibited at all meetings and social events except in the designated areas.

DISCLAIMER
The Pathological Society of Great Britain & Ireland cannot be held responsible for any injury or loss sustained during the Meeting.

SOCIAL ACTIVITIES
Thursday 6 January
Society Dinner, The Great Hall, St. Bartholomew's Hospital.
Please reserve your ticket when registering (fee £50).

Local Places of Interest
St. Paul's Cathedral and the Museum of London are a short walk away. Please refer to the Internet for further information.

Future Meetings

2005 (5–8 July) *Newcastle-upon-Tyne*
Newcastle Pathology 2005
3rd Joint Meeting of the Pathological Society of Great Britain & Ireland and the British Division of the IAP.

2006 (4–7 July) *Manchester*
2006 Centenary Meeting.

2007 (3–6 July) *Glasgow*
Glasgow Pathology 2007.
4th Joint Meeting of the Pathological Society of Great Britain & Ireland and the British Division of the IAP.

2008 (1–4 July) *Belfast*

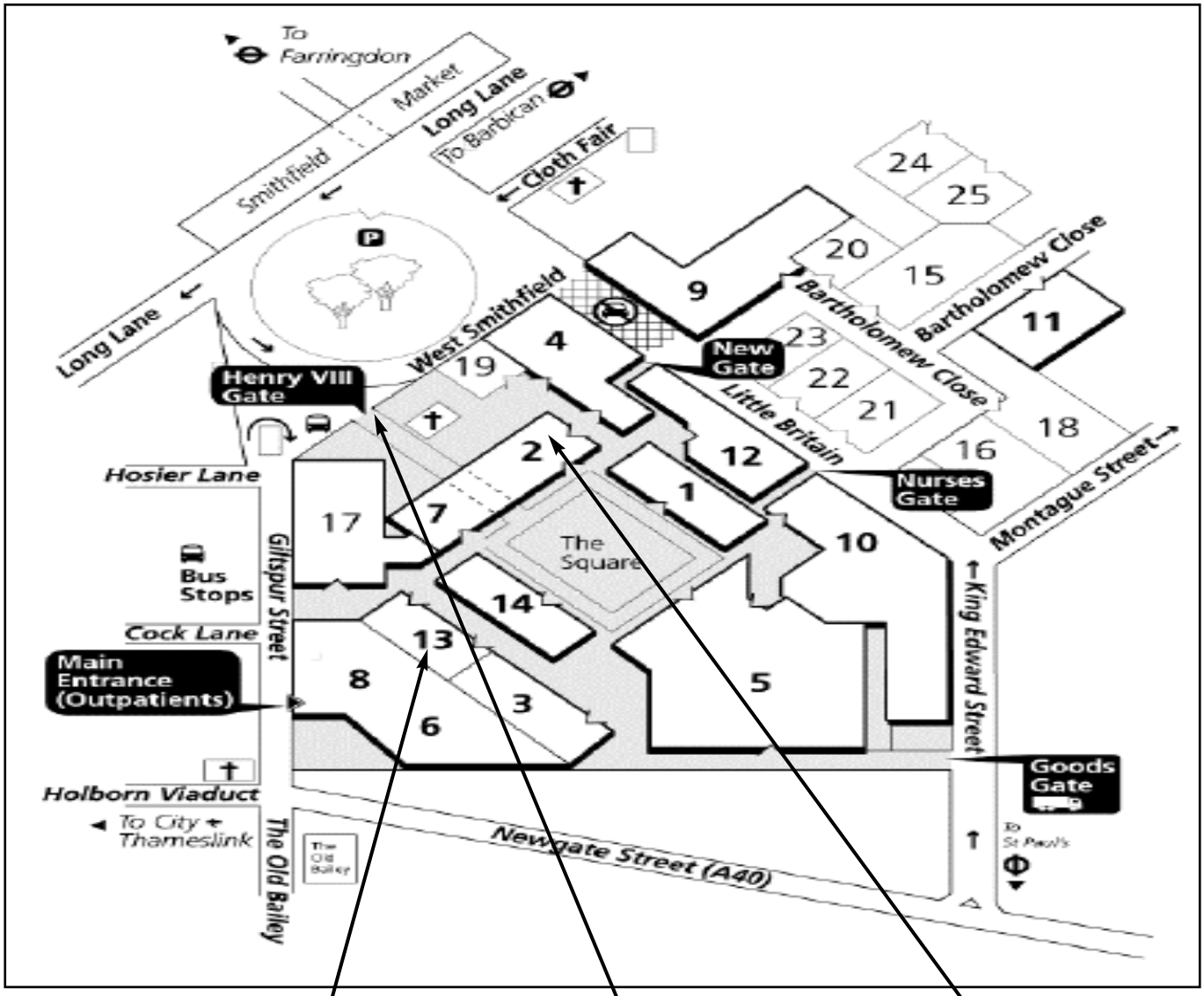
2009 (July) *St. Andrews*

Notice to Members

PROGRAMME

Members of the Pathological Society
attending the Meeting
must bring this programme with them
as only a limited number
will be available at the Meeting.

ST. BARTHOLOMEW'S HOSPITAL MAP



Robin Brook Centre

Great Hall

Entrance
It is recommended you enter using the Henry VIII Gate.

The Pathological Society of Great Britain and Ireland wishes to acknowledge the support of the following companies

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(as at the time of going to press)

Summary Programme – Thursday 6 January 2005

09.00–10.30 Foyer, Robin Brook Centre
Registration and Coffee

10.30 Morris Lecture Theatre
MINI-SYMPOSIUM: *New Developments in Skin Pathology*
PLENARY LECTURE

12.30 Wykeham Balme Room
LUNCH

13.30 Bainbridge and Boyle Rooms
POSTER VIEWING (All categories)
[Abstracts 29–71]

15.00 The Foyer
TEA

15.30 Morris Lecture Theatre
PLENARY ORAL SESSION
[Abstracts 1–4]

16.45 Morris Lecture Theatre
The Pathological Society of Great Britain & Ireland's
1ST GOUDIE LECTURE
"Modelling multi-step human tumorigenesis in vitro: the importance of cellular context"
Professor David Wynford-Thomas [Abstract 74]

19.30 The Great Hall
RECEPTION AND SOCIETY DINNER

Summary Programme – Friday 7 January 2005

09.00 Morris Lecture Theatre ORAL COMMUNICATIONS Gastrointestinal [Abstracts 5–8] Hepatobiliary/Pancreas [Abstracts 9–10]	**09.00 Paterson Ross Lecture Theatre** ORAL COMMUNICATIONS Genitourinary/Renal [Abstracts 11–12] Osteoarticular/Soft Tissue [Abstracts 13–14] Head and Neck [Abstract 15] Pulmonary [Abstract 16]
10.30 The Foyer COFFEE	
11.00 Morris Lecture Theatre ORAL COMMUNICATIONS Breast [Abstracts 17–19] Gynaecological [Abstracts 20–21] Skin [Abstract 22]	**11.00 Paterson Ross Lecture Theatre** ORAL COMMUNICATIONS Cellular/Molecular [Abstracts 23–24] Experimental Tumour Pathology [Abstract 25] Technical Advances [Abstract 26] Education and Audit [Abstracts 27–28]
12.30 Wykeham Balme Room LUNCH	
14.00 Morris Lecture Theatre MINI-SYMPOSIUM: *"Cancer Cell Biology"* PLENARY LECTURE	

Detailed Programme – Thursday 6 January 2005

Presenter = {P} Abstract numbers are shown in bold type and in square brackets eg **[259]**

10.30 – 12.30 **Morris Lecture Theatre**
MINI-SYMPOSIUM: NEW DEVELOPMENTS IN SKIN PATHOLOGY
Chairman: Professor NA Wright,
St. Bartholomew's & The Royal London School of Medicine & Dentistry

10.30 **Mechanisms of skin cancer metastasis**
Professor F Nestlé, Centre of Medical Research, University of Zurich, Switzerland

11.00 **[72]** **Prenatal diagnosis of skin disorders**
Professor J McGrath, St John's Institute of Dermatology, London

11.30 **PLENARY LECTURE**
Genetic skin disorders
Professor I Leigh, Cancer Research UK, London

12.30 – 13.30 **Wykeham Balme Room**
LUNCH

13.30 – 15.00 **Bainbridge and Boyle Rooms**
POSTER VIEWING
CATEGORIES
Autopsy & Forensic **[29]**
Breast **[30–37]**
Cardiovascular/Pulmonary **[38–39]**
Cellular/Molecular **[40–44]**
Education & Audit **[45–47]**
Endocrine **[48]**
Gastrointestinal **[49–54]**
Genitourinary/Renal **[55–60]**
Gynaecological **[61–64]**
Head & Neck **[65–66]**
Hepatobiliary/Pancreas **[67]**
Lymphoreticular **[68]**
Neuropathology/Ophthalmic **[69]**
Osteoarticular/Soft Tissue **[70]**
Technical Advances **[71]**

15.00 – 15.30 **The Foyer**
TEA

15.30 – 16.30 **Morris Lecture Theatre**
PLENARY ORAL SESSION
Chairman: Professor M Pignatelli,
University of Bristol and Bristol Royal Infirmary

15.30 **[1]** **1Mb Resolution Array-CGH Identifies Small Chromosome Aberrations in Chromosome Unstable and Microsatellite Unstable Colorectal Cancers**
G Poulogiannis , K Ichimura , NGA Miller , IM Frayling , RG Morris , DJ Harrison , VP Collins , A Ibrahim , AH Wyllie , **{P}** MJ Arends

15.45 **[2]** **Epithelial-mesenchymal transition in portal fibrosis**
{P} L Zhao, H Robertson, JA Kirby, DEJ Jones, AD Burt

16.00 **[3]** **Analysis of Serial Plasma DNA Samples during follow-up of women with Primary Breast Cancers**
{P} JA Shaw , K Dampier , M Tamburo De Bella , L Primrose , ML Slade , T Powles , RA Walker , RC Coombes

Detailed Programme – Thursday 6 January 2005

Presenter = {P} Abstract numbers are shown in bold type and in square brackets eg **[259]**

16.15 **[4]** **Why is there a Unique Pattern of Differentiation in FAP-Associated Thyroid Tumours?**
{P} E D Williams, N A Dathan, H Bardwell

16.45 – 17.30 **PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S 1ST GOUDIE LECTURE**
Chairman: Professor PA Hall
Queen's University, Belfast and Belfast City Hospital

[74] **Modelling multi-step human tumorigenesis *in vitro*: the importance of cellular context**
Professor D Wynford-Thomas, University of Wales, College of Medicine, Cardiff

19.30 **The Great Hall** *(St. Bartholomew's Hospital, London)*
SOCIETY DINNER

Detailed Programme – Friday 7 January 2005

Presenter = {P} Abstract numbers are shown in bold type and in square brackets eg **[259]**

09.00 – 10.30 **Morris Lecture Theatre**
ORAL COMMUNICATIONS
Gastrointestinal; Hepatobiliary/Pancreas
Chairmen: Dr RFT McMahon, University of Manchester
and Professor DJ Harrison, University of Edinburgh

09.00 **[5]** **The TFF2/MUC6-Secreting Cell Lineage in Mucous Transformation of the Human Gastric Mucosa**
{P} NA Wright, JR Goldenring

09.15 **[6]** **Clusters of Phenotypically-Related Human Colonic Crypts develop through Crypt Fission: Implications for Colorectal Carcinogenesis**
{P} SAC McDonald, SL Preston, L Greaves, P Tadrous, P Saseini, M Novelli, D Oukriff, J Jankowski, D Turnbull, N Wright

09.30 **[7]** **Quality Assurance in a Multidisciplinary Study – The MERCURY Experience**
{P} S Fisher , IR Daniels, P Quirke

09.45 **[8]** **Detection of Non-Pathogenic E.Coli within Granulomas and Bowel Wall in Patients with Crohn's Disease using Laser Capture Microdissection and PCR**
{P} P Ryan, RG Kelly, G Lee, JK Collins, GC O'Sullivan, JO'Connell, F Shanahan

10.00 **[9]** **Ductular reaction in the post-transplanted liver: a component of cellular rejection**
{P} A Ahmed El-Refaie, AD Burt

10.15 **[10]** **The Natural History of Hepatitis C with Severe Hepatic Fibrosis**
A Lawson, N Taguri, K Rye, S Hagan, **{P}** AM Zaitoun, K Neal, W Irving, MMc Kendrick, D Gleeson, M Wiselka, S Ryder

10.30 – 11.00 **The Foyer**
COFFEE

11.00 – 12.30 **Morris Lecture Theatre**
ORAL COMMUNICATIONS
Breast; Gynaecological; Skin
Chairmen: Dr JJ Going, University of Glasgow
and Dr TP Rollason, Birmingham Women's Hospital

11.00 **[17]** **Apoptotic Regulation within Normal and Cancer Containing Breasts**
{P} RA Walker, A Gordon-Weeks, J Luckett

11.15 **[18]** **The Expression of Mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their Prognostic Significance in Human Breast Cancer**
{P} B Boyce, E Rakha, DAbd El-Rehim, A Green, C Paish, I Ellis

11.30 **[19]** **Oestrogen Receptor Variant Expression as Potential Selectors for Adjuvant Endocrine Therapy in Breast Cancer Patients**
{P} AR Green, EC Paish, JM Gee, RI Nicholson, KL Cheung, JF Robertson, IO Ellis

11.45 **[20]** **Mast cells in human decidua and trophoblast: alternations in placental pathology**
{P} XXu, JN Bulmer, AD Burt

12.00 **[21]** **Histological grading of epithelial ovarian carcinoma – does it matter which grading system is used?**
{P} N Singh, A Ayhan

Detailed Programme – Friday 7 January 2005

Presenter = {P} Abstract numbers are shown in bold type and in square brackets eg **[259]**

12.15 **[22]** **Transplanted Bone marrow cells engraft within the mouse epidermis and proliferate with no evidence of cell fusion**
{P} M Brittan, KM Braun, LM Reynolds, FJ Conti , AR Reynolds, R Poulsom, MR Alison, NA Wright, KM Hodivala-Dilke

12.30 – **Wykeham Balme Room**
LUNCH

09.00 – 10.30 **Paterson Ross Lecture Theatre**
ORAL COMMUNICATIONS
Genitourinary/Renal; Osteoarticular/Soft Tissue; Head and Neck; Pulmonary
Chairmen: Professor S Fleming, University of Dundee
and Professor AM Flanagan, University College, London

09.00 **[11]** **Prediction of Response to a Topoisomerase I Inhibitor in Renal Cell Carcinomas**
{P} DM Berney, P Lobo, J Shamash, RTD Oliver

09.15 **[12]** **Detection of human papillomavirus and expression of p16INK4A in penile carcinoma**
{P} DM Prowse, S Youshya, E Ktori, P Agarwal, RT Oliver, SI Baithun

09.30 **[13]** **Direct induction by melanoma, prostate cancer and Ewing's sarcoma tumour cells of osteoclast formation by a RANKL-independent mechanism**
{P} YS Lau, A Sabokbar, H Giele, CLMH Gibbons, V Cerundolo, NA Athanasou

09.45 **[14]** **Ape/Ref-1 Expression in Torn Rotator Cuff**
{P} TR Helliwell, A Sheth, SP Frostick, A Rawal, J Gibson, V Rayner , MM Roebuck

10.00 **[15]** **Artificial Neural Networks and Survival Prediction in Laryngeal Cancer**
{P} TR Helliwell, AS Jones, AGF Taktak, JE Fenton, AC Fisher

10.15 **[16]** **Genome-wide Analysis of LOH by SNP Microarrays to Demonstrate Chromosomal Loss in Lung Cancer Precursor Lesions**
{P} DRJ Snead, DP Dhillon, H Vohra, N Briffa, A Blanks, H Bird

10.30 – 11.00 **The Foyer**
COFFEE

11.00 – 12.30 **Paterson Ross Lecture Theatre**
ORAL COMMUNICATIONS
Cellular/Molecular; Experimental Tumour Pathology; Technical Advances; Education and Audit
Chairmen: Dr MJ Arends, University of Cambridge
and Professor P Domizio, St. Bartholomew's Hospital, London

11.00 **[23]** **Myofibroblasts Associated with Liver Tumours are Frequently of Bone Marrow Origin**
{P} P Vig, F Russo, B Bigger, N Wright, H Thomas, M Alison, S Forbes

11.15 **[24]** **fl-Dystroglycan is Constitutively Expressed at the Intercellular Junctions of Epithelial Cells and this Expression is Frequently Absent in Common Cancers**
{P} SS Cross, FE Hollingbury, JM Lippitt, JW Catto, FC Hamdy, SJ Winder

Detailed Programme – Friday 7 January 2005

Presenter = {P} Abstract numbers are shown in bold type and in square brackets eg **[259]**

11.00 – 12.30 **Paterson Ross Lecture Theatre**
ORAL COMMUNICATIONS — *continued*

11.30 **[25]** **Markers of Adenocarcinoma Characteristic of the Site of Origin – Development of a Diagnostic Algorithm**
{P} JL Dennis, TR Hvidsten, EC Wit, J Komorowski, AK Bell, I Downie, J Mooney, C Verbeke, C Bellamy, WN Keith, KA Oien

11.45 **[26]** **Morphological Characterisation of Tissues by Optical Coherence Tomography**
{P} PJ Tadrous, G Dobre, R Cucu, AGh Podoleanu, S Shousha, EMA Lalani, GWH Stamp

12.00 **[27]** **Career choices for pathology: national surveys of graduates of 1974–2000 from UK medical schools**
{P} P Domizio, C du Boulay, T Lambert

12.15 **[28]** **Improving National Outcomes in Rectal Cancer – The Development of an English National MDT-TME Project**
{P} J Jessop, P Quirke

12.30 – 14.00 **Wykeham Balme Room**
LUNCH

14.00 – 16.00 **Morris Lecture Theatre**
MINI-SYMPOSIUM: CANCER CELL BIOLOGY
Chairman: Professor P Quirke, University of Leeds

14.00 **Targeting viral gene therapy for cancer**
Professor N Lemoine, Cancer Research UK, London

14.30 **Genetic pathology and its application to cancer diagnosis and therapy**
Professor I Tomlinson, Cancer Research UK, London

15.00 **PLENARY LECTURE**
[73] **Cadherins, catenins and cancer**
Professor M Mareel, Ghent University Hospital, Belgium

Abstracts

1

1Mb Resolution Array-CGH Identifies Small Chromosome Aberrations in Chromosome Unstable and Microsatellite Unstable Colorectal Cancers

G Poulogiannis , K Ichimura , NGA Miller , IM Frayling , RG Morris , DJ Harrison , VP Collins , A Ibrahim , AH Wyllie , **{P}** MJ Arends
Department of Pathology, Cambridge, United Kingdom

Colorectal cancers (CRC) show at least two patterns of genomic instability: chromosomal instability (identifiable as aneuploidy) or microsatellite instability (usually occurring in near-diploid CRC with inactivation of the DNA mismatch repair system). Sometimes both or neither type of instability coexists. Here, we have used a high resolution array, with clones spaced on average 1Mb apart, for array-CGH analysis to identify DNA copy number changes in over 80 colorectal adenocarcinomas. In addition the samples were analysed for their microsatellite status (by interrogation of 10 loci), expression of mismatch repair proteins hMLH1 and hMLH2 (by immunohistochemistry), DNA ploidy (by flow cytometry) and clinical and pathological parameters. FISH using a centromeric probe on chromosome 6 was used to adjust the copy number of the other chromosomes. As expected, large regions of DNA copy number changes were confirmed at the loci of genes known to show aberrations in CRC such as *APC* on chromosome 5*, SMAD4* on chromosome 18*,* and *p53* on chromosome 17. Both microsatellite stable cancers with gross aneuploidy, and near-diploid cancers with microsatellite instability showed small regions of DNA copy number change involving one or a few genes only. We found several previously undetected heterozygous and homozygous deletions of genes related to the *K-ras* and *Wnt* pathways, as well as high-level amplifications of genes involved in cell cycle control. We conclude that, as well as chromosome and microsatellite instabilities, other small genomic abnormalities may afford selective growth advantage in colorectal neoplasia.

2

Epithelial-mesenchymal transition in portal fibrosis

{P} L Zhao [1], H Robertson [1], JA Kirby [2], DEJ Jones [2], AD Burt [1]
[1] School of Clinical and Laboratory Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom, [2] Immunobiology Research Group, University of Newcatle upon Tyne, Newcastle upon Tyne, United Kingdom

Intra-hepatic bile duct damage, with portal tract fibrosis, is characteristic of chronic ductopaenic diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Previously, it was thought that this process involved T cell mediated death of intra-hepatic biliary epithelial cells (IBEC) and was irreversible. Partial resolution of vanishing bile duct syndrome has been observed, suggesting that IBEC loss may be reversible.

The mechanisms of progressive fibrosis in such ductopaenic processes remain poorly defined. In chronic renal transplant rejection, TGFβ-driven tubular epithelial to mesenchymal transition (EMT) is a major source of fibroblasts; this is consistent with chronic immune injury in which T cell processes are themselves modulated by regulatory T cells. Induction of the antigen S100A4 is used to identify epithelial cells undergoing EMT, which is reversed by TGFβ antagonists. We hypothesised that a similar phenomenon could be responsible for portal fibrosis.

S100A4 was absent from cytokeratin19- positive IBEC in normal human liver but was expressed in IBEC in biopsy sections from both PBC and PSC. Hepatocytes adjacent to portal tracts were S100A4-positive as were most immune cells and some fibroblast-like cells. Cells within the ductular reaction showed coexpression of CK19 and S100A4. Colocalisation studies showed CD3+ T cells in contact with viable S100A4-expressing IBEC and nuclear pSmad 2/3 in S100A4-expressing IBEC, suggesting intracellular signalling in response to activated TGFβ.

The results suggest that EMT involving bile ducts and the ductular reaction may contribute to portal tract fibrosis. Intervention therapies could prevent bile duct loss and reduce portal tract fibrosis.

3

Analysis Of Serial Plasma DNA Samples During Follow Up Of Women With Primary Breast Cancers

{P} JA Shaw , K Dampier , M Tamburo De Bella , L Primrose , ML Slade , T Powles , RA Walker , RC Coombes
University of Leicester, Leicester, United Kingdom

The aim of this study was to investigate the utility of plasma DNA analysis for follow up of primary breast cancer patients. Blood samples were taken over a period of 2 years after diagnosis for 10 primary patients and over 6 months for 10 metastatic controls. DNA was extracted from lymphocytes, plasma and foci of tumour cells isolated by laser capture microdissection, and analysed for LOH at four loci (BRCA1, BRCA2, PTEN, DM-1). PAGE and DNA Bioanalyser Chips were used to determine the size range of plasma DNA.
The metastatic patients all showed consistent tumour specific LOH in plasma DNA, Two primary breast cancers had plasma DNA at diagnosis that showed LOH. Both patients relapsed within 1 year; one subsequently died and one developed metastases. The other primary patients remain disease free more than 2 years later. However, all have detectable plasma DNA, 5 with LOH in at least 2 of 3 subsequent samples. Qualitative gel analyses revealed plasma DNA fragments of > 1000bp in size, consistent with generation by tumour necrosis and amenable to full analysis using SNP Arrays. This pilot study suggests that identifying plasma DNA with LOH at diagnosis in primary breast cancers may be a useful prognostic indicator and warrants investigation in a larger series of patients.

4

Why Is There A Unique Pattern Of Differentiation In FAP-Associated Thyroid Tumours?

{P} E D Williams [1], N A Dathan [2], H Bardwell [1]
[1] Strangeways Research Laboratory, Cambridge, United Kingdom, [2] Istituto di Biostrutture e Bioimmagini, Naples, Italy

Thyroid tumours associated with FAP are differentiated slow-growing carcinomas with unique morphological features ('cribriform-morular'). Somatic mutations in APC, PTC, and ß-catenin occur, but germline APC mutation must be the common feature in the genotype-phenotype correlation. Thyroidectomy specimens from 24 FAP patients showed 134 thyroid tumours, 131 with the typical morphology. To investigate the link between thyroid transcription factor expression and differentiation we have studied TTF1, TTF2 and PAX8 expression, as well as thyroglobulin and ß-catenin, in 44 thyroid carcinomas from FAP patients and 32 controls. In normal thyroid tissue and in papillary and follicular carcinomas PAX8 as well as TTF1 showed consistent nuclear positivity, ß-catenin showed variable cell membrane positivity. In contrast FAP-associated tumours showed a lack of PAX8 expression, absent thyroglobulin and consistent strong nuclear localization of ß-catenin.

The Wnt signal transduction pathway, controlled by APC, is important in differentiation, leads to overexpression of ß-catenin, and interacts with PAX8 (necessary for thyroglobulin production). Activation of the Wnt pathway leads to transdifferentiation in the lung. Our findings suggest that loss of APC leads to activation of the Wnt pathway in thyroid follicular cells and 'transdifferentiation' with the development of differentiated carcinomas lacking the morphological and functional characteristics typical of follicular cells.

5

The TFF2/MUC6-Secreting Cell Lineage In Mucous Transformation Of The Human Gastric Mucosa

{P} NA Wright [1], JR Goldenring [2]
[1] *Cancer Research UK, London, United Kingdom,* [2] *Vanderbilt University, Tennessee, United States*

In several experimental and clinical situations, attention has been drawn to the presence of mucous transformation of gastric body glands; such changes are associated with H. pylori and H. felis infection, and it has been proposed that such a lineage, which expresses TFF2/hSP, and also MUC6, called Spasmolytic Polypeptide-Expressing Metaplasia (SPEM), is the precursor of gastric dysplasia and carcinoma. Gastric excision specimens were studied in which glands lined with cells of mucous phenotype were present in body mucosa. 23 cases showed H. pylori in the antrum, and 10 in the body mucosa. Hyperplasia of the mucous neck cells was a frequent finding, and mucous cell transformation occurred within the lower neck area of the gland, closely associated with MNC hyperplasia. The mucous cells expressed TFF2/hSP, MUC-6, lysozyme, PSTI and PDX-1. It is possible that MNCs proceed to differentiate into the cells seen in mucous transformation, but since in fully-developed mucous transformation MNCs are not seen, differentiation may be directly from stem cell progeny. Both MNC hyperplasia and mucous transformation in body glands appear closely associated with the presence of H. pylori-induced gastritis, suggesting that they represent a mucosal defence reaction to H. pylori. The TTF2/MUC6 secreting lineage (TMUCSL), which includes, inter alia, mucous neck cells, basal antral gland cells, Brunner's gland cells, PPM, SPEM and basal UACL gland cells, is an important phenotype which occurs indigenously in the gastrointestinal mucosa and is frequently induced in conditions where damage and regeneration is occurring. The relationship between mucous cell lineages, TMUCSL and the evolution of gastric carcinoma in H. pylori infection certainly warrants a great deal more attention.

6

Clusters Of Phenotypically-Related Human Colonic Crypts Develop Through Crypt Fission: Implications For Colorectal Carcinogenesis

{P} SAC McDonald [1], SL Preston [1], L Greaves [2], P Tadrous [1], P Saseini [1], M Novelli [4], D Oukriff [4], J Jankowski [3], D Turnbull [2], N Wright [1]
[1] *Cancer Research UK, London, United Kingdom,* [2] *University of Newcastle, Newcastle, United Kingdom,* [3] *University of Leicester, Leicester, United Kingdom,* [4] *University College Hospital, London, United Kingdom*

Introduction: It has been proposed that colorectal cancer is caused by the accumulation of DNA mutations in colonic stem cells. Crypt fission (a crypt splitting to form two daughter crypts) is a likely method by which mutations spread through the colon. Crypts within close proximity to each other should therefore be related. We have used mitochondrial (mt) cytochrome c oxidase subunit I (cox) as a marker of crypt clonality due to the relatively high mutation rate of mtDNA. Aim: To investigate whether colonic crypts with cox mutations are clustered. Methods: Immunohistochemistry for cox was performed on en face sections of normal colonic tissue (from 14 patients undergoing resection). Sequencing of the mt genome was performed on laser-captured cox-negative crypts and their closest cox-positive neighbours. In a further experiment, all crypts within images of each section were labelled as cox-negative or cox-positive, and a computer program was used to identify clusters of negative crypts by comparison to 1000 iterations of a random assignation of these labels. Results: In one patient, all crypts had the same mtDNA sequence except that the cox-negative crypts had a 6277 G-A transition, which predicts a Gly125Asp amino acid substitution within cox. In another patient, a 7275 T-C transition predicting a Ser458Pro substitution within cox was found. All 14 patients exhibited clustering of cox-negative crypts with each having a relative prevalence (a negative crypt having a negative neighbour) of greater than 1. When compared to the random iterations, 12/14 patients attained significance ($p<0.05$). Conclusions: Related colonic crypts are clustered. The existence of clusters supports crypt fission as a mechanism by which DNA mutations spread through the gut. This has important implications for colorectal tumourigenesis.

7

Quality Assurance in a Multidisciplinary Study - The MERCURY Experience.

{P} S Fisher , IR Daniels , P Quirke
Pelican Cancer Foundation, Basingstoke, United Kingdom

Introduction: The MERCURY Study aimed to develop a multidisciplinary, pre-treatment MRI-based staging system for rectal cancer.
Methods: Prior to commencement, specialist workshops were held for radiologists and pathologists to standardise the assessment and audit. Proforma based reporting was used for collection of all multidisciplinary data. Digital records of the MRI scans and photographs of excised specimens were collected for audit.
Results: 714 consecutive patients were recruited from 11 centres. 92.7% of data proformas were complete. Of patients registered, 97% underwent an MRI scan and 94.3% were technically satisfactory. 95% of patients treated were discussed pre-operatively at an MDT (range 66-100%). Decisions made pre-operatively - 48% primary surgery, 10% SRT + surgery, 22.5% chemo-radiotherapy, 10.8% long-course radiotherapy, 5.8% palliative care and 2.6% were observed. Operations performed - 63% an anterior resection, 22.8% an abdomino-perineal resection, 2.4% local excisions and 11.8% others. Macroscopic assessment was available on 404 specimens. The median node count was 12.
Conclusion: A high standard was achieved in quantity and quality of the data. Training via specialist workshops ensured standardisation of procedures and reporting. The multidisciplinary, proforma-based method of data collection provided concise and accurate information for audit, quality assurance and data analysis.

8

Detection Of Non-Pathogenic *E.Coli* Within Granulomas And Bowel Wall In Patients With Crohn's Disease Using Laser Capture Microdissection And PCR

{P} P Ryan [2], RG Kelly [1], G Lee [2], JK Collins [3], GC O'Sullivan [4], J O'Connell [1], F Shanahan [1]
[1] *Departments of Medicine, National University of Ireland, Cork., Cork, Ireland,* [2] *Departments of Histopathology, National University of Ireland, Cork., Cork, Ireland,* [3] *Departments of Microbiology, National University of Ireland, Cork., Cork, Ireland,* [4] *Departments of Surgery, National University of Ireland, Cork., Cork, Ireland*

BACKGROUND: We have previously shown that *Mycobacterium paratuberculosis* DNA is detectable in granulomas in 40% of surgical cases of Crohn's disease. The significance of this finding is influenced by the degree to which inflamed bowel wall, including granulomas, is infiltrated by luminal bacteria. *E.coli* is a gram-negative commensal bacterium, not suspected to be of aetiological significance in Crohn's disease. AIMS: To examine Crohn's disease granulomas and whole sections of bowel for presence of *E.coli* DNA. METHODS: Archival tissue from 15 surgical cases of Crohn's disease and 10 non-Crohn's granulomatous bowel disease controls were examined. Discrete granulomas were isolated using laser capture microdissection. DNA from granulomas and full-thickness sections were examined for presence of E.coli DNA by PCR amplification of a 200 base-pair segment of the uidA gene. RESULTS: In Crohn's disease patients *E.coli* DNA was detected in 12/15 microdissected granulomas and in 8/15 full thickness sections. In non-Crohn's granulomatous controls *E.coli* was detected in 1/10 microdissected granulomas and 4/10 full thickness sections. CONCLUSIONS: *E.coli* DNA may be detected more frequently in Crohn's disease granulomas than in other non-Crohn's bowel granulomas. This may indicate a general tendency for luminal bacteria to colonise inflamed tissue, or it may be due to increased uptake of bacterial DNA by gut antigen presenting cells. In the light of previous detection of *M.paratuberculosis* DNA in Crohn's granulomas, the non-specific nature of the type of bacterial DNA present in granulomas is evidence against any one bacterium having a significant causative role in Crohn's disease.

9

Ductular reaction in the post-transplanted liver: a component of cellular rejection

{P} A Ahmed El-Refaie , A D Burt
School of Clinical and Laboratory Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom

Background: The ductular reaction in human liver is most commonly seen as a consequence of cholestasis or as a response to hepatic necrosis. It can be observed in biopsies from orthotopic liver transplants but in this setting its significance is uncertain. It has often been taken to be a sign of biliary obstruction and an indication for invasive investigative procedures such as ERCP but often such approaches yield no obvious cause. Here, we assess whether it may be associated with the process of rejection.
Methods: Post-transplant liver biopsies (n = 444) were retrieved from liver transplant patients from 1994 to 2002. They included those with histological/clinical evidence of: (i) acute rejection – mild (n = 136), moderate (n = 134) and severe (n = 50), (ii) chronic rejection (n = 16), (iii) bile duct obstruction (n = 57), (iv) ischaemic damage (n = 21), (v) chronic non-specific hepatitis (n = 36), (vi) chronic HCV recurrence (n = 20) and (vii) post-transplant 'normal liver' (n = 4). The degree of ductular reaction was assessed using a semi-quantitative scoring system and image analysis.
Results: The degree of ductular reaction was significantly correlated with acute liver allograft rejection severity ($p < 0.001$) and in particular with the Banff grade of portal inflammation ($r^2 = 0.288$; $P < 0.001$). Ductular reaction was also observed (as expected) in cases where there was clinical evidence of extrahepatic biliary obstruction but it was not associated with ischaemic injury.
Conclusion: The ductular reaction is part of the tissue response in acute cellular rejection; its presence does not necessarily indicate an acute cholestatic problem in post-transplanted livers grades.

10

THE NATURAL HISTORY OF HEPATITIS C WITH SEVERE HEPATIC FIBROSIS

A Lawson , N Taguri , K Rye , S Hagan , **{P}** A M Zaitoun , K Neal , W Irving , M Mc Kendrick , D Gleeson , M Wiselka , S Ryder
Queen's Medical Centre, Nottingham, United Kingdom

Introduction: There is limited data on the natural history of Hepatitis C (HCV) infection, and in particular on the prognosis for those patients with severe liver fibrosis. **Aim:** To examine the morbidity and mortality of patients with severe fibrosis secondary to HCV infection, within the Trent HCV Study.
Methods: 90 HCV infected patients (60 male, mean age 50 (range 30-79)) were identified from the Trent HCV study group database as having had a liver biopsy prior to January 1, 2001 demonstrating severe fibrosis (Ishak stage 4,5 or 6), and no evidence at the time of biopsy of hepatocellular carcinoma. Follow-up data was extracted from the database and the patient's hospital records, from the date of biopsy until either their death or the end of the observation period (December 31, 2003). **Results:** Median follow-up was 57 months (range 2-171). 29 (32%) patients died (n=26) or were transplanted (n=6, 3 died) at a median time from biopsy of 27 months. 19 (73%) deaths attributable to HCV infection. Survival probability was 81% (n=90), 71% (n=62) and 43% (n=7) at 3, 5 and 10 years, respectively. 9 (10%) patients developed Hepatocellular carcinoma (HCC) after a median of 39 months (range 10-106). For patients with no previous history of decompensated cirrhosis (n=82), the survival probability was 88%, 78% and 40% at 3, 5 and 10 years. A history of decompensated cirrhosis ($p<0.001$) and the patient's age at biopsy ($p=0.009$) were independent predictors of death (Cox regression analysis). A protective effect was seen for both decompensation and death in patients who received interferon and ribavirin therapy (n=65)($p=0.017$).
Conclusion: This study demonstrates a worse prognosis for HCV infected patients with severe liver fibrosis than has previously been shown. It suggests, however, that intervention with IFN/ Ribavirin may improve survival.

11

Prediction of Response To A Topoisomerase I Inhibitor in Renal Cell Carcinomas

{P} DM Berney , P Lobo , J Shamash , RTD Oliver
St Bartholomew's and The Royal London Hospital, Queen Mary University, London, United Kingdom

Renal cell carcinomas are usually unresponsive to conventional chemotherapy regimes. However, administration of a Topoisomerase I (Topo I) inhibitor to patients with metastatic renal cell carcinoma (RCC) has shown responses, even in patients with cytokine refractory tumours. At present it is not possible to identify which patients would be responsive to such treatment before administration. We therefore investigated the immunohistochemical expression of Topo I and Topoisomerase II (Topo II) in a series of RCCs including patients who had been given a Topo I inhibitor (Irenotecan). Cases were selected from patients with cytokine refractory RCC. Patients had active progressive disease, and were commenced on a treatment course of Irinotecan under a phase II trial. Tissue blocks were sought from all cases, and obtained for 23 cases of RCC. Sections were stained for Topo I and II and Ki-67 to examine the proliferative index of the tumours.

Survival data was available for 15 patients. Relative risks for a favourable outcome were calculated in relation to marker status. Strong staining for Topo I increased the chance of a favourable outcome by 2.5 times although this was not significant, within this small cohort (p=0.119). The stronger staining patterns appear to lean towards an increase in progression free survival, in particular Topo I (RR =0.423, CI 0.123-1.35). Neither Ki-67 nor Topo II expression was associated with survival, though Topo II expression was highly positively correlated with Ki-67.

The expression of Topo I in this series suggested that it may be helpful in selecting patients likely to benefit from Irinotecan. Immunochemistry may be a viable method of testing for likely Topo I sensitivity in RCC's.

12

Detection of human papillomavirus and expression of p16INK4A in penile carcinoma

{P} DM Prowse , S Youshya , E Ktori , P Agarwal , RT Oliver , SI Baithun
Queen Mary University of London, London, United Kingdom

Human papillomavirus (HPV) are double stranded DNA viruses that are associated with the development of epithelial lessons. Specific high-risk HPV are important sexually transmitted human carcinogens recognised to cause cervical carcinoma and implicated in other anogenital cancers including penile cancer. An analysis of a series of 67 tissue samples from 33 penile carcinomas was performed to determine HPV presence and expression of $p16^{ink4a}$. We used a degenerate nested PCR technique to detect a broad spectrum of HPV types in archival specimens. Sixteen (48%) of the carcinomas were found to harbour HPV DNA, eleven contained Alpha HPV (33%) and five Beta HPV (15%). High-risk HPV 16, which is associated with malignant mucosal lesions, was detected in ten (30%) of the penile carcinomas. HPV 18, another high-risk mucosal HPV was detected in one (3%) case. Beta HPV types 5, 14 and 17, previously associated with both benign and malignant cutaneous lesions, were also found in a small number of the samples. Strong immunostaining for p16INK4a was observed in a high frequency of carcinomas and this often associated with HPV expression. This increased expression of p16INK4A is consistent with an active role for HPV in interfering with the Rb pathway during penile carcinogenesis.

13

Direct induction by melanoma, prostate cancer and Ewing's sarcoma tumour cells of osteoclast formation by a RANKL-independent mechanism

{P} YS Lau [1], A Sabokbar [1], H Giele [2], CLMH Gibbons [2], V Cerundolo [1], NA Athanasou [1]
[1] University of Oxford, Oxford, United Kingdom, [2] Nuffield Orthopaedic Centre, Oxford, United Kingdom

Osteolysis due to metastasis or invasion by malignant tumours is associated with an increase in osteoclast formation and resorption. Osteoclasts are known to be formed from monocyte/macrophage precursors by both RANKL- and TNFα-induced mechanisms. We added conditioned medium from cultures of tumour cell lines of neoplasms known to cause bone destruction, including melanoma (A375), prostate cancer (LNCap) and Ewing's sarcoma (TC71), to human monocyte cultures incubated in the presence and absence of M-CSF, RANKL and TNFα/IL-1 and found that this resulted in the formation of mature bone resorbing osteoclasts. Osteoclast formation was assessed by expression of tartrate-resistant acid phosphatase, vitronectin receptor and the ability to carry out lacunar resorption. Osteoclast formation required the presence of M-CSF but occurred in the absence of RANKL or TNFα. The addition of RANK:Fc or an antibody to TNFα did not block osteoclast formation, indicating that this process was not RANKL- or TNFα-induced. This is the first report of tumour cells directly inducing osteoclast formation through the release of a soluble factor that appears to operate by a RANKL-independent mechanism. As primary and secondary bone tumours contain macrophages, this factor is likely to play a role in tumour osteolysis.

14

Ape/Ref-1 Expression in Torn Rotator Cuff

{P} TR Helliwell , A Sheth , SP Frostick , A Rawal , J Gibson , V Rayner , MM Roebuck
University of Liverpool, Liverpool, United Kingdom

Pressure from the head of the humerus results in a functionally avascular zone that is the site of degeneration and tears in the rotator cuff. Fluctuating levels of tissue oxygenation lead to oxidative stress, the production of reactive oxygen species (ROS) from mitochondria and the potential for DNA damage.

The expression of the multifunctional DNA repair enzyme, APE/ref-1, was determined in torn rotator cuff tissue and correlated with morphology and measures of shoulder function in 20 patients undergoing surgical repair for rotator cuff tears.

Rounded fibroblasts in areas of degenerate tendon show strong nuclear and cytoplasmic (mitochondrial) APE/ref-1 positivity, while spindle shaped fibroblasts in relatively intact tendon show cytoplasmic positivity with occasional weak nuclear staining. Nuclear expression in endothelium was intense and focal. Vascular smooth muscle showed widespread cytoplasmic positivity, most intense in tissue with strong fibroblast positivity. Patients with strong fibroblast expression of APE/ref-1 showed a significant reduction in the pre-operative shoulder function, compared with those with focal weaker expression.

APE/ref-1 is a potential defence against ROS and its expression in degenerate rotator cuff tissue from patients with reduced shoulder function may indicate attempted repair of hypoxia-induced nuclear or mitochondrial DNA damage in fibroblasts and vascular smooth muscle.

15

Artificial Neural Networks and Survival Prediction in Laryngeal Cancer

{P} TR Helliwell , AS Jones , AGF Taktak , JE Fenton , AC Fisher
University of Liverpool, Liverpool, United Kingdom

The process of carcinogenesis involves many variables with highly complex interactions and significant non-linearity. The most appropriate mathematical method to model such chaotic systems is not known.

This paper describes a retrospective comparison of an artificial neural network model and Cox's proportional hazards model in predicting survival, using data on 873 treated patients with squamous carcinoma of the larynx. The results were tested against the Kaplan-Meier observed survival. The binary covariates were age, sex, performance status, subsite, grade and stage. The models were tested in turn on randomly-allocated training and study datasets.

There was no significant difference in overall survival between the three methods. Both models gave a greater separation of binary survival curves than suggested by univariate analysis (X^2 for trend p=0.0087). The differences were significant for Cox's model and for the neural network by ANOVA (p=0.0061 and p=0.0008) and the log-rank test (p=0.0053 and p=0.0012). Greater separation of the curves was seen with the neural network.

The neural network gives qualitatively similar, but quantitatively different, prediction of survival compared with Cox's model. We suggest that this approach indicates the validity of using artificial neural network analysis to study the complex, interacting systems involved in carcinogenesis.

16

Genome-wide Analysis of LOH by SNP Microarrays to Demonstrate Chromosomal Loss in Lung Cancer Precursor Lesions

{P} DRJ Snead [1], DP Dhillon [1], H Vohra [1], N Briffa [1], A Blanks [2], H Bird [2]
[1] University Hospitals of Coventry and Warwickshire NHS Trust, Coventry, United Kingdom, [2] University of Warwick, Coventry, United Kingdom

Cancer develops by stepwise accruement of genetic and epigenetic events. To some extent, individual mutational events can be matched to recognizable pre-malignant lesions. Until recently, such investigations required relatively large amounts of DNA, ensuring much of the work has been carried out on established tumours and cell lines, and relatively little investigation has been done on precursor lesions. In such samples the key mutational steps may be masked by secondary events. We have used single nucleotide polymorphisms (SNP) microarray chips, to perform genome wide analysis of loss of heterozygosity (LOH) on paired samples of microdissected invasive lung carcinoma, and its epithelial origin, to demonstrate sequential chromosomal loss from the pre-cursor lesion, to the invasive tumour. In a proof of principle experiment, we harvested samples of 200 cells, from a cryostat section of fresh frozen tumour, and the overlying epithelium showing epithelial hyperplasia, by laser capture microdissection (LCM). DNA was extracted and subjected to whole genome amplification and hybridized to the Affymetrix 10,000 SNP microarray. DNA from lymph node cryostat sections was used as the patient's normal control. In this experiment, the DNA was amplified in three separate reactions and pooled prior to hybridization. The concordance of the normal sample with the hyperplasia was 99.48%, the 0.52% non-concordance being mainly attributable to the LOH observed between the normal and hyperplastic epithelium. The concordance of the tumour sample with the normal lymph node was only 87.17%, and this was attributable to many regions of LOH seen in the invasive tumour sample. Both putative and known tumour suppressor genes are located in the regions of loss.

17

Apoptotic Regulation within Normal and Cancer Containing Breasts

{P} R A Walker , A Gordon-Weeks , J Luckett
University of Leicester, Leicester, United Kingdom

We have previously identified differences between the normal breast of women with and without cancer, with respect to apoptosis, epidermal growth factor receptor expression and beta4 integrin expression by myoepithelial cells, supporting the hypothesis that alterations in growth regulation of normal breast can predispose to the development of breast cancer.

To evaluate this further the expression of the apoptotic regulatory proteins bcl-2, bax and caspase-3 was examined in 119 age-matched tissues (57 non-involved tissue from cancer-containing breast (NTCCB) and 62 normal from women without cancer) using immunohistochemistry. Apoptosis and proliferation indices (AI and PI) were determined using M30 and MIB-1 immunohistochemistry.

There was a significant decrease in AI in NTCCB compared to controls, but PIs were similar. AI increased and PI decreased with age in controls but no changes were seen with age in NTCCB. Expression of bcl-2 was significantly greater in NTCCB, with a slight difference for bax but no differences for the 2 groups for caspase-3.

Preliminary studies of AI and PI of MCF-7 and T 47-D breast cancer cells following culture with conditioned medium from myoepithelial cells from normal and NTCCB showed that the latter increased PI and decreased AI in comparison to that from normal.

There are differences in the apoptotic regulation in cancer-containing breasts and preliminary data suggest that the myoepithelial cell may be an important factor in this.

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The Expression of Mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC And MUC6) and their Prognostic Significance in Human Breast Cancer

{P} B Boyce , E Rakha , D Abd El-Rehim , A Green , C Paish , I Ellis
Nottingham University, Nottingham, United Kingdom

Mucins are a large family of glycoproteins expressed by many epithelial cells and their malignant counterparts. Much interest has been focused on expression of its members in breast cancer because of their potential role as prognostic indicators and their involvement in cancer therapy. In this study we have examined 1447 cases of invasive breast carcinoma with a long-term follow up, using tissue microarray (TMA) technology and immunohistochemistry to evaluate the expression profiles of several mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and to assess their prognostic value. We detected MUC1 expression in 90.4% of tumours. MUC1 over-expression was associated with a lower histological grade, smaller tumour size and positive higher oestrogen receptor (ER) phenotype. The sub-cellular localization but not the level of expression had a prognostic value in predicting outcome. The aberrant cytoplasmic and /or membranous localization of MUC1 was associated with poor overall survival compared with apical localization, which is the normal physiological site of expression. MUC2 expression was noticed in only 8.3% of all cases and was restricted to the cytoplasm of the tumour cells. An inverse trend was identified between its expression and lymph node stage and vascular invasion status. On excluding cases of mucinous carcinoma from the analysis, an inverse association with ER status emerged. MUC3 expression was detected in the cytoplasm (91%) and membranes (17%) of the tumour cells but only its membranous expression was found to be a potentially poor prognostic feature; associated with higher grade, presence of vascular invasion, negative ER expression, poorer Nottingham Prognostic Index and development of recurrence. Positive expression of MUC4 and MUC5AC was detected in 95% and 37% of the studied cases respectively. Apart from an association between MUC4 expression and tumour grade, no association with other clinicopathological variables was detected. MUC6 expression was detected in 20% of cases and its expression was associated with ER negative tumours.

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Oestrogen Receptor Variant Expression As Potential Selectors For Adjuvant Endocrine Therapy In Breast Cancer Patients

{P} AR Green [1], EC Paish [2], JM Gee [3], RI Nicholson [3], KL Cheung [4], JF Robertson [4], IO Ellis [2]
[1] Department of Histopathology, School of Molecular Medical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, United Kingdom, [2] Department of Histopathology, University of Nottingham, Nottingham City Hospital, Nottingham, United Kingdom, [3] Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, United Kingdom, [4] Professorial Unit of Surgery, Nottingham City Hospital, Nottingham, United Kingdom

Disease-free interval differs amongst invasive breast cancer patients, who had not received any prior adjuvant systemic therapy, with oestrogen receptor (ER) positive cancers treated by endocrine therapy at the time of relapse. This suggests the presence of biological factors inherent in tumours which affect responsiveness to subsequent endocrine therapy. ER splice variants result from exon deletions and can repress wild-type receptors and modulate anti-oestrogen activity. We hypothesise ER splice variants contribute to the differences seen with therapeutic response in these patients. We have characterised variant ER expression in primary invasive breast cancer patients that either responded or not responded to endocrine therapy at the time of relapse. Breast tumour cells were isolated from formalin-fixed archival tumour sections using laser microdissection. Total RNA was extracted and expression of ERα, ERαΔ2-3, ERαΔ3, ERαΔ5, ERβ1, ERβ2 was quantified using real-time PCR. Gene expression was normalised against 18s expression. Expression of ERα wild-type and variants were detected in most breast tumours although levels differed. ERβ1 was highly expressed in tumours with low ERα expression. ERβ2 was less commonly expressed. An association between disease-free interval and ER variant expression remains to be elucidated although there was a tendency for tumours expressing ERα also expressed ERαΔ3.

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Mast cells in human decidua and trophoblast: alternations in placental pathology

{P} X Xu , JN Bulmer , AD Burt
School of Clinical and Laboratory Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom

The semi-allogeneic foetus is allowed to develop until term during a normal pregnancy. The precise functions of the various immune cell types at the implantation site and within decidua and trophoblasts are not well defined. However, it is thought that certain sub-populations of leucocytes, such as mast cells (MC), play a role in pregnancies which do not progress to term.
The purpose of this study was to map MC populations in decidua during normal pregnancy and to compare the numbers of such cells with that in 'pathological' pregnancy. Tissues were obtained with fully informed consent from women during normal pregnancies, Caesarean section, ectopic pregnancies and first trimester spontaneous abortions.
Mast cells were detected in tissue sections using anti-tryptase antibody and the density of tryptase+ cells was evaluated by light microscopy. Tryptase+ mast cells were located among smooth muscle cells and around blood vessels in myometrium and near decidual glands or blood vessels in decidua.
Quantitatively there were significant differences between mast cell densities in myometrium for 1st and 2nd trimester placental bed versus the 3rd trimester placental bed. There were significantly more tryptase$^+$ mast cells in myometrium than in decidua of 1st and 2nd trimester placental bed. In 3rd trimester, there were statistically significant more tryptase$^+$ mast cells in placental bed than in non-placental bed. Compared with normal pregnancy, there was a higher density of mast cells in spontaneous abortion deciduas. There was no difference between normal and ectopic pregnancy.
The data supports previous suggestions that mast cells may play a role in spontaneous abortion.

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Histological grading of epithelial ovarian carcinoma - does it matter which grading system is used?

{P} N Singh [1], A Ayhan [2]
[1] *Barts and the London NHS Trust, London, United Kingdom,* [2] *Hacettepe University School of Medicine, Ankara, Turkey*

The histological grade of ovarian epithelial cancers is reported to correlate closely with survival. There is, however, some variability in grading systems and in many publications it is not clear which grading system has been applied. In this study we compared tumour grade based on conventional criteria with nuclear grading and the Shimizu-Silverberg system.

The original histology slides of 182 primary serous (n=142) and mucinous (n=40) ovarian carcinomas were graded with conventional criteria, nuclear grading alone and Shimizu-Silverberg grading. When considered as a group there appeared to be no significant difference between different grading systems ($p>0.2$), although it is important to note that in an individual case a difference in grade could determine whether or not adjuvant treatment is given. In serous invasive tumours, however, there was a significant difference between systems ($p<0.02$). This was primarily in the grade 1 and 2 categories, that were generally downgraded in the Silverberg system, while grade 3 carcinomas were generally graded as such with any system.

We conclude that Grade 1 and 2 serous carcinomas can be graded differently depending on the system applied. There appears to be a need to consider either the universal application of a comprehensive system such as that of Shimizu-Silverberg or the move to a two-tier system.

22

Transplanted Bone marrow cells engraft within the mouse epidermis and proliferate with no evidence of cell fusion

{P} M Brittan , KM Braun , LM Reynolds , FJ Conti , AR Reynolds , R Poulsom , MR Alison , NA Wright , KM Hodivala-Dilke
Cancer Research UK, London, United Kingdom

Introduction. Bone marrow (BM) cells engraft in non-haematopoietic tissues and form adult cell lineages. We investigated the contribution of BM to epidermal regeneration. Method. Lethally-irradiated female mice were rescued by BM transplant from GFP male mice. Epidermal wounding was induced 6 weeks post-transplant. Epidermis was harvested 4, 7 and 30 days post-wounding, or keratinocytes were isolated from the epidermis and re-suspended in growth medium. BrDU was injected 2 hours before sacrifice. Donor cells were detected by GFP immunohistochemistry (IHC), or *in situ* hybridisation (ISH) for Y chromosome, combined with IHC. For fusion studies, a similar model was employed using male wild type (WT) recipients. Results. BM contributes to 7.2% of keratinocytes in normal epidermis, increasing significantly to 11.5% in wounded epidermis. BMDKs were present in clusters in the regenerating epidermis. BMDKs frequently engrafted epidermal stem cell zones and often expressed CD34, an epidermal stem cell marker. In the epidermis, BM-derived cells, morphologically typical of keratinocytes, were negative for macrophage marker F4/80, and neutrophil marker, Ly6G. Fluorescent IHC for GFP, keratin-14 (k14), and BrDU showed that BMDKs proliferate: supported by the presence of GFP+, k14+ keratinocyte *in vitro* colonies from WT epidermis transplanted with GFP+ BM. ISH for Y chromosome combined with GFP and k14 IHC in epidermis from male WT mice transplanted with male GFP+ BM, showed that GFP+ cells express k14, but do not fuse with pre-existing keratinocytes. Conclusion. We demonstrate for the first time, BMDKs capable of proliferation *in vivo*, without fusion with native keratinocytes.

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Myofibroblasts Associated with Liver Tumours are Frequently of Bone Marrow Origin

{P} P Vig [1], F Russo [2], B Bigger [2], N Wright [1], H Thomas [2], M Alison [1], S Forbes [2]
[1] *Cancer Research UK, London, United Kingdom,* [2] *Imperial College, London, United Kingdom*

Aim: Myofibroblasts produce the tumour capsule seen around certain liver cancers and may have a pro-angiogenic role in liver metastasis. We have recently found that a significant proportion of hepatic myofibroblasts are of bone marrow (BM) origin in human liver fibrosis. Our aim was to identify whether myofibroblasts associated with liver tumours had a BM origin in a murine model of chronic liver injury and hepatocellular carcinoma.
Methods: Hepatitis B surface antigen transgenic female mice (HBsAg-tg) received lethal irradiation followed by a BM transplant with whole or lineage-depleted (Lin^-) BM from a wild-type male donor. Prior to transplantation the BM cells were transduced with a HIV vector carrying the GFP marker gene under the control of a spleen focus forming virus (SFFV) promoter. After 6 weeks mice were treated with retrorsine to block hepatocyte regeneration. After this, half of them received splenocytes from females immunized with HBsAg DNA plasmid. After 6 months the animals were sacrificed and BM derived cells were tracked using *in-situ* hybridisation (ISH) for the Y chromosome and immunohistochemistry for GFP.
Results: Tumours were seen within the livers. The hepatocytes within the liver cancers were not BM derived. There were numerous Y chromosome positive myofibroblasts. Smooth muscle cells within large vessel walls were frequently of BM origin.
Conclusions: Many myofibroblasts associated with liver tumours are of BM origin in this model. Both the whole and Lin^- fraction of BM contains cells with a myofibroblast potential. These circulating cells may have a role in the pathogenesis of liver tumour development.

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β-Dystroglycan Is Constitutively Expressed At The Intercellular Junctions of Epithelial Cells And This Expression Is Frequently Absent In Common Cancers

{P} SS Cross [1], FE Hollingbury [1], JM Lippitt [2], JW Catto [2], FC Hamdy [2], SJ Winder [3]
[1] *Academic Unit of Pathology, School of Medicine & Biomedical Sciences, University of Sheffield, Sheffield, United Kingdom,* [2] *Academic Unit of Urology, School of Medicine & Biomedical Sciences, University of Sheffield, Sheffield, United Kingdom,* [3] *Department of Biomedical Science, School of Medicine & Biomedical Sciences, University of Sheffield, Sheffield, United Kingdom*

Dystroglycan is a protein with extracellular α and transmembrane β subunits which link the extracellular matrix and cytoskeleton by binding to laminin and other matrix molecules. Previously published studies have shown reduced expression of α-dystroglycan in many cancers but β-dystroglycan has only been investigated in a few cases of prostate, breast and oral cancer. We have performed an immunohistochemical survey of β-dystroglycan expression on custom made tissue arrays containing 389 tissue cores representing 29 human tissue types and 23 different tumour types. Immunohistochemistry for dystroglycan was performed using a monoclonal antibody raised against the cytoplasmic domain of β-dystroglycan. In normal glandular and transitional epithelium there was strong dystroglycan expression at the intercellular junction between the epithelial cells, and between epithelial cells and the basement membrane. In squamous epithelium there was strong intercellular staining in the basal epithelial layers in skin and cervix but this was absent in the oesophagus. β-dystroglycan was completely absent or very weakly expressed in the majority of cancers including 98% of colorectal cancers (81 cases), 100% of ureteric transitional cell cancers (57 cases), 100% of oesophageal cancers (10 squamous, 10 adenocarcinoma) but it was present in some malignant tumours including cutaneous basal cell cancers. This is the first comprehensive survey of β-dystroglycan expression in human tissues and cancers. The absence of this important transmembrane protein in the majority of cancers may play a significant role in tumour progression and the mechanisms for this require further investigation.

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Markers Of Adenocarcinoma Characteristic Of The Site Of Origin – Development Of A Diagnostic Algorithm

{P} JL Dennis [1], TR Hvidsten [2], EC Wit [1], J Komorowski [2], AK Bell [1], I Downie [1], J Mooney [1], C Verbeke [3], C Bellamy [4], WN Keith [1], KA Oien [1]

[1] University of Glasgow, GLASGOW, United Kingdom, [2] University of Uppsala, UPPSALA, Sweden, [3] St. James' Hospital, LEEDS, United Kingdom, [4] University of Edinburgh, EDINBURGH, United Kingdom

BACKGROUND: Patients with metastatic adenocarcinoma of unknown origin are a common clinical problem. Knowledge of the primary site is important for their management, but histologically such tumours appear similar. Better diagnostic markers are needed to enable the assignment of metastases to likely sites of origin on pathological samples.
METHODS: Expression profiling of 27 candidate markers was performed using tissue microarrays and immunohistochemistry. In the first round, we studied 352 primary adenocarcinomas, from seven main sites (breast, colon, lung, ovary, pancreas, prostate and stomach) and their differential diagnoses. Data were analysed in Microsoft® Access and the Rosetta system and used to develop a classification scheme. In the second round, we studied 100 primary adenocarcinomas and 30 paired metastases.
RESULTS: In the first round, we generated expression profiles for all 27 candidate markers in each of the seven main primary sites. Data analysis led to a simplified diagnostic panel and decision tree containing 10 markers only: CA125, CDX2, CK7, CK20, ER, GCDFP-15, lysozyme, mesothelin, PSA and TTF1. Applying the panel and tree to the original data provided correct classification in 88%. The 10 markers and diagnostic algorithm were then tested in a second, independent, set of primary and metastatic tumours and again 88% were correctly classified.
CONCLUSIONS: This classification scheme should enable better prediction on biopsy material of the primary site in patients with metastatic adenocarcinoma of unknown origin, leading to improved management and therapy.

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Morphological Characterisation of Tissues by Optical Coherence Tomography

{P} P.J. Tadrous [1], G. Dobre [2], R. Cucu [2], A.Gh. Podoleanu [2], S. Shousha [1], E.M.A. Lalani [1], G.W.H. Stamp [1]

[1] Imperial College, London, United Kingdom, [2] University of Kent, Canterbury, United Kingdom

Optical coherence tomography (OCT) produces cross-sectional images of intact tissue (at histological resolutions) using infra-red light interferometry. It has potential for in vivo histological diagnosis. However the OCT morphology of general human tissues has not been systematically investigated. Here we present a systematic comparison of OCT images with routine histology which aims to define rules for OCT image interpretation.

Breast tissue samples (both 5mm^3 chunks and 30micron frozen sections) from 14 patients were imaged with OCT using 850nm illumination. The same tissues were then processed and stained with H&E and precise correspondencies identified between H&E and OCT images of the same tissue using 2-dimensional and 3-dimensional comparison techniques.

We found that there are characteristic OCT appearances of certain tissue types but these characteristics differ according to 3-dimensional configuration and the surrounding context e.g. epithelial cords appear dark while epithelium with intricate 3D configurations (e.g. cribriform/papillary) is bright. Also the infra-red absorptive/scattering effects of overlying necrotic tissue can alter the appearance of deeper structures. Furthermore, observing how the OCT pattern changes with depth/movement can give more information than static OCT images.

These data provide an evidence-based foundation and benchmark for future OCT studies with a view to in vivo histological diagnosis.

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Career choices for pathology: national surveys of graduates of 1974-2000 from UK medical schools

{P} P Domizio [1], C du Boulay [2], T Lambert [3]

[1] Queen Mary, University of London & Royal College of Pathologists, London, United Kingdom, [2] University of Southampton & Royal College of Pathologists, Southampton, United Kingdom, [3] UK Medical Careers Research Group, Oxford, United Kingdom

Background : In the past 10 years there has been increasing concern about recruitment of junior doctors into the pathology specialties, particularly histopathology, in the UK. Vacancies across the country have been rising sharply and in some areas now reach 25%.
Aim : To report career choices for and career progression in pathology.
Method : Postal questionnaire surveys of qualifiers from all UK medical schools in nine qualification years since 1974.
Results : 74% of doctors responded at one year (24623/33198) and three years (17741/24044) after qualification. Between 1983 and 1993 the percentage of doctors choosing pathology more than halved from 4.8% to 2.1% and has remained static ever since. 57% of doctors who chose pathology one year after qualification were still working in pathology at year 10. Hours and conditions of work, the doctor's personal assessment of their aptitudes, and their experience of the subject as a student influenced long-term career choices for pathology.
Conclusions : Recruitment of UK graduates into the pathology specialties must increase to meet demand in the new and expanding subspecialties. This depends on raising the profile of pathology to medical students and junior hospital doctors. Innovative ways of doing this must be developed.

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Improving National Outcomes in Rectal Cancer - The Development of an English National MDT-TME Project

{P} J Jessop , P Quirke

Pelican Cancer Foundation, Basingstoke, United Kingdom

Introduction: Based upon the success of European national training programmes for total mesorectal excision, and the demonstration of an improvement in the outcome for rectal cancer, a Multidisciplinary Team-Total Mesorectal Excision (MDT-TME) development programme was introduced in April 2003 in England. This involves a three-year post-graduate programme aimed at all colorectal MDTs, consisting of a two-day workshop based upon the principles of the diagnosis and treatment of rectal cancer, with additional mentoring courses for specific disciplines.
Methods: Consultant Surgeons, Radiologists, Pathologists, Oncologists, Colorectal Nurse Specialists and MDT Co-ordinators from every colorectal MDT have been invited to attend a multidisciplinary workshop. Teams are encouraged to attend with their cancer network. 10 workshops per year are held with capacity for 5 MDTs.
Results: Courses began in June 2003. To date 112 teams of the 190 identified have registered with 41 teams trained by 31 March 2004. 73 surgeons, 23 pathologists, 27 radiologists, 55 nurses, 18 oncologists and 13 MDT co-ordinators have participated in the programme.
Discussion: There has been enormous enthusiasm from the MDTs to be involved. The anticipated reluctance of teams to attend a "training development" programme has been avoided, with broad acceptance of an ethos to "share and improve best practice". Only time will assess success.

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Seasonal Variation In Mortality From Myocardial Infarction And Haemopericardium. A Post Mortem Study.

{P} O Biedrzycki , S Baithun
The Royal London Hospital, London, United Kingdom

The seasonal variation in the incidence of and mortality from myocardial infarction (MI) has been well documented in the past. Death due to haemopericardium (HP) after MI should follow a similar trend as this is a usually a delayed complication. We analysed the case load of several pathologists working at two mortuaries in London to see if a seasonal variation in mortality due to HP could be established, and also looked at mortality from MI during the same period, in the same geographical patient cohort for comparison. 2266 post mortem cases of MI and 135 cases of HP were included over a 5 year period from 1999 to 2004. These were subdivided into "winter" if they occurred from 1st November to the end of March (5 months) and "summer" if the occurred between 1st April and the end of October (7 months). 83(61.5%) cases of HP and 1051(46.4%) cases of MI occurred in the 5 winter months. An independent samples T-Test was performed on both groups. The MI group showed a mean difference of 36.63 cases between the winter and summer months (95% confidence interval 8-65) and this difference was statistically significant. (2-tailed value 0.016). The HP group showed a mean difference of 8.97 cases between the winter and summer months (95% confidence interval 3.64-14.30) and this difference was also statistically significant (2-tailed value 0.0004.) There was no statistically significant difference in the age of patients dying in summer or winter as has been reported previously, and neither was there a difference in the ages of patients dying from myocardial infarction or haemopericardium. We conclude that, as would be expected from the known seasonal variation in MI mortality, there is also seasonal variation in mortality from Haemopericardium.

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Loss Of Intercellular Expression Of β-Dystroglycan Is A Common Event In Breast Cancer

{P} SS Cross [1], FE Hollingbury [1], JM Lippitt [2], CA Evans [5], SP Balasubramanian [3], MWR Reed [3], FC Hamdy [2], SJ Winder [4]
[1] Academic Unit of Pathology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [2] Academic Unit of Urology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [3] Academic Unit of Surgical Oncology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [4] Department of Biomedical Science, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [5] Department of Histopathology, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Trust, Sheffield, United Kingdom

Dystroglycan is a protein with extracellular α and transmembrane β subunits which link the extracellular matrix and cytoskeleton by binding to laminin and other matrix molecules. One previous published study has shown reduced expression of β-dystroglycan in breast cancer but only 6 cases were studied. We have investigated the expression of β-dystroglycan in 343 cases of breast cancer. Immunohistochemistry for dystroglycan was performed using a monoclonal antibody raised against the cytoplasmic domain of β-dystroglycan. In the epithelium in normal breast tissue there was strong dystroglycan expression at the intercellular junction between the epithelial cells. Only 6% of the breast cancers retained this pattern of staining, in 69% it was completely absent and in 25% it was weak. Retention of dystroglycan staining was significantly associated with negative oestrogen receptor status (p=0.039), smaller tumour size (p=0.025) and tubular histological type (p=0.038) but there was no significant relationship with tumour grade, axillary lymph node status, vascular invasion, or Nottingham Prognostic Index. These results show that loss of dystroglycan expression is a common and early event in breast cancer and that only small tubular cancers tend to retain normal patterns of expression.

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S100A9 Protein Is Overexpressed In Some Breast Cancers And Is Significantly Associated With Increasing Tumour Grade And Negative Oestrogen Receptor Status

{P} SS Cross [1], SP Balasubramanian [2], CA Evans [4], MWR Reed [2], FC Hamdy [3], I Rehman [3]
[1] Academic Unit of Pathology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [2] Academic Unit of Surgical Oncology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [3] Academic Unit of Urology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [4] Department of Histopathology, Royal Hallamshire Hospital, Sheffield Teaching Hospitrals NHS Trust, Sheffield, United Kingdom

S100A9 (calgranulin B) is a member of the EF-hand type calcium binding protein family which includes some proteins that have abnormal patterns of expression in human cancer. S100A9 commonly forms a heterodimer with S100A8 and is expressed in neutrophils and macrophages but it may be expressed as a homodimer. We have previously shown in a small series that the homodimer is not expressed in normal breast tissue but is expressed in some breast cancers. In this study we investigate a larger series of breast cancers. 400 invasive breast cancers (20% grade 1, 50% grade 2, 30% grade 3) were sampled in triplicate in tissue microarrays and immunohistochemistry for S100A9 was performed with a monoclonal antibody. There was strong immunohistochemical expression of S100A9 in 12% of tumours. There was a strong positive association between the following factors and S100A9 expression: increasing tumour grade ($p<0.0005$), increasing Nottingham Prognostic Index ($p<0.0005$), decreasing expression of oestrogen receptor ($p<0.0005$). There was no significant association with tumour size, axillary nodal status, vascular channel invasion and histological tumour type. This study shows that S100A9 is expressed in about 10% of breast cancers and that these tend to be high grade oestrogen receptor negative tumours. Whether this expression is a specific feature of these tumours or whether it represents the generalised genomic/transcriptomic derangement in such tumours requires further investigation.

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Routine Sampling Of Nipple In Mastectomy Specimens: Pathology Of Limited Clinical Value?

{P} M S GILL , M J STAUNTON
Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, United Kingdom

Aim: The Royal College of Pathologists recommends pathologists to review locally their specimens of "no or limited value". It is standard practice to sample the nipple when assessing mastectomy specimens. Studies have shown malignant involvement of the nipple in 5.6% to 43% of mastectomy specimens, however there has been a great variation in the sampling technique. We aimed to evaluate the utility of nipple sampling in mastectomy specimens (we routinely examine one block of nipple in all mastectomies)
Methods: We reviewed all nipple-bearing mastectomy specimens over a 30-month period to end of June 2004. Review of reports identified all nipples with either macroscopic or microscopic evidence of disease. We excluded prophylactic and skin-sparing mastectomy specimens and specimens from males. 457 mastectomy specimens from 446 patients were reviewed.
Results: Occult nipple involvement was seen in 17 (3.71%) cases (5 DCIS, 4 Paget's, 6 invasive carcinoma, 1 angiosarcoma and 1 lymphovascular invasion). Apart from Paget's, the remaining 13 cases showed the same disease in the nipple as had already been demonstrated in other blocks. Sampling did not provide any additional information of prognostic significance.
Conclusion: If a nipple is macroscopically normal, sampling for microscopy is of limited value.

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Genotypic And Phenotypic Characteristics Of Human Breast Carcinoma

{P} E Rakha , J Armour , S Pinder , C Paish , I Ellis
Nottingham University, Nottingham, United Kingdom

Loss of chromosomal material at 16q is one of the most frequent genetic events in breast cancer. The smallest region of deletion at 16q has been localised to 16q22.1, indicating the presence of a tumour suppressor gene (TSG) at this region. Multiplex Amplifiable Probe Hybridisation (MAPH) is a simple, accurate and a high-resolution technique that provides an alternative approach to DNA copy-number measurement. The aim of this study was to apply MAPH to measure the genomic copy number alteration at 16q22.1 in malignant breast tissues and to examine the expression of the most likely candidate genes at this region by immunohistochemistry. RESULTS: We identified deletion of the whole 16q22.1 region in 13 cases (37%) and interstitial deletion in 9 cases (26%). We delineated the smallest region of deletion at 16q22.1 to a 3Mb region centromeric to the P-cadherin gene. A significant correlation was found between E-cadherin protein expression and gene copy number changes, as well as with histological tumour type. No correlation was detected between the expression of P-cadherin, E2F-4, CTCF or TRF2 genes with tumour type or with copy number changes. No expression of Ksp-cadherin or VE-cadherin was detected in normal and/or malignant epithelial tissues of the breast in these cases. CONCLUSIONS: We have demonstrated that MAPH is suitable for the assessment of genomic imbalances in malignant tissues. The smallest region of deletion in invasive ductal tumours of the breast at 16q22.1 is located between the VE-cadherin and P-cadherin genes. Although our results support E-cadherin as the TSG in invasive lobular carcinoma, they argue against the candidacy of E2F-4, CTCF, TRF2, P-cadherin, Ksp-cadherin or VE-cadherin as TSGs in ductal carcinoma of the breast.

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Application of Immunohistochemistry Reduces Inter-observer Variation in Classification of Papillary Breast Lesions on Core Biopsy

{P} AG Douglas-Jones [1], JM Morgan [1], V Shah [3], M Rashid [4], NS Dallimore [2]
[1] University of Cardiff, Cardiff, United Kingdom, [2] Llandough Hospital, Penarth, United Kingdom, [3] Singleton Hospital, Swansea, United Kingdom, [4] Royal Gwent Hospital, Newport, United Kingdom

This study investigates agreement on core biopsy diagnosis of papillary breast lesions, which is acknowledged as a difficult area and determines the effect of the use of immunohistochemistry (IHC) to assist diagnosis.
H&E sections of 129 core biopsies of breast papillary lesions were circulated to four observers who categorised each case as: B2 (benign), B3a (epithelial proliferation, probably benign but requiring biopsy), B3b (epithelial proliferation with cytological or architectural atypia), 4 (probably malignant but insufficient material or artefact to allow diagnosis), 5 (malignant papillary lesion). Immunostaining was performed for calponin, p63 and CK5/6 and slides recirculated.
There was unanimous agreement in 48% of cases on H&E which rose to 93% after the use of IHC. Overall unweighted Kappa (Ku; 5 categories) rose from 0.5 to 0.91 and weighted (Kw) rose from 0.76 to 0.95 after the use of IHC. The main effect of IHC was to reduce the use of intermediate categories (particularly B3a) and allow definitive diagnosis (B2 or B5).
Agreement on H&E sections alone in papillary core biopsies of breast is only 48% (Ku=0.50; Kw=0.76) but is significantly increased to 93% (Ku=0.91; Kw=0.95; $p<0.0001$) by the use of IHC for CK5/6, calponin and p63.

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Accuracy of Classification of Breast Papillary Lesions in Needle Core Biopsies

{P} AG Douglas-Jones [1], V Shah [3], M Rashid [4], NS Dallimore [2]
[1] Cardiff University, Cardiff, United Kingdom, [2] Llandough Hospital, Penarth, United Kingdom, [3] Singleton Hospital, Swansea, United Kingdom, [4] Royal Gwent Hospital, Newport, United Kingdom

Interpretation of needle core biopsy (NCB) specimens from papillary breast lesions is regarded as difficult and currently they are designated as B3 and excised for diagnosis. This study compares NCB with excision diagnosis in these lesions.
NCBs of 129 papillary breast lesions were identified and immunostained for calponin, p63 and CK5/6. The consensus opinion of four breast pathologists on H&E and immunostained slides were recorded as B2: benign; B3: atypical; B4: suspicious for papillary carcinoma; B5: papillary carcinoma. On follow-up, excision specimens were available in 107 (83%) cases which were reviewed and a consensus diagnosis (B2-B5) recorded The diagnoses on NCB and excision were compared.

NCB Diagnosis	Excision specimen diagnosis				Total
	B2	B3	B4	B5	
B2	34 (87%)	4 (10%)	0	1 (3%)	39
B3	0	7 (70%)	0	3 (30%)	10
B4	0	0	0	2 (100%)	2
B5	0	1 (2%)	0	55 (98%)	56

The two cases with major discordance (NCB-B2/excision-B5 and NCB-B5/excision-B3) were both due to sampling error. The first case (radiologically R5) was related to the presence of multiple papillomas while in the second case (radiologically R3) the lesion was not represented in the excision specimen.
The study shows that most breast papillary lesions can be accurately diagnosed on NCB. Benign papillary lesions diagnosed on NCB may not require excision in the absence of suspicious clinical/radiological findings.

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Three-Dimensional Reconstruction Of A Human Breast Carcinoma Using Routine Laboratory Equipment And Immunohistochemistry

{P} T Kurien [1], R Boyce [1], CE Paish [1], J Ronan [1], J Maddison [2], EA Rakha [1], AR Green [1], IO Ellis [1]
[1] Breast Cancer Pathology Research Group, Department of Histopathology, Nottingham City Hospital NHS Trust and University of Nottingham, Nottingham, United Kingdom, [2] Medical Solutions PLC, 1 Orchard Place, Nottingham Business Park, Nottingham, United Kingdom

The aim of this study was to establish a three-dimensional (3D) reconstruction of an invasive breast carcinoma using basic laboratory equipment to evaluate and characterise the spatial arrangement of parenchymal cells. 128 sequential 4µm tumour sections (20µm apart) were immunohistochemically stained with an epithelial (AE1/AE3) or tumour (c-erbB-2) specific marker in order to reconstruct two 3D images of the normal and malignant parenchymal cells. Sections were imaged using an automated microscope based scanning system with integrated digital camera. Accurate alignment of the images was carried out using a semi-automatic graphical method and an automatic search algorithm employing the Fibonacci search algorithm. The volume was reconstructed using maximum, minimum point projection and Back To Front opacity blending. The quality of the reconstructed images was distinct and provided a comprehensive and explicit view of the normal and malignant breast parenchymal tissues specifically showing the spatial arrangement of the tumour cells and their relation to the surrounding tissues at a high resolution. This simple and reproducible approach has enabled us to understand the spread and infiltration of invasive carcinoma and could also be used to analyse spatial relationship between atypical hyperplastic and malignant *in situ* lesions of the breast.

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Data structures describing human breast duct systems in 3D

JJ Going
University of Glasgow, Glasgow, United Kingdom

Morphological and molecular studies of human breast cancer precursors are rarely able to place these lesions in a breast-wide developmental context. Tools for doing so are poorly developed, but there is no obvious reason why such mapping should not be achieved.
All the ducts in an autopsy breast had been traced in a stack of 25 x 2mm serial 'subgross' cleared sections stained with haematoxylin. As a feasibility study 3D data describing the largest duct system (representing about 25% of the whole breast) were extracted manually from the slice images and successfully encoded in a format readable by the open-source neuron modelling software 'CVAPP' (University of Southampton). This file format ('swc') can handle highly branched dendritic structures. In the digital model the branching ducts could be inspected and rotated freely in 3D. More efficient data extraction from subgross sections would facilitate qualitative and quantitative studies of relationships between breast cancer precursor lesions and developmental features of the parenchyma in which they develop.

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Expression And Prognostic Significance Of Activated Caspase-3 (CASP3) In Malignant Pleural Mesothelioma (MPM)

{P} A Klabatsa [1], DA Fennell [2], MT Evans [2], JPC Steele [2], J Shamash [2], RM Rudd [2], MT Sheaff [1]
[1] Institute of Cell and Molecular Science; Pathology, Barts and the London Queen Mary School of Medicine and Dentistry, London, United Kingdom, [2] Department of Medical Oncology, Barts and the London NHS Trust, London, United Kingdom

Malignant pleural mesothelioma (MPM) is an aggressive tumour characterised by resistance to apoptosis (Fennell DA and Rudd RM, 2004). However the underlying apoptotic mechanisms are poorly understood. Activated Caspase-3 (CASP3) is a key-executioner of apoptosis involved both in the intrinsic and the extrinsic pathways. Defining the functionality of CASP3 is a prerequisite to understanding apoptosis resistance in this disease and development of novel therapeutic strategies.
CASP3 expression was measured in 61 formalin-fixed, paraffin-embedded specimens of MPM. Immunohistochemistry was performed using the rabbit polyclonal Cleaved Caspase-3 (Asp175) antibody, and following the standard avidin-biotin complex technique. Slides were scored semi-quantitatively (0, 1, 2, 3) according to extent and intensity of staining.
CASP3 expression was observed in 20 MPM specimens (32.78%) with a focal distribution of mainly cytoplasmic staining. However, only 1 MPM specimen exhibited significant expression of immunostaining (score=3). 41 of the cases studied (67.2%) did not express the antibody.
MPM cells exhibit low expression levels of CASP3, consistent with a low level of spontaneous apoptosis. Ongoing work is in progress to correlate the findings with clinical data and define expression and prognostic significance of apical caspases 7, 8, and 9.

1. Fennell DA and Rudd RM. Lancet Oncol 2004: 5 (354-362)

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A Comparison of two Cytologic Methods for Analysis of Bronchial Brush Specimens in Lung Cancer

{P} O Sivakumaran [1], TC O'Shaughnessy [2], MT Sheaff [1]
[1] CYTOLOGY DEPARTMENT, BARTS AND THE LONDON NHS TRUST, LONDON, United Kingdom, [2] RESPIRATORY MEDICINE DEPARTMENT, NEWHAM GENERAL HOPSITAL, LONDON, United Kingdom

Cytological examination of bronchial brush specimens is effective in diagnosing bronchogenic carcinoma. The potential pitfalls of the direct smear technique (DST) are loss of informative material and distortion of cellular detail. Before the brush is discarded it can be agitated in saline solution and the dislodged cells centrifuged and cytospun to produce a further specimen (saline brush wash; SBW). These two techniques were compared.
Specimens were taken from 160 different patients at bronchoscopy. Forty-one specimens were found to contain malignant cells. In each of these 41 specimens, the cells in a designated area on each slide were counted and the total number of malignant cells/6.5 mm diameter circle recorded.
Cytology was positive in 40 cases using DST and 41 using SBW**.** DST slides had significantly fewer cells than SBW for the same unit area. The cellular detail was superior with SBW in 33 cases and equivalent in 5. In 1 case the DST specimen was so poorly preserved that the malignant cells were not identified although the SBW was positive. In only 3 DST cases was the presentation better than SBW.
The results suggest that SBW is superior to DST. If both techniques are performed then this may very well improve the presentation of the material and also increase diagnostic sensitivity.

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Comparative Gene Expression Pattern of Indicator Genes in Parallel Human Bone Marrow And Peripheral Blood Samples in Acute Myeloid Leukaemia Using Real-Time PCR

{P} E Sakhinia [1], M Farahangpour [1], J Lin Yin [2], G Brady [3], J Hoyland [1], R Byers [1]
[1] Laboratory Medicine Academic Group, Faculty of Medicine,University of Manchester, Manchester, United Kingdom, [2] Department of Haematology, MRI, Manchester, United Kingdom, [3] Epistem Ltd., Manchester, United Kingdom

Accurate and rapid diagnosis and monitoring of haematological malignancy is vital for optimal treatment. Recent microarray studies have identified Indicator genes that may provide more precise prognostication (Golub et al, 1999). Bone marrow (BM) aspiration is painful and not inexpensive and it would be preferable if these genes could be measured in peripheral blood (PB) samples. We have developed a quantitative gene expression-profiling method which we have used to compare expression of these Indicator genes in human BM and PB samples. Parallel whole bone marrow aspirate and peripheral blood samples were obtained from 19 patients with AML and mononuclear cells (MC) isolated from both sample types by density gradient centrifugation. The mRNA was then globally amplified using a PolyA RT-PCR method and the expression profile of the 17 top ranked genes from Golub et al (1999), were measured by real-time PCR. All values were calibrated against control standards and normalised to the mean of three housekeeping genes (IF2-beta, GAPDH and human ribosomal protein S9) and obtained data were statistically analysed and compared using SPSS software. The results demonstrate similar expression levels between BM and PB for some genes (Leptin receptor, fumarylacetoacetate, CD33, Adepsin, Proteoglycan 1, MB-1, Cyclin D3, hSNF2b, RBAp48, Proteasome iota, HKrT-1 and E2A) indicating the possibility of their routine use in monitoring disease activity in PB samples rather than BM; conversely there was significant difference in expression in BM and PB samples for C-myb, Hox-A9, LYN, Cystatin c and LTC4 ($P<0.05$) and these genes would not be good PB markers.

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Validation And Clinical Application of Microarray Indicator Genes From Acute Leukaemia in Human Bone Marrow Samples Using Real-Time PCR

{P} E Sakhinia [1], M Farahangpour [1], J Lin Yin [2], G Brady [3], J Hoyland [1], R Byers [1]
[1] Laboratory Medicine Academic Group, Faculty of Medicine, University of Manchester, Manchester, United Kingdom, [2] Department of Haematology, Manchester Royal Infirmary, Manchester, United Kingdom, [3] Epistem Ltd, Manchester, United Kingdom

Cancer subtype discovery and classification using microarray signature has the potential to transform pathological diagnosis but measurement of Indicator genes in routine practice remains difficult. We tested the use of real-time PCR measurements of indicator genes for AML and ALL (Golub et al, 1999) as a method for validation and application of microarray gene signatures. Mononuclear cells (MC) were isolated from the whole bone marrow (BM) aspirates and sorted into unselected (total), CD34+ve and CD34-ve fractions. The mRNA of each fraction was globally amplified using a PolyA PCR method. We measured the expression profile of the 17 top-ranked genes from Golub et al. (1999) using real-time PCR. All values were normalised to the mean of three housekeeping genes and obtained data were statistically analysed and compared using SPSS software. The data for all 17 genes was obtained for 5 ALL, 26 AML, 12 AML remission, 4 CGL and 9 morphologically normal BM samples, each further fractionated into three fractions (total MC, CD34+ve and CD34-ve MC). C-myb gene was significantly increased in ALL in the total BM fraction, whilst Cystain c was increased in the CD34-ve fraction of the AML group. hSNF2b was significantly increased in the ALL total B.M fraction and Hox-A9 was significantly increased in the AML CD34+ve B.M fraction. LYN and CD33 were both significantly increased in AML compared to remission AML, indicating the ability of the method to determine the activity status of the disease. The results also demonstrate the ability to validate gene expression signatures through a simple, sensitive, robust and independent method, allowing translation into routine clinical use. In principle the method could be extended to any other tumour types for which gene signatures exist.

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Nuclear transcriptional factors and hypoxia-inducible genes mediate the hepatic vascular adaptive response to chronic hypoxia

{P} TYH Lau [1], GL Tipoe [1], ML Fung [2], E Liong [2], H So [3], LC Liao [4], KM Leung [1], AA Nanji [5]
[1] Department of Anatomy, Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [2] Department of Physiology, Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [3] Laboratory Animal Unit, The University of Hong Kong, Hong Kong, China, [4] Department of Analytical Toxicology, West China University of Medical Sciences, West China, China, [5] Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, PA, United States

We determined the hepatic expression of transcriptional factor HIF-1α in hypoxia and genes possessing hypoxia response element (HRE) such as iNOS, VEGF and ET-1 that modulate the vascular response in liver. We also evaluated the concomitant expressions of NF-κB and AP-1. Blood and liver samples from adult SD rats were collected at specific time-points after exposure of animals to 10% oxygen for a period of 28 days. Samples from the normoxic and hypoxic rats were analyzed for serum ALT, hematocrit, 8-isoprostane, immunohistochemistry, RT-PCR, Western Blotting and EMSA.

Our results showed a significant increase in the hematocrit and a significant weight loss in the hypoxic rats. The liver morphology and serum ALT were normal. Total free 8-isoprostane levels and nitrotyrosine protein were not elevated. iNOS mRNA peaked at day 21 whereas eNOS, VEGF and ET-1 mRNAs progressively increased from day 7 to day 28 in hypoxic liver. Similar trends were observed at the protein level by Western blotting. HIF-1α, NF-κB and AP-1 were upregulated in hypoxic liver. We conclude that the vascular adaptive ability of the liver in chronic hypoxia triggers compensatory nitric oxide-dependent mechanisms for cell survival towards a vasodilatory response through upregulation of transcription factors, HRE and eNOS genes

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NITRIC OXIDE IN AN ACUTE LIVER INJURY MODEL

TM Leung [1], GL Tipoe [1], **{P}** TYH Lau [1], E Liong [2], H So [5], KM Leung [1], WM Tom [3], ML Fung [2], ST Fan [4], AA Nanji [5]
[1] Department of Anatomy, Faculty og Medicine, The University of Hong Kong, Hong Kong, China, [2] Department of Physiology, Faculty of Medicine, The University of Hing Kong, Hong Kong, China, [3] Department of Pharmacology, Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [4] Department of Surgery, Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [5] Laboratory Animal Unit, The University of Hong Kong, Hong Kong, China, [6] Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, PA, United States

We investigated whether a high level of nitric oxide (NO) is protective or injurious in acute liver injury. An acute phase ICR mice model was used by injecting CCl_4 with or without NO inhibitors (SMT and L-NIL) and NO donor (SNP). Blood and liver tissues were collected. Immunocytochemistry, RT-PCR, Western Blotting, EMSA, serum ALT and total 8-isoprostane analyses were performed. Our results showed high levels of ALT with liver cells necrosis, increased total 8-isoprostane and nitrotyrosine protein after CCl_4 administration. NO inhibitors and SNP abrogated these effects. Protein and mRNA levels in CCl_4–treated mice demonstrated upregulation of TNF-α, iNOS, and COX-2. NO inhibitors with CCl_4 diminished the expression of these proinflammatory mediators. NF-κB was also upregulated in CCl_4 treated mice but was reversed in NO inhibitors pretreated mice. CCl_4 with SNP showed slightly lower expression of COX-2 when compared with CCl_4 treated mice but not for TNF-α, iNOS and NF-κB activity. Partial protection of SNP from lipid peroxidation and oxidative stress is due to its scavenging action. We conclude that high level of NO is detrimental in acute liver injury and can be ameliorated by decreasing the NO level with NO inhibitors and NO donor.

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Tissue Microarray Is A Useful Tool In The Evaluation Of Genes Implicated In Transformation Of Follicular Lymphoma

{P} A Lee [1], AJ Davies [2], A Clear [1], M Calaminici [1], J Matthews [2], A Norton [1], G Wright [3], M L M Staudt [3], J Fitzgibbon [2], TA Lister [2], LK Goff [2]
[1] Dept of Histopathology,St Bartholomew's Hospital, London, United Kingdom, [2] Dept of Medical Oncology, St Bartholomew's Hospital, London, United Kingdom, [3] The Lymphoid Malignancies Section, National Cancer Institute, Bethesda, Maryland, United States

A subset of follicular lymphoma (FL) patients (pts.) transform to a more aggressive histological sub-type, typically diffuse large B-cell lymphoma (DLBCL) associated with poor response to therapy and short survival. Gene expression profiling of tissue pre- and post-transformation (Tx) has provided insight into genes involved. To validate these genetic changes a Tx-tissue microarray (Tx-TMA) was created comprising serial samples from 35 pts. The Tx-TMA was used to investigate the phenotype of transformed DLBCL according to the germinal centre (GC) (associated with better prognosis) versus non-GC-like gene signature model of *de novo* DLBCL. Immunohistochemical staining with CD10, BCL6 and MUM1 is used to discriminate between the two subclasses. This panel defined 31/35 (89%) transformed DLBCL pts. as GC phenotype and 3/35 (9%) as non-GC (1/35 was equivocal). Gene expression profiles show transcript reduction of follicular dendritic cell (FDC) markers CD21 and CD23 on Tx. This was corroborated by Tx-TMA; samples from 71% (20/28) of pts. lost FDC meshwork on transformation ((CD21 loss 15/28 (54%); CD23 loss 17/28 (61%)). These preliminary studies suggest that TMA of serial biopsies from pts. with transformed FL provides a powerful means of assessing the relevance of gene expression, both within the tumour and the microenvironment.

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Neuroendocrine Tumours – Reporting Prognostic Factors and Terminology

{P} I P CHANDLER , C FINLAYSON
St George's Hospital, London, United Kingdom

We undertook both a workload review of neuroendocrine tumours between 1990 and 2003, and an audit of how these tumours were reported between 2000 and 2003. The case review examined the range of anatomical sites involved in a group of 110 tumours over 14 years. These included 65 in the lung, 9 in the foregut, 40 in the midgut, 9 in the hindgut and 4 in the pancreas. The main conclusion of the audit was that the text reporting of the main prognostic factors in predicting neuroendocrine tumour behaviour could be improved upon. All factors (such as size, atypia and necrosis) were mentioned in just 16/31 lung tumours, 6/10 appendiceal tumours and 3/4 ileal tumours. We highlight here what those prognostic factors are, and why they are important to both look for in surgical pathology specimens and include in reports. We also discuss changing trends in the terminology of these tumours, hoping to shed light on a potentially confusing area.

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NHShistopathology.com – E-strategies To Optimise Recruitment To Histopathology Training In Great Britain

{P} P Naik [1], S Cossins [2], NR Griffin [1]
[1] *Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom,* [2] *National Histopathology SHO Schools, Leeds, United Kingdom*

The United Kingdom Department of Health announced significant changes to post-graduate medical education in a policy statement entitled `Modernising Medical Careers.' The NHS Histopathology Schools are dedicated to modernising training to facilitate such changes, and to alleviate personnel shortages. The NHS Histopathology training schools started in 2000 with the aim of improving and standardising SHO training across England, and have grown rapidly to a proposed intake of approximately ninety-six trainees in 2005, across twelve sites. In order to increase number and diversity of applicants to this unique programme, the Schools have developed e-strategies to stimulate the recruitment process through a web portal – http://www.nhshistopathology.com. The site utilises material modified from the previously published brochure and now enables real-time updating. The site was designed using Macromedia Studio MX and PHP, and within a week of completion, was listed in the Google.com search engine and Alexa.com site position analysis tool at rank 54,208 (from a total of 39.6mn URLs). We believe this will increase the global awareness and expansion of the training programme to better assist prospective applicants and recruitment to the schools. Up to date traffic analysis will be presented in comparison to other UK health sites together with a real-time demonstration.

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Cases Of Twin Molar Pregnancies Are Being Misdiagnosed As Partial Molar Pregnancies

{P} C A Evans [1], M Wells [2], B W Hancock [2]
[1] *Sheffield Teaching Hospitals, Sheffield, United Kingdom,* [2] *University of Sheffield, Sheffield, United Kingdom*

Hydatidiform molar pregnancies are abnormal gestations with a reported incidence of 1 in 1000 to 1 in 2000 pregnancies. They are characterised morphologically by the presence of hydropic change within the placental villi accompanied by variable trophoblastic proliferation. Cases of twin gestations where there is a normal foetus together with a molar gestation are less common arising in 1 in 20000 to 1 in 100000 pregnancies. Twin molar pregnancies are reported, usually comprising a normal foetus with a complete mole. Rarely, cases of a normal foetus with a partial mole or two complete moles have been reported.

The pathology reports and the original histology with immunohistochemistry for $p57^{KIP2}$ (a paternally imprinted, maternally expressed gene) of nine reported twin molar pregnancies have been reviewed. Two cases of complete twin molar pregnancy had been misinterpreted as partial moles. Four cases were confirmed as complete moles and three cases were rejected from review as there was no proof of a twin pregnancy. It is important to distinguish the types of pregnancy present, particularly within the context of twin molar pregnancies with a complete mole. These pregnancies are known to have a higher incidence of persistent trophoblastic disease and maternal complications such as pre-eclampsia.

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Galectin-3 Immunohistochemistry In Fine Needle Aspirates Of Thyroid Nodules

{P} AJ Marker , S Rassool , L Happerfield , VKK Chatterjee , SK Thomas , GC Wishart
Addenbrooke's NHS Trust, Cambridge, United Kingdom

Introduction: Galectin-3 expression was assessed immunohistochemically in clots prepared from cytological material aspirated from thyroid lesions to determine whether galectin-3 is a useful marker for the recognition of malignancy.

Methods: Sections of paraffin-embedded clot preparations were prepared from 32 archival cases, with number of cases in parenthesis, as follows: papillary carcinoma (10); follicular carcinoma (6); follicular adenoma (11), multinodular goitre (5). Sections were pre-treated with Dako high pH retrieval solution for fifteen minutes, then treated with galectin-3 antibody (Novocastra 9C4 clone) at 1/75 dilution for one hour. The detection system used was Dako EnVision. Staining was scored on the following scale: 0, absent; +, weak; ++, moderate; +++, strong (Table 1).

Results: Table 1. Expression of galectin-3 in thyroid tissues:

Tissue	Cases	0	+	++	+++
Papillary carcinoma	10	-	1	2	7
Follicular carcinoma	6	2	1	1	2
Follicular adenoma	11	5	5	0	1
Multinodular goitre	5	5	0	0	0

Discussion: The results show that papillary carcinomas express galectin-3 in keeping with previous reports. Most benign follicular lesions are either negative or show only weak expression. However, galectin-3 appears to be of limited use as a marker for the recognition of malignancy in cytological material aspirated from follicular lesions since half of the follicular carcinomas were either negative or only weakly positive.

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Colorectal Serrated Adenomas – A Clinico-Pathological Study of 46 Cases

A Chandra [1], A Cerar [2], **{P}** A. A. Sheikh [1], I.C Talbot [1]
[1] St Mark's and Northwich Park Hospital, London, United Kingdom, [2] Institute of Pathology, Medical Faculty, Korytkova, Ljubljana, Slovenia

Background: Serrated adenomas (SA) are thought to be the missing link between hyperplastic polyps and invasive carcinoma in the colorectum.
Aims: We were interested in the association of colorectal SA with invasive carcinoma, local recurrence, synchronicity and metachronicity of lesions.
Subjects: We studied 4536 polyps from 1096 patients retrospectively over an eight year period (1987-1995).
Methods: Adenomas showing at least 50% of serrated architecture were called SA by three reviewing pathologists.
Results: Ninety-one (2%) of all polyps were called SA, which were found in 46 patients. Invasive carcinomas were seen in 3 out of 46 (6.4%) patients of whom one was a case of FAP. A male preponderance (70%) was noted and features of a mild degree of dysplasia seen in a majority (83%) of serrated adenomas. Follow up ranged form 1-12 years with mean time being 5.75 years. Recurrences of SA were seen in 3(6.4%) cases, synchronous SA in 16 (34.8%) cases and metachronous SA in 9 (19.6%) cases.
Conclusion: Invasive carcinoma arising in serrated adenoma is rare, accounting for 2 (4.3%) cases studied in this series.

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The Acute Effect Of Naproxen On The Gastric Inflammatory Cell Infiltrate In *H. Pylori* Infection In Healthy Human Subjects

{P} MW James [1], AM Zaitoun [2], CT Atherton [1], CJ Hawkey [1], JC Atherton [1]
[1] Wolfson Digestive Diseases Centre, Nottingham, United Kingdom, [2] Division of Histopathology, Nottingham, United Kingdom

Introduction
NSAIDs reduce gastric cancer by unknown mechanisms. We have previously shown that NSAIDs reduce *H. pylori*-induced interleukin-8 (a neutrophil chemokine) *in vitro* and animal models of *H. pylori* infection demonstrate a reduction in neutrophils with NSAIDs. Acute histopathological studies in humans are lacking.
Methods
16 volunteers (12 positive and 4 negative for *H. pylori*) entered a placebo-controlled, blinded crossover study of naproxen, a non-selective NSAID, on the acute and chronic inflammatory cell infiltrate in the gastric mucosa. Gastric mucosal biopsies taken at baseline and 3, 12 and 48 hours after starting treatment were graded blindly by the Sydney system and fully quantitative histopathology.
Results
Neutrophils and lymphocytes were significantly higher in those infected with *H. pylori* with or without naproxen therapy ($p<0.01$). Mean neutrophils in *H. pylori* positive subjects were significantly lower during naproxen therapy than placebo by 3 hours (by quantitative histopathology but not Sydney system, $p=0.05$) persisting at 48 hours ($p=0.03$). Naproxen had no effect on lymphocytes, eosinophils or mast cells in *H. pylori* positive subjects, or on any cell population in those without *H. pylori* infection.
Conclusion
Naproxen acutely reduces *H. pylori*-associated neutrophilic infiltration in humans and this may contribute to a reduction in cancer risk.

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Histopathological Screening of Gastrointestinal Biopsies by Non-medical Staff

{P} KP West , A Wilkin
Department of Histopathology, University Hospitals of Leicester, Leicester, United Kingdom

Endoscopic biopsies represent a substantial component of histopathology workload. Even with the use of strict guidelines, a substantial proportion of these biopsies is likely to be normal e.g duodenal biopsies in suspected cases of coeliac disease. If large numbers of normal specimens continue to be received it may be possible for non-medical staff to separate normal from abnormal biopsies in a fashion analogous to cervical screening. In order to test this concept a fourth year medical student, with no significant histology experience, was given 12 hours training and asked to distinguish between normal and abnormal mucosal samples. A total of 125 samples was reviewed. Cases were classified as normal or abnormal by the student and the diagnosis was reviewed by an experienced histopathologist. The samples comprised 11 oesophageal biopsies, 34 gastric biopsies, 24 duodenal biopsies and 56 colorectal biopsies. The student was able to distinguish between normal and abnormal with a sensitivity of 97% and specificity of 70% (positive predictive value 97% and negative predictive 90%). The main problems encountered were malorientation and crush artefact. The results suggest that screening of gastrointestinal biopsies may merit further consideration.

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MRI Predicts Surgical Resection Margin Status in Patients with Rectal Cancer. - Results from The MERCURY Study Group

{P} G Brown , IR Daniels , P Quirke
Pelican Cancer Foundation, Basingstoke, United Kingdom

PURPOSE: The presence of tumour at the circumferential resection margin (CRM) profoundly affects prognosis in rectal cancer making accurate pre-operative prediction of CRM status crucial. Currently there is no agreement for the cut-off value that should be employed for pre-operatively defining patients as CRM -ve.

METHOD AND MATERIALS: A multi-centre, multidisciplinary European collaboration (MERCURY) was initiated to prospectively evaluate MRI in rectal cancer staging. Prediction of CRM status was based a 1mm cut-off. Of 714 consecutive patients enrolled between January 2002 - October 2003, 325 were eligible for assessing mesorectal fascia involvement on MRI compared with CRM involvement on histopathology. Standardised reporting proformas were employed. The minimum distance of tumour to the mesorectal fascia was noted for each patient. The potential CRM was defined as involved if the distance on MRI was <1mm.On axial whole-mount histopathological sections of the rectal cancer specimens, the CRM was involved if the distance of tumour to the margin was <1mm.

RESULTS: Agreement between MRI and histopathology assessment of CRM status when MRI defined CRM involvement as tumour <1mm from the mesorectal fascia was 266/325 (82%, 95% CI 77-85%). Increasing this cut-off to 2mm worsened accuracy 200/325 (61%, 95% CI 56% - 67%).

CONCLUSIONS: Our study confirms that MR accurately predicts CRM status if a cut-off of 1mm is used.

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High Spatial Resolution MRI Predicts Tumour Spread in Patients with Rectal Cancer - Results from The MERCURY Study Group

{P} IR Daniels , G Brown , P Quirke
Pelican Cancer Foundation, Basingstoke, United Kingdom

Accurate preoperative assessment allows prognostic stratification and targeting of pre-operative therapy. A multicentre European collaboration (MERCURY) was initiated to prospectively evaluate High Spatial Resolution MRI in determining extent of local tumour spread in rectal cancer.

The primary endpoint was equivalence between MRI measurement of tumour invasion beyond the bowel wall and histopathology. Equivalence was considered if the difference was within +/- 0.5mm. Based on an expected standard deviation of 2.121mm, 277 patients were required to demonstrate equivalence. The primary hypothesis was that the 95% CI should lie within -0.5 and 0.5mm. A total of 714 consecutive patients from 11 European centres were registered in the study between January 2002 - October 2003, 295 were eligible for the primary end-point assessment of depth of invasion beyond the bowel wall following primary surgery.
Mean depth of tumour invasion beyond the bowel wall was 2.77mm (sd 4.60) and 2.81mm (sd 4.28) for HSR MRI and histopathology respectively. The difference between HSR MRI and histopathology assessment of the depth of tumour invasion beyond the bowel wall was -0.046mm and the 95% confidence interval was -0.487 - 0.395. Therefore, HSR MRI and histopathology assessment of extramural depth showed equivalence, thus allowing accurate pre-operative prognostication.

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Magnetic Resonance Imaging of Low Rectal Cancers: A Multicentre, Multidisciplinary European Study of 282 Tumours Located Within 6cm of the Anal Verge.

{P} IR Daniels , S Fisher , P Quirke
Pelican Cancer Foundation, Basingstoke, United Kingdom

Objectives: It is recognized that the treatment of low rectal cancer is technically challenging due to the inaccessibility of the rectum in the depths of the pelvis. Despite complete rectal excision by APE, the involved margin rates are high, local recurrence is common and survival rates are worse. We have defined low rectal tumours as being within 6cm of the anal verge.
Methods: In a multicentre, multidisciplinary European study, pre-operative MRI was used to stage 712 rectal cancers. Of these tumours 282 have a lower edge 6cm of less from the anal verge.
Results: 55 patients went to palliative care. 85 underwent an AR, and 87 an APE. Overall CRM +ve rate was 12.6% (AR) and 32.9% (APE). Using Quirke grading, AR resections were complete/moderate (89%), but APE specimen grades were poor/standard in 96%. Only 4% had enhanced resections.

Conclusion: There appeared to be a crossover point at 4cm from the anal verge where the AR rate exceeded the APE rate. This large study of low rectal cancer confirms the difficulty in obtaining clear margins in the lower rectal cancers and indicates worse results in those who required an APE.

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Unusual Relapses Of Testicular Germ Cell Tumours

{P} DM Berney [1], I Roberts [2], SI Baithun [1], RTD Oliver [1]
[1] St Bartholomew's and The Royal London Hospitals,Queen Mary University of London, London, United Kingdom, [2] John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom

Over 200 resections from metastatic germ cell tumours have been examined in referral. Most of these show either necrosis, teratoma, or malignant germ cell elements, but there remain cases which are difficult to classify.
These include:
1. Five sarcomatous transformations including two rhabdomyosarcomas, a leiomyosarcoma and two carcino-sarcomas. These prove unresponsive to chemotherapy and surgury remains the only treatment.
2. Two adenocarcinomas in patients with residual teratoma. One of these patients had teratoma present for 15 years before the adenocarcinoma arose. Complete surgical resection is vital.
3. Three cases of primitive neuroectodermal transformation have been seen. These tumours are unresponsive to germ cell regimens but may respond to neuroectodermal type regimens. The possibility of significant remission is possible.
4. Two cases of cystic trophoblastic disease both showed cysts lined by viable trophoblast but with degenerative changes within the cells. These tumours are cured by excision and require no further chemotherapy
5. Leukaemic transformation has not been reported in a testicular germ cell tumour. A case of teratoma has been seen with bone containing hypercellular marrow, highly suspicious of chronic myeloid leukaemia. No Philadelphia chromosome was present, and the appearances were interpreted as secondary to the administration of granulocyte colony stimulating factor.
Correct interpretation of metastatic germ cell tumours is vital is correct therapy to be administered.

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Erythropoietin And Erythropoietin Receptor Expression In Renal Cell Carcinomas

{P} DM Berney , K Argyle , M Cheang , T Powles , RTD Oliver
St Bartholomews and The Royal london Hospital, Queen Mary University, London, United Kingdom

Renal cell carcinomas (RCC) are associated with increased serum erythropoietin (Epo) levels. Epo is known to have a powerful anti-apoptotic effect in bone marrow and may play a role in carcinogenesis. Previous studies have tried to correlate serum EPO levels with prognosis in RCC, however the findings are inconclusive.
We investigated the immunohistochemical expression of Epo and erythropoietin receptor (EpoR) in 60 cases of RCC using tissue micro array, with three cores of each tumour. 11 RCC's had been treated with adjuvant chemotherapy. 8 breast carcinoma cases were included for comparison.
EPO and EPOR were strongly expressed in renal cell carcinomas. EPO expression was significantly increased in clear cell tumours compared with papillary tumours ($p<0.013$) but was not associated with grade,stage or prognosis. EPOR expression was significantly higher in RCC compared to breast cancer ($p<0.015$) and was positively associated with tumour grade ($p<0.01$). EpoR was also strongly expressed in stromal cells and this was negatively correlated with the staining in tumour cells. Stromal staining was not seen in the breast tumours.
EPOR is over-expressed in RCC, both within tumour cells and in stromal tissue. The finding of EPOR expression in stromal cells has not been reported previously and may indicate a separate pathway by which tumour kinetics are modified. EPO levels were not over-expressed. This may be because the level of EPO is regulated systemically, and depends on patient factors, such as anaemia and hypoxia. EPOR is unlikely to be altered to such a degree by patient factors, and therefore may be a more important factor in RCC tumorigenesis.

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Which Basal Cell Marker? A Survey Of Prostatic Basal Cell Markers Used In The UK.

{P} DM Berney [1], M Varma [2], B Jasani [2], A Rhodes [3]
[1] St Bartholomews and The Royal London Hospital, Queen Mary University, London, United Kingdom, [2] University Hospital of Wales, Cardiff, United Kingdom, [3] University of West of England, Bristol, United Kingdom

A questionnaire was sent to all laboratories registered with the United Kingdom National External Quality Assurance Scheme for immunohistochemistry enquiring about the immunohistochemical methods routinely used for the diagnosis of prostate cancer. Responses were received from 220 (68%) of laboratories. Basal cell marker immunohistochemistry was performed by 115 (87%) of 133 responding UK laboratories. Most (60%) of these laboratories used a single basal cell marker. The most commonly used markers were high molecular weight cytokeratin antibody 34betaE12 (77%), cytokeratin 5/6 (42%) and LP34 (26%). The more recently described basal cell marker, p63 was available in only 4% of laboratories. All the basal cell markers were consistently used after pre-treatment, with heat induced epitope retrieval the most commonly used method used by UK laboratories for all the markers. There is considerable variation in the choice of basal cell markers used by UK laboratories to distinguish benign prostate glands from prostate cancer, with most centres using only a single marker. Since none of these markers react with all benign glands, use of a combination of basal cell markers is suggested to help resolve this important differential diagnosis.

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Tissue Micro Array Of A Historical Archive: The British Testicular Tumour Panel Series.

{P} DM Berney , K Argyle , A Clear , RTD Oliver
St Bartholomews and The Royal London Hospital, Queen Mary University, London, United Kingdom

The British Testicular Tumour Panel (BTTP) reviewed difficult testicular cases from 1950 to 1980. The archive includes human and animal tumours and each case has a detailed case history. Macroscopic photographs, slides and some paraffin embedded material is also available. Detailed examination of these cases has not been performed in the era of immunohistochemistry.
The cases were placed on a computerised database, with diagnoses, listing disagreements, where they occurred. 4000 archive cases were reviewed and 2795 paraffin blocks were found constituting 758 cases. 200 cases originally diagnosed as seminoma were distilled using tissue micro array (TMA) into six recipient blocks. The H and E appearance of the 200 tumours was reviewed. Immunochemistry for placental alkaline phosphatase (PLAP) and inhibin was performed.
193 cases (96.5%) showed strong PLAP staining and 6 cases showed weak PLAP staining. A single case was PLAP negative and inhibin positive. This case was composed of clear cells, which showed some spindling and a lymphocytic infiltrate. This was interpreted as a clear cell sertoli cell tumour, which has not been described until recently.
The results have confirmed the accuracy of the BTTP. No significant loss of antigenicity was seen despite the age of some of the specimens. TMA is an excellent technique for saving important tumour archives, and can rapidly and cheaply identify rare tumours. This TMA work may prove of further value in testing novel antibodies, discovering rare cases for future series, and for education and training.

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Loss Of Intercellular Expression Of β-Dystroglycan Is A Common Event In Prostate Cancer And Is Significantly Associated With Loss Of Differentiation

{P} SS Cross [1], FE Hollingbury [1], JM Lippitt [2], KM Feeley [3], A-R Azzouzi [4], SJ Winder [5], FC Hamdy [2]
[1] Academic Unit of Pathology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [2] Academic Unit of Urology, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [3] Department of Histopathology, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Trust, Sheffield, United Kingdom, [4] Centre de Recherche pour les Pathologies Prostatiques, Universite Paris VII, Paris, France, [5] Department of Biomedical Science, School of Medicine & Biomedical Science, University of Sheffield, Sheffield, United Kingdom

Dystroglycan is a protein with extracellular α and transmembrane β subunits which link the extracellular matrix and cytoskeleton by binding to laminin and other matrix molecules. One previous published study has shown reduced expression of β-dystroglycan in prostate cancer but only 15 cases were studied. We have investigated the expression of dystroglycan in 41 cases of benign prostatic hyperplasia and 113 prostate cancers. Immunohistochemistry was performed using a monoclonal antibody raised against the cytoplasmic domain of β-dystroglycan. In the epithelium in benign prostatic hyperplasia and the morphologically normal background of cancer cases there was strong β-dystroglycan expression at the junction between the epithelial cells and the basement membrane, and at the intercellular junctions of epithelial cells. The intercellular expression was lost in 41% of cancers and was only weak in a further 37%. The basal expression was lost in 19% of cancers and was only weak in a further 47%. In the 9 cases of high grade PIN there was strong staining in both sites in 3 cases, weak staining in 4 cases and intercellular absence in 2 cases. Increasing Gleason tumour grade was significantly associated with the reduction in intercellular expression of dystroglycan (Jonckheere-Terpstra test, $p=0.027$) but not with the basal expression ($p=0.233$). These results show that loss of intercellular expression of β-dystroglycan is a very common phenomenon in prostate cancer and occurs with increasingly frequency in less differentiated tumours. These data point to previously unrecognised roles for dystroglycan in cell-cell interactions and normal tissue morphology.

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Laser Capture Microdissection, an Essential Tool for Studying Gene Expression Levels in Heterogeneous Tissues

{P} L J Christie , J Patterson , J Mullins , S Fleming
University of Dundee, Dundee, United Kingdom

We aimed to investigate gene expression in a transgenic mouse model showing mineralocorticoid receptor driven hypertrophy and hyperplasia of the distal tubule and associated hypertension. The 11β-hydroxysteroid dehydrogenases determine the availability of glucocorticoids to activate their receptors and modulate target gene transcription. 11βHSD2 knockout mice show hypertension, polyuria and hypokalaemia arising from illicit activation of mineralocorticoid receptors by glucocorticoids in the absence of the protective action of 11βHSD2. This phenotype is comparable to the Syndrome of Apparent Mineralocorticoid Excess, seen in humans with mutations in the 11βHSD2 gene.

RNA extracted from whole kidney sections was compared to RNA from microdissected distal tubule from both wild type and knockout mice. Using real time RT-PCR of whole kidney for expression levels of the steroid hormone inducible kinase gene SGK-1 no significant difference between the genotypes was demonstrated. However, in laser captured distal tubules these experiments demonstrated a 10-fold down-regulation of SGK-1 in 11βHSD2 knockout mice at 5 months of age but not in comparable mice at 3 months of age.

These data show that significant differences in gene expression levels may be masked by “contaminating” cells in whole tissue, particularly when the cells of interest represent a small proportion of the tissue volume.

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A Review of the Pathology and Management of Uterine Serous Papillary Carcinoma (USPC) and Correlation with Outcome

{P} D Faratian [1], A Stillie [3], R M C Busby-Earle [2], V J Cowie [3], H Monaghan [1]
[1] Department of Pathology, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom, [2] Simpson's Centre for Reproductive Health, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom, [3] Edinburgh Cancer Centre, Western General Hospital, Edinburgh, United Kingdom

Uterine serous papillary carcinoma (USPC) is a highly aggressive gynaecological cancer accounting for 10% of endometrial carcinomas. It is clinicopathologically distinct from the more common endometrioid endometrial carcinoma (EEC), with a propensity for early local and peritoneal spread, which frequently results in upstaging at the time of operation. While it has been suggested that the term USPC should be reserved for endometrial carcinomas containing at least 25% of tumour with USPC morphology, no studies to date have addressed how much USPC pattern is actually required to confer the poor prognosis phenotype of 'pure' USPC. There are continuing uncertainties regarding the optimal surgical and oncological management of USPC, largely due to the relative rarity of the disease precluding large scale clinical trials.

In order to address some of these uncertainties, we performed an audit of the pathology and management of 67 patients with USPC treated between 1994 and 2004. The pathology of the original biopsy and resection were reviewed to determine the percentage of histological subtypes of endometrial carcinoma present in each tumour, and the medical records reviewed to compile the clinical data. The clinicopathological characteristics were correlated with overall survival (OS) and progression-free survival (PFS), with particular emphasis on the effect of 'pure' and 'mixed' histological morphologies on outcome. In brief, we found that mixed histology tumours containing USPC showed a similar survival curve to pure USPC tumours. Patients with Stage 1 and Stage 2 USPC had a markedly better survival to those with Stage 3 and Stage 4 disease. This study contributes to the growing body of data on USPC.

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RETROSPECTIVE AUDIT OF SMEAR AND BIOPSY CORRELATIONS

{P} S. I. Baithun , G. Curran , M. Sheaff
Barts and The London NHS Trust, London, United Kingdom

The computer files at Barts and The London NHS Trust were examined for the calendar year 2002 and correlation was made between cytology reports and histological outcomes and the positive predictive values for both low- and high-grade lesions were calculated. There were 1421 patients with abnormal cytology confirmed by histology. Of those, 717 were called high-grade and 704 low-grade on cytology alone. Comparison of the histology and cytology showed concordance in 581/717 (81%) in high-grade and 566/704 (80%) for low-grade. Therefore, the positive predictive value is above 80% for both groups. Further analysis of the mismatches for the 136 high-grade cytology group found 24/136 normal, HPV 26/136 and CIN1 86/136. Further analysis of the 138 low-grade cytology group shows that 50/138 high-grade, 85/138 no CIN and 3/138 cervical carcinomas. We also examined the ethnic origin of the patients with abnormal biopsies and found the prevalence of disease to be lower in the Asian and Moslem population compared with the non-Asian community. This study shows the value of cytology in predicting both low- and high-grade lesions. Our positive predictive value for both the high- and low-grade lesions is above 80%, which is well above the expected average set by the NHSCSP. Further work is ongoing to review both cytology and histology for the non-matching cases and also to establish whether specialisation in histological and cytological reporting demonstrates increase specificity in reporting.

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THE INCIDENCE OF MORPHOLOGICAL 'TRANSITION' IN SEROUS AND MUCINOUS OVARIAN NEOPLASIA

A Ayhan [3], **{P}** N Singh [1], U Menon [2], S Gayther [2], IJ Jacobs [2]
[1] St Bartholomew's and the Royal London School of Medicine and Dentistry, London, United Kingdom, [2] Institute of Women's Health, University College London, London, United Kingdom, [3] Hacettepe University School of Medicine, Ankara, Turkey

Morphological 'transition' from benign to malignant epithelium is a recognised feature of ovarian epithelial neoplasia. We aimed to characterise the frequency of transition, the relative proportions of benign-appearing and invasive epithelia, and the association of transition with clinicopathological features.

Histopathological analysis was performed in 241 primary ovarian tumors composed of 37serous borderline tumours (SBT), 22 mucinous borderline tumours (MBT); 142 serous invasive and 40 mucinous invasive neoplasms. Transition was present in 79.25% of tumors. This included all SBT, MBT and invasive mucinous and 65% of serous invasive tumors. Transition correlated with tumor weight ($p=0.005$) and diameter ($p=0.017$), and inversely correlated with grade ($p<0.001$) and stage ($p<0.001$). The proportion of benign appearing epithelium correlated with serum Ca125 ($p=0.033$), weight ($p=0.001$), diameter ($p=0.007$), grade ($p<0.001$) and stage ($p<0.001$). Widespread stromal invasion in malignant tumors correlated with absence of transition (>50% of tumor; $p<0.001$) and small size ($p=0.003$ for weight, $p<0.001$ for diameter).

In conclusion, transition and areas of benign-appearing epithelium occurred frequently in ovarian epithelial neoplasms. Benign-appearing epithelium may represent a mature or differentiated form of malignant epithelium. Alternatively such areas may reflect a still recognisable benign precursor. Serous invasive malignancies appear to fall into two different subgroups with striking differences in terms of transition and clinicopathological parameters. These observations need further investigation by molecular techniques.

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Unusual ovarian tumours presenting with raised serum alpha-fetoprotein

{P} N Singh , DM Berney , S Arif , A Faruqi , SI Baithun
Barts and the London NHS Trust, London, United Kingdom

The oncofetal antigen alpha fetoprotein (AFP) is a 70 kD protein expressed in fetal liver. Elevation of serum AFP levels occurs in germ cell tumours, particularly yolk sac tumour, and hepatocellular carcinoma. In young women with pelvic masses and elevated serum AFP the most obvious clinical possibility is that of a malignant germ cell tumour. We present three cases of unusual ovarian tumours in young women presenting with raised AFP and pelvic masses in whom the diagnosis was of Sertoli-Leydig tumour with heterologous elements (n=2) and metastatic gastric signet ring carcinoma with undifferentiated areas (n=1). Women with malignant germ cell tumours are currently managed with conservative surgery and chemotherapy with excellent survival results. Accurate histological diagnosis is crucial for successful treatment.

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Odontogenic Tumours in the v-Ha-ras (Tg.AC) Mouse, a model for Man

{P} A Hargreaves , C Sadler , S Barratt , L Bishop , J Wright
Syngenta CTL, Macclesfield, United Kingdom

The Tg.AC transgenic mouse carries a v-Ha-ras oncogene linked to a zeta globin promoter. The model has been shown to develop a range of epithelial and mesenchymal tumours, including neoplasms of odontogenic origin. 120 Tg.AC hemizygous mice were housed for a period of six months as part of a strain assessment study. Upon histopathological examination 27 odontogenic tumours were identified. Three 3 main types were identified: 1) Mesenchymal cells in a dense fibrous-like matrix, (2) Loose stroma surrounded by anastomosing cords of epithelial cells that exhibited squamous differentiation, and (3) Odontomas forming mineralised tooth structures surrounded by well-differentiated odontoblasts and ameloblasts. There were similarities between the tumours recorded in this study and human odontogenic tumours. Representative sections of each tumour type were selected for various stains, both conventional and immunohistochemical. These included trichrome, reticulin, elastin, cytokeratin, vimentin, desmin, ki 67 and a number of neural markers. The 3 main groups of mouse odontogenic tumour showed variations in their staining characteristics and antigenic expression suggesting progression from 1 to 3. Evidence of cell proliferation was not apparent. It is suggested that the Tg.AC mouse may prove a suitable model for its human counterpart, in the study of odontogenic tumorigenesis.

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Immunocytochemical Labelling in the Diagnosis of Sinonasal Malignant Melanoma

S Prasad, AS Jones , **{P}** TR Helliwell
University of Liverpool, Liverpool, United Kingdom

Sinonasal melanoma is a rare malignancy, the immunocytochemical diagnosis of which often requires the use of a panel of antibodies. The antigenic profile of 18 primary sinonasal melanomas has been characterised as part of a study to examine their immunophenotype.

A tissue microarray was constructed using two cores of tumour tissue from archival wax blocks. The percentage of tumour cells showing immunocytochemical expression of each of 9 antigens was determined, together with the intensity of expression.

There was a close correlation between the results from the two cores. Using an arbitrary cut-off point of 10% cells, vimentin was positive in 15 cases, S-100 protein in 18 cases, melan-A in 14 cases, HMB45 in 13 cases, NK1C3 in 11 cases and tyrosinase in 6 cases. The Ki-67 labelling index ranged from 0-30% and p53 index from 0-65%. No cases were cytokeratin-positive (CAM5.2) and only 4 cases showed weak labelling for EGFR.

Two cases contained melanin. Melanomas with a predominantly spindle cell morphology were more often negative for HMB45 and positive for NK1C3 and tyrosinase than epithelioid melanomas.

A panel of S-100 protein, HMB45 and melan-A would have identified all of the melanomas in this series. Cytokeratin-positivity was not observed.

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Pathogenesis of peribiliary gland hamartomas revisited

{P} F Stark [1], D Smith [2], A D Burt [1]
[1] School of Clinical and Laboratory Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom, [2] Northumbria NHS Trust, North Tyneside, United Kingdom

Benign lesions composed of ductular structures set in a mature extracellular matrix are a common incidental finding in the liver at surgery or autopsy. These were previously referred to as bile duct adenomas but the demonstration of expression of unique proteins found in peribiliary glands led to the suggestion that these lesions may be hamartomatous. They are most often seen in a subcapsular location and are distinct from ductal plate malformations.

We identified a case in which a 'peribiliary gland hamartoma' had arisen in the liver of a patient with α1 antitrypsin deficiency. Intracytoplasmic globules (α1 AT positive) were found in the ductular structures of the lesion indicating that they had arisen from hepatocytes. There was evidence of atrophy in the surrounding parenchyma. Review of further cases of similar lesions also revealed areas of atrophy.

Parenchymal extinction or atrophy generally occurs because of local intrahepatic flow abnormalities. This may be accompanied by a localised regenerative response in the form of a ductular reaction. We hypothesise that so-called peribiliary gland hamartomas are not hamartomatous or neoplastic but the residuum of a response to parenchymal extinction.

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Caspase-3 Expression in Hodgkin's Lymphoma

{P} M Kandil , N Assad , K Amer , A El Naggar
Faculty of Medicine-Menoufeya University, Shebin el Kom, Egypt

Caspase-3 is a member of caspases family which mediates several complex proteolytic cascades responsible for execution of various stimuli of apoptotic cell death. The aim of this study was to evaluate caspase-3 expression in Hodgkin's lymphoma patients, using immunohistochemical technique, and correlate it with other clinicopathological factors aiming at estimating its predictive and prognostic value. The study was carried out on 51 cases of Hodgkin's lymphoma that comprises 46 cases of classical Hodgkin's lymphoma (CHL) and 5 cases of nodular lymphocyte predominance Hodgkin's lymphoma (NLP-HL) as well as 10 cases of reactive follicular hyperplasia (RFH) as a control group. In RFH, caspase-3 expression was detected in germinal centres' while mantle zones were mostly negative. In CHL cases, 93.5% of cases were caspase-3 positive while all cases of NLP-HL cases were negative. The increased caspase-3 expression was found to be statistically significantly correlated with apoptotic count and survival, where the cases with increased caspase-3 expression ($\geq$10%) had a better survival than those with (<10%) expression. In Cox multiple regression test, stage at presentation of CHL followed by caspase-3 expression were the most independent indicators of survival. Caspase-3 expression could be used as a good predictive and prognostic marker in CHL.

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The Unfolded Protein Response in Vanishing White Matter Disease

{P} J.P. van der Voorn [1], B. van Kollenburg [1], G. Bertrand [1], K. VanHaren [2], G.C. Scheper [1], J.M. Powers [2], M.S. van der Knaap [1]
[1] VU University Medical Center, Amsterdam, Netherlands, [2] University of Rochester Medical Center, Rochester, United States

Leukoencephalopathy with Vanishing White Matter (VWM) is an autosomal recessive white matter disorder. Episodes of major and rapid neurological deterioration are provoked by minor head trauma and particularly febrile infections. Characteristic pathological findings include, in addition to cystic white matter degeneration, foamy oligodendrocytes, dysmorphic astrocytes, oligodendrocytosis, and apoptotic losses of oligodendrocytes. VWM is caused by mutations in any of the five genes encoding the subunits of the eukaryotic initiation factor (eIF) 2B (eIF2B). eIF2B activity plays an important role in the regulation of protein synthesis under stress conditions. Mutant eIF2B in VWM may impair the ability of cells to regulate protein synthesis in response to cell stress and perhaps even under normal physiological conditions. An overload of misfolded or denaturated proteins in the endoplasmic reticulum activates the unfolded protein response (UPR), a compensatory mechanism that inhibits synthesis of new proteins and can induce both prosurvival and proapoptotic signals. We have studied the activation of the UPR in VWM through the immunohistochemical expression and Western blot analysis of its upstream components PERK and phosphorylated eIF2α (eIF2αP) as well as the downstream effector proteins activating transcription factor-4 (ATF4) and C/EBP homologous protein (CHOP) in the brains of 3 VWM patients and age-matched controls. We demonstrated activation of the UPR in glia of VWM patients. Our findings may point to a possible explanation for the dysmorphic glia, the increased numbers of oligodendrocytes and the apoptotic loss of oligodendrocytes in VWM.

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To Assess The Prognostic Significance Of PCNA Index, Mib-I Labeling Index And CD68 Labelled Fraction In The Stromal Component Of Giant Cell Tumours Of Bone

{P} Safaya Rajni , K Iyer Venkateshwaran
All India Institute of Medical Sciences, Delhi, India

Giant cell tumour of bone (GCT) is composed of osteoclastic Giant cells and stroma which comprises of both neoplastic as well as reactive histiocytic cells. 24 primary and 25 recurrent giant cells tumours were analysed for histological and immunohistochemical features. Since grading system in giant cell tumours does not predict the behaviour or the clinical outcome therefore, an attempt is made to localize the antigens and study the difference in proliferative index of the stromal components by using PCNA, MiB-I and CD68. Mean PCNA index of primary GCT was 23.54% while it was 37.33% in recurrent GCT. Mean MiB-1 labelling index was 6.15% in primary and 6.02% in recurrent GCT. Antigen co-localisation for PCNA and CD68 using two different chromogens for the two antibodies showed both reactive and neoplastic cells to be proliferating. Recurrent GCT showed less of reactive histiocytic cells and more of neoplastic stromal cells with proliferative activity when compared to primary GCT. These differences in the nature of the neoplastic component may indicate more aggressive behaviour in primary GCT as well as in recurrent GCT.

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Cell counting made easier

JJ Going
University of Glasgow, Glasgow, United Kingdom

Counting cells in histological sections is tedious, but the labour can be reduced by a sound but insufficiently known method (Human Pathology 1994;25:333-336).
Cells are usually counted within a square ‘frame’ divided as a 10 x 10 grid into 100 small squares, over as many fields as necessary. Counting the number of cells in a random 10% subset of the small squares and multiplying that number by 10 gives an unbiased estimate of the number of cells in the large square, provided a correct counting rule is used. In practice, averaged over 10 microscope fields the error of the estimate was < ±10% in 19 cases out of 20. In a ‘Monte Carlo’ simulation 2.5 million cells were counted in 10,000 fields. Estimated total was 2,500,750 cells (error 0.03%) and 95% confidence limits of 10-field averages were ±12%.
This method reduces the number of cells to be counted by 90% with no loss of accuracy and a small loss of precision irrelevant to most projects, in which greater sources of variance are routinely ignored. It is ideally suited to projects in which automated cell counting is impractical, and can be implemented using an eyepiece graticule or on digital images.

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PRENATAL DIAGNOSIS OF INHERITED SKIN DISORDERS

JA McGrath
Genetic Skin Disease Group, St John's Institute of Dermatology, GKT Medical School, St Thomas' Hospital, London, United Kingdom

Although there has been great progress in discovering the precise molecular pathology in several of the severe genodermatoses, new and effective therapies for these disorders have yet to materialise. As a result, one of the most important practical interventions for families at risk for recurrence of genetic skin diseases is prenatal diagnosis. Twenty-five years ago, ultrastructural examination of fetal skin biopsies was established in a limited number of conditions. These biopsies were taken during the second trimester (~16 weeks for epidermolysis bullosa but up to 22 weeks for some subtypes of ichthyosis). Skin samples could also be examined by immunolabelling with antibodies to the defective/missing proteins, an approach which has proved to be both reliable and quick. However, testing for most of these disorders has gradually been superseded by DNA-based analysis, which can also be applied to a much broader range of genetic disorders. Since 1979, the Genetic Skin Disease Group at St John’s has performed 265 prenatal tests by a variety of methods (191 fetal skin biopsies, 72 chorionic villus samples and two preimplantation genetic diagnostic tests). The major indications for fetal skin sampling have been epidermolysis bullosa (EB) (138 cases, including 88 junctional and 36 dystrophic), ichthyosis (37 cases, including 22 tests for harlequin ichthyosis) and oculocutaneous albinism (12 cases). Of the chorionic villus samples, 71 tests were for EB (38 junctional, 33 dystrophic) and the other was for EEC syndrome. Both of the preimplantation genetic diagnostic procedures were for skin fragility-ectodermal dysplasia syndrome. All tests provided accurate diagnoses. Over the last decade, the number of fetal skin biopsy tests has declined sharply: 77 biopsies were performed in 1990-95, compared to only 11 biopsies in 1996-2000. However, for disorders such as harlequin ichthyosis, for which the gene is not yet known, fetal skin biopsy (at 20-22 weeks’ gestation) remains the method of choice, although three-dimensional ultrasound may be another (and slightly earlier) option. For EB testing, fetal skin biopsy has been superseded by chorionic villus sampling in most cases. In general, this can be carried out at a much earlier gestation (10-12 weeks) but it is crucial to ensure that informative gene markers/mutations have been determined before any analysis is undertaken. Increasing knowledge of gene mutations has also led to new requests for prenatal testing in clinically less severe genodermatoses, such as localized recessive dystrophic EB or EB simplex. This has raised new ethical issues about precisely which conditions should be tested for, especially given the lack of treatment available if an affected fetus is diagnosed. Likewise, the feasibility of preimplantation genetic diagnosis represents both a new choice for prenatal testing as well as an ethical challenge. Nevertheless, prenatal testing represents a very important option for many families at risk of inherited skin disorders.

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Cadherins, catenins and cancer

MMK Mareel
University Hospital, Ghent, Belgium

Cancer is caused by the activation of promoter genes and the inactivation of suppressor genes; it is characterized by invasion and eventual metastasis. E-cadherin is an invasion-suppressor protein in primary cancers, as evidenced by observations on cell lines, on transgenic mice tumors and on human cancers. This statement necessitates the following considerations: One, other members of the family, e.g. N-cadherin and P-cadherin, may act as invasion-promoters. Two, E-cadherin pertains to a protein complex comprising the catenins and actin that also act as invasion regulators. Three, the cadherin/catenin/actin complex participates at invasion signaling networks with several other protein complexes. Four, a single protein, e. g. β-catenin, may act as a suppressor or as a promoter of invasion pending its proteomic complex. Acquisition of invasion may result from E-cadherin loss or deregulation at various levels: gene mutation; promoter methylation; activation of transcription factors like SIP and snail; protein phosphorylation and glycosylation; endocytosis; proteolysis. Loss of E-cadherin will not convert a normal epithelial cell into an invasive cancer cell, unless this loss is complemented by activation of invasion promoters, such as Ras. This illustrates that an altered cadherin/catenin complex is only one of the elements participating at the cancer cell invasion program.

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Modelling Multi-Step Human Tumorigenesis *In Vitro*: The Importance Of Cellular Context

D. Wynford-Thomas
Cancer Research UK Laboratories, Department of Pathology, School of Medicine, Cardiff University.

Although an essential starting point, analysis of clinical tissue samples is, for most cancers, insufficient to fully establish the sequence and functional significance of the underlying genetic and epigenetic events, not least because each phenotypic "stage" often requires multiple events. To fully understand multi-step tumorigenesis therefore requires experimental models, of which the transgenic mouse has been the pre-eminent example. In recent years however there has been a resurgence of interest in models aimed at reconstructing tumourigenesis starting from normal human cells. This has been driven in part by the realisation that the genetics underlying a key feature of the malignant phenotype – escape from replicative senescence (whether "physiological" or oncogene-induced) – differ signficantly between mouse and man. Several such models have now been published which claim to have "reconstructed" the malignant phenotype starting with mesenchymal or epithelial cell types. Unfortunately, however, many of these are potentially misleading since the nature and order of genetic events used in the model fails to reflect that seen in the corresponding real human tumours, a good example being the artificiality of fibroblast transformation by a combination of ras activation plus abrogation of Rb and p53. We have been developing in vitro human models which attempt to avoid the above pitfalls, focussing on two aspects of tumorigenesis: (i) initiation and (ii) escape from senescence.

(a) Epithelial cells

Using retroviral gene transfer into primary thyroid epithelial cells in monolayer we have shown that ras mutation, which is one of the putative initiating events in vivo, induces rapid proliferation in this otherwise quiescent cell type, leading to a phenotype closely resembling that of a benign thyroid tumour (follicular adenoma). This proliferogenic effect contrasts markedly with the growth inhibitory response to ras in primary fibroblasts, a difference which is not explicable by trivial factors such as expression levels. Even in thyroid cells, however, ras-induced proliferation in vitro is eventually self-limiting, but only after 20 - 25 population doublings (PD). The ensuing senescence-like state is associated with marked elevation of $p16^{ink4a}$ expression, which correlates well with our recent finding of high p16 protein levels in follicular adenoma in vivo. Contrary to prediction however, we have found that most well-differentiated follicular carcinomas also retain high p16 expression (which is consistent with their surprisingly low proliferative rate). New data from our in vitro model now suggest that thyroid cells possess an additional growth suppressor mechanism, mediated by inteferon signalling, which acts as a "back-up" to p16. Such redundancy would neatly explain why both loss of p16 and progression to "rapid" tumour growth occur so rarely in cancers derived from follicular cells.

(b) Astrocytes

Astrocytes are normally proliferatively quiescent in vivo but proliferate rapidly on exposure to appropriate growth factors in vitro. Proliferation eventually ceases, however, in a viable state which resembles fibroblast senescence, but after a much smaller number of PD. This state is dependent on normal p53 function, as in fibroblasts, but in contrast to the latter is independent of telomere erosion. Expression of mutant p53 confers temporary escape leading, after a further 20 PD, to a second senescence-like arrest associated with high levels of p16 expression. Astrocytes show a surprisingly frequent spontaneous escape from this second arrest, in all cases accompanied by loss of p16 expression, leading eventually to cell death ("crisis") through critical telomere erosion, from which immortal clones can be rescued by hTERT expression. The molecular "stages" defined by this model thus correlate remarkably well with in vivo analyses of glioma progression which indicate a sequence of: 1) activation of mitogenic pathways (e.g. autocrine PDGF signalling) and 2) p53 mutation in low grade tumours, followed by 3) p16 loss (or equivalent disprution of the Rb "pathway") and 4) activation of telomerase during the progression to high grade astrocytomas.

In summary, we believe that such models offer unique oppportunities for "dissecting" multi-step tumorigenesis, but only if they adhere to the principle of: "the right genes, in the right order, in the right cell".