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GLUT1 STAINING PATTERN IN CONTIGUOUS AND NON CONTIGUOUS COLORECTAL ADENOMAS AND THEIR CORRESPONDING TUMOURS

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Background: Glucose Transporter 1 (GLUT-1) is a high affinity glucose transporter. Increased glucose uptake at cell membranes is a metabolic characteristic of tumour cells. Glut1 at the membrane represents the active form of the protein in cancers.

Elevated levels of Glut1 have been reported in many human malignancies however the correlation between levels in contiguous and non-contiguous adenomas in colorectal cancer has not yet been elucidated.

Design: Using tissue microarrays and immunohistochemistry, the aim of this study was to evaluate the localisation of Glut1 in 31 adenomas and corresponding tumours. 14/31 adenomas were contiguous to the cancer. Four 6mm cores from each sample were scored and assessed for Glut1 staining pattern (either supranuclear, mixed or membranous).

Results: In contiguous adenomas (4/14), a mixed or membranous Glut1 pattern was detected. All these adenomas showed high-grade dysplasia. All corresponding cancers for these cases showed the same Glut1 staining pattern. However, no non-contiguous adenomas showed membranous Glut1 staining.

Conclusion: Membranous or mixed pattern of Glut1 correlates with high-grade dysplasia in adenomas and their corresponding cancers.

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Lymph Node Examination in Oesophago-gastric Carcinoma: Should Three Levels be Examined?

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The presence of lymph node metastases are one of the most important prognostic factors in oesophago-gastric carcinoma and is one of the most significant independent factors on multivariate analysis. Standard practice is to take one section from every lymph node found in oesophago-gastric carcinoma resection specimens to examine for metastatic carcinoma. There are currently no guidelines in the Royal College of Pathologists Minimum Data Set for oesophageal or gastric cancers on whether one section is sufficient for the assessment of lymph node metastases. In this audit 60 oesophago-gastric carcinoma cases were identified and two additional levels cut on all blocks containing lymph nodes without metastases. Per cancer an average of 14 lymph nodes were identified with 38 cases originally N0. In total 721 lymph nodes had two additional levels examined. 10 extra metastases were identified in 9 cases. In 3 cases this led to upstaging from N0 to N1. In conclusion we recommend examining two additional levels in all oesophago-gastric cancer resections that on initial single section examination are N0.

P55

A study of tumour cell budding in early stage colorectal carcinoma: unique molecular characteristics and value in prognosis.

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INTRODUCTION : Tumour cell budding at the invasive margin of early colorectal cancer (CRC) may provide a simple, reproducible method for predicting lymph node involvement. The unique protein expression of these cells may explain their invasive phenotype.

MATERIALS and METHODS : A consecutive series of 52 pT1 and pT2 CRC cases was identified from a pathology database spanning 1997-2004. All tumours were scored independently by two observers for the presence of tumour cell budding : BD-1 (minimal budding) and BD-2 (marked). Immunohistochemistry was performed on 36/52 randomly selected tumours for CD44 HCAM, CD44variant6, C-Met and Osteopontin. Protein expression in budding cells and change in expression from central tumour to budding cells was scored. Fishers Exact Test was used to compare budding and immunohistochemistry scores with three clinico-pathological features.

RESULTS : Mild budding occurred in 39/52 (75%) tumours with inter-observer agreement in 47/52 cases (90.4%). Marked budding was associated with poor tumour differentiation ($p=0.001$), but not with pT stage. There was a trend towards a higher incidence of lymph node metastasis in BD-2 cases. CD44v6 expression in budding cells was found in 28/36 (77.8%) cases and was associated with the absence of lymph node metastasis ($p=0.04$). Down-regulation of CD44v6 in budding cells was associated with lymph node metastasis. Significant down-regulation of CD44 HCAM was observed in budding cells compared to the central tumour ($p < 0.05$).

DISCUSSION : Alterations in CD44 expression in tumour budding cells may play a role in the acquisition of a migratory, invasive phenotype and contribute to tumour progression and metastasis.

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Dysplasia and Invasive Adenocarcinoma in Giant Inflammatory Colonic Polyps.

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Giant inflammatory polyps are a rare complication of chronic inflammatory bowel disease. It is generally believed that such lesions, if asymptomatic, do not need surgical removal. We report three patients with chronic inflammatory bowel disease who developed invasive adenocarcinoma within giant inflammatory polyps.

A 43-year-old male with a nine-year history of Crohn's Disease had two lesions in the colon that macroscopically resembled giant inflammatory polyps. Microscopically both polypoid areas showed low grade dysplasia and well differentiated adenocarcinoma (pT2 and pT4). The second patient was a 50-year-old male with a four-year history of ulcerative colitis. Macroscopic examination revealed two polypoid areas consistent with giant inflammatory polyps. Microscopically both showed low-grade dysplasia and one a well differentiated mucinous adenocarcinoma, (pT3). The third case was a 52-year-old male with a fifteen-year history of ulcerative colitis. Macroscopically two polypoid areas were identified consistent with giant inflammatory polyps. Both showed low-grade dysplasia and one contained an area of well differentiated adenocarcinoma, (pT3).

In total six giant inflammatory polypoid areas were identified, two in each patient, all of which showed foci of low-grade dysplasia with four showing evidence of well-differentiated adenocarcinoma. In light of these cases we recommend removal of giant inflammatory polyps.

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Inter-Observer Variation In The Reporting Of Extramural Vascular Invasion In Colon Carcinoma

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Introduction: Extramural Vascular Invasion (EMVI) is a prognostic indicator of metastasis and survival in colon carcinoma, independent of Dukes's stage and the degree of tumour differentiation. However, the reporting of EMVI has a variable prevalence of 10-90%. This has been attributed in part to variations in the use of an elastin stain to demarcate extramural blood vessels. This study aimed to determine for the first time the degree of inter-observer agreement in the reporting of EMVI in colon carcinoma without the use of an elastic stain.

Methods: 20 cases of pT3 and pT4 Dukes's B colon cancers were identified from a pathology database and 49 H&E slides representing extramural tumour invasion were selected. Slides were anonymised and distributed to four specialist gastrointestinal pathologists who independently assessed the presence or absence of EMVI. Kappa values were performed to assess the degree of inter-observer agreement.

Results: Kappa values ranged from 0.23 (poor agreement) to 0.45 (moderate agreement).

Conclusions: The reporting of EMVI in Dukes's B colon carcinomas on H&E stained slides has only poor – moderate agreement when assessed by specialist gastrointestinal pathologists. The introduction of standardised histological criteria or the use of an elastin stain in the diagnosis of EMVI may assist in improving inter-observer agreement, and thus more accurate assessment of individual patient prognosis in colon carcinoma.

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The Human And Mouse Gastric Glands Do Possess a Periglandular Myofibroblastic Sheath

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Introduction: In the gastrointestinal tract the intestinal subepithelial myofibroblasts (ISEMF) form a sheath around the stem cell compartment thus creating the stem cell niche. They also play a role in the response of the stomach to injury. However, Mutoh et al have suggested that the periglandular fibroblast sheath is generated in the stomach only when intestinal metaplasia occurs, and could not demonstrate the presence of this sheath in normal gastric mucosa. Here we show that the myofibroblast sheath exists in normal gastric mucosa, which is consistent with its expected physiological role in provision of the stem cell niche.

Methods: Paraffin sections of normal mouse and human stomach were immunostained for α -sma and α -sma and vimentin respectively. Myofibroblasts were identified on the basis of their morphology and positive immunoreactivity for α -SMA.

Results: Myofibroblasts were seen surrounding the stomach glands in normal mouse and human stomach sections, both in the en-face and cross sectional planes.

Conclusions: We have shown that the periglandular myofibroblast sheath is present in normal human and mouse gastric mucosa. The presence of the myofibroblast sheath underlines its importance as a component of the stem cell niche.

P60

A Review of Gastro-Intestinal Stromal Tumours (GISTs) Presenting At a Single Hospital In a Twenty Year Period

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The purpose of the study was to identify all cases of gastro-intestinal stromal tumours, which had presented in this department between 1984-2004. Positive staining with CD117 was deemed necessary for a definite diagnosis. Mesenchymal tumours, which presented before 1989 were all considered to be of smooth muscle origin, at the time of reporting. The review was prompted by the advent of Imatinib, which is successful in treating GISTs, and to identify potential beneficiaries from the treatment. 66 of the 78 cases identified showed positivity with CD117. These included 32 cases, which had previously been designated to be of smooth muscle origin. The median age of the patients was 71 (range 16-88) of which 31 were male and 35 were females. Our study revealed 33 patients who were alive and these included 4 in whom the diagnosis had been altered. Review of the patient record was available in 49 of the patients. Although 6 died of the disease and 10 from other causes, 10 patients were identified who had been lost to follow-up, two with advanced disease, who may benefit from targeted therapy. This highlights the need to search more widely for such patients at a national level.

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Beneficial Effects of Carbon Monoxide-Releasing Molecules (CO-RMs) on Stress-Induced Renal Injury

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We have recently identified a class of compounds, CO-releasing molecules (CO-RMs), which exert important pharmacological activities by carrying and delivering CO to biological systems. Here, we examined the possible protective effects of CO liberated from CO-RMs on stress-induced renal damage. Exposure of LLC-PK₁ cells to cisplatin (CP) caused significant apoptosis as evidenced by caspase-3 activation and increased number of floating cells (>70%). Treatment with CO-RMs resulted in a remarkable decrease in CP-mediated caspase-3 activation and cell detachment. In a model of CP-induced nephrotoxicity in rats, administration of CO-RMs resulted in amelioration of renal dysfunction, decreased number of apoptotic tubular cells as evidenced by TUNEL assay and improved histological profile. In addition, a model of hypothermic hypoxia was used to measure haemodynamic, biochemical and pathological parameters in isolated perfused rabbit kidneys. We found that kidneys flushed with a preservation solution supplemented with CO-RMs and stored at 4°C for 24 h displayed at reperfusion a significant protective vasodilatory effect, improved mitochondrial integrity and protected against ischaemia-reperfusion injury. Inactive compounds (iCO-RMs), which do not liberate CO, failed to prevent renal injury. Thus, CO-RMs are effective therapeutic agents that can be used to ameliorate renal injury and protect against nephrotoxicity from different pathological stresses.

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Juxtaglomerular apparatus tumours – A report of two cases

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Juxtaglomerular apparatus tumours, also called 'reninomas' are rare renal neoplasms causing hypertension in young adults. Approximately 70 cases have been reported since it was described by Robertson et al in 1967. The majority are considered benign and only one malignant case (presenting with lung metastasis) has been reported to date. These tumours are usually positive for rennin immunohistochemistry and have characteristic appearance on electron microscopy.

We present two renal tumours referred to our department in the period 2001 to 2005. Both the patients were adults and were hypertensive – a 34 year old man and a 45 year old woman. The tumours measured 6.5 cm and 4.5 cm respectively. A preliminary morphological diagnosis of Juxtaglomerular apparatus tumour was made in both cases. Renin immunohistochemistry was performed and electron microscopy carried out in one case.

We discuss the clinical features, morphology, immunohistochemical findings and electron microscopy of these tumours with specific regard to our cases.

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Transitional Cell Carcinoma of the Urinary Tract in Renal Transplant Recipients

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Introduction: Renal transplant recipients are prone to develop a variety of malignancies in a greater rate than the general population as a result of decreased immunosurveillance produced by prolonged post-transplant immunosuppressive therapy. The data about urinary tract transitional cell carcinoma (TCC) in these patients is not well established.

Objectives: To determine the incidence and characteristics of TCC in renal transplant recipients and compare it with that of the general population.

Patients and methods: We reviewed the records of 681 renal transplant recipients at Bart's and the London hospitals between 1984 and 2005 to identify patients developing post-transplant TCC. The incidence has been calculated and the characteristics of these tumours have been evaluated.

Results: 8 transplant recipients developed TCC (3 in the native kidney/ureter, 3 in the transplanted kidney/ureter and 2 in the bladder) resulting in a total incidence of 1.17% and an incidence of TCC in native tissue of 0.7%. Both are significantly higher than that of the general population (1 in 100,000) ($p < 0.0001$ & $p < 0.0001$ respectively). The average time of diagnosis after transplantation was 10.6 ± 2.6 years. All cases presented with haematuria. All tumours were invasive at the time of presentation and of a high grade.

Conclusions: TCCs incidence is higher in renal transplant recipients compared to the general population. They occur late and are mainly seen in the upper urinary tract with a high grade and stage.

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Renal Cell Carcinoma Metastasis Presenting As Rectal Bleeding

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A 74 year old man underwent a nephrectomy for a left sided clear cell renal cell carcinoma in 1990. He presented with shortness of breath and a multiple nodules on CT chest thirteen years later. He was treated with interferon and 9 months later had a normal chest x-ray.

Fourteen months later, February 2004, he presented with 3 to 4 months of minor rectal bleeding. Rigid sigmoidoscopy to 15cms showed small haemorrhoids. April 2004 interferon was stopped.

October 2004 he was admitted with persistent rectal bleeding and a low haemoglobin. Again, rigid sigmoidoscopy showed only small haemorrhoids.

June 2005, he collapsed and was admitted. CT brain was normal. Full body CT showed a polypoid rectosigmoid mass and enlarged inguinal and common iliac nodes.

August 2005, the polyp was removed endoscopically and histology revealed a clear cell renal cell carcinoma metastasis.

P65

Bone marrow-derived haematopoietic and mesenchymal stem cells colonise kidney but fail to produce collagen I in renal fibrosis

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Clinical trials involving injection of bone marrow (BM)-derived stem cells are under way in patients suffering from ischaemic heart disease. In rodents, BM-derived stem cells attenuate acute tubular necrosis. However, BM cells may also generate interstitial fibroblasts in models of renal fibrosis. The role of BM-derived cells in promoting renal fibrosis needs to be investigated.

Wild type female mice were transplanted with whole BM (n=14) or mesenchymal stem cells (n=10) from male transgenic mice expressing luciferase reporter molecule under the control of the COL1A2 gene (coding for the alpha 2 chain of collagen I). Fibrosis was induced by unilateral ureteric obstruction (UO). UO was also performed in untransplanted transgenic mice and wild type mice.

BM-derived myofibroblasts were identified by in situ hybridisation (ISH) for the Y chromosome and immunohistochemistry for alpha-SMA. Fibrosis was evaluated by ISH for collagen I mRNA. Reporter molecule expression was assessed by chemiluminescent assays and ISH.

Collagen I mRNA levels were increased in all obstructed kidneys.

Wild type mice had no significant luciferase activity or mRNA. The obstructed kidneys of transgenic mice showed markedly increased luciferase activity and mRNA. In obstructed kidneys of BM-transplanted mice, 8.6% of SMA-positive interstitial cells were BM-derived, but luciferase activity was not increased and very rare tubular cells and a single interstitial cell in all animals tested expressed luciferase mRNA.

Our results indicate that although BM-derived cells infiltrate the kidney, they are not major contributors of collagen I.

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Retrospective Audit Of Cervical Cytology And Histology Correlation

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In this study our aim was to establish the departmental level of concordance between low grade (borderline nuclear changes or mild dyskaryosis) cervical smears and corresponding histological outcome. The method involved retrieving cervical smears (using an Aylesbury spatula) and subsequent histology slides using computer records for the calendar year 2003. There were 3345 low-grade smears from 3094 women. The study looked at the 212 patients with low-grade cytology who turned out to have high-grade histology (CIN2 or CIN3). An internal control group was included of those with negative or low-grade histology. Review of all the cytology by two consultants and one specialist registrar, revealed 123/212 (58%) concordance between the original and reviewed diagnoses. However in 89/212 (42%) the lesions were upgraded into high grade. This study seeks the possible reasons why these smears are under-called. The conclusions of this search include: the presence of micro-biopsies in cytological smears which may be misinterpreted as being low grade; a scanty sample where it might be difficult to state, with confidence, that cytology is high grade based on so few cells; the difficulty of interpreting pale or small cells; and the possibility of high grade dyskaryotic cells being misinterpreted as innocent glandular changes.

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HISTOLOGICAL FEATURES OF LEUPROLIDE-TREATED LEIOMYOMAS

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We investigated the histopathological effects of the Gonadotrophin Releasing Hormone (GnRH) agonist leuprolide on uterine leiomyomas. Leiomyomas from 13 women who underwent surgery following treatment with leuprolide were compared with leiomyomas from age-matched patients who had not received any hormonal treatment. The interval between leuprolide treatment and hysterectomy ranged from 10 days to 139 days (mean 60 days). The treated leiomyomas showed necrosis (3/13), increased mitotic activity (6/10), increased cellularity (5/13), haemorrhage (5/13), collections of foamy macrophages (7/13), haemosiderin deposition and hyalinisation (11/13). In the control group, haemorrhage and foamy macrophages were seen in the leiomyomas from one patient and hyalinisation was present in 5 of the 13 cases. Haemorrhage, necrosis and increased mitoses tended to occur in leiomyomas of most recently treated patients. Leiomyomas from the patient who had received leuprolide 10 days prior to surgery showed coagulative necrosis and mitotic activity up to 20/10 high power fields. These features initially raised the possibility of leiomyosarcoma, but their occurrence in multiple tumours from the same patient militated against a diagnosis of leiomyosarcoma. We conclude that GnRH agonists shrink leiomyomas by inducing necrosis followed by fibrosis. The changes induced in the early stages of treatment may simulate the histopathological features of leiomyosarcoma.

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UNOPPOSED OESTROGEN REPLACEMENT MAY INDUCE TUMOURS OR TUMOUR-LIKE BEHAVIOUR IN ENDOMETRIOSIS

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Prolonged unopposed oestrogen replacement is contra-indicated in women with an intact uterus because of an increased risk of subsequent endometrial hyperplasia and carcinoma. It is, however, commonly used following hysterectomy and bilateral salpingo-oophorectomy (TAHBSO). We present 4 patients who received unopposed oestrogen replacement following TAHBSO, 3 of whom developed malignant tumours arising in endometriosis (1 carcinosarcoma, 2 endometrioid stromal sarcomas). The fourth patient developed recurrent endometriosis that behaved in an aggressive tumour-like way. The literature contains 25 other instances of tumours occurring in extragonadal endometriosis following long term oestrogen replacement after TAHBSO. These include 14 endometrioid adenocarcinomas, 3 adenocarcinomas, 2 adenofibromas and 1 endometrioid stromal sarcoma. Given that many cases of endometriosis are asymptomatic, we conclude that unopposed oestrogen replacement following hysterectomy and salpingo-oophorectomy is contraindicated in all women whether they are known to have endometriosis or not.

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Immunohistochemical Analysis of Malignant Hepatic Tumours

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Introduction: In clinical practice the malignant hepatic tumours commonly encountered are: primary hepatocellular carcinoma, metastatic carcinoma and primary cholangiocarcinoma. Histologically, the distinction between them is often difficult. The objective of this study was to identify a panel of antibodies to successfully differentiate these tumours.

Materials & Methods: Liver tumour blocks were retrieved from the pathology archives of two Bahraini hospitals. Tissue slides from each case were tested separately with each of ten antibodies (HepPar1, CD10, MOC31, Villin, AFP, pCEA, mCEA, Ck7, Ck19, and Ck20).

Results: Liver biopsy tissue from 51 patients was examined. The diagnoses were: Hepatocellular carcinoma (n=23); metastatic adenocarcinoma (n=15); cholangiocarcinoma (n=5); metastatic small cell carcinoma (n=7); and unclassified (n=1).

Sensitivity and specificity values for different antibodies in relation to their positive staining of specific tumours: HepPar1 for HCC - 81.8% and 100%; MOC31 for MA - 73.3% and 92.1%; pCEA (canalicular) for HCC - 82.6% and 83.3%; mCEA for MA - 93.3% and 75.6%; CK7 for CC - 100% and 68%; CK19 for MA and CC as a combined group - 90% and 86.3%.

Conclusions: An antibody panel consisting of HepPar1, pCEA, CK19 and CK7 together with either MOC31 or mCEA is recommended to differentiate these tumours

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The Immunophenotyping of Diffuse Large B-Cell Lymphomas of the Gastrointestinal Tract

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Studies have identified that antigens expressed in nodal Diffuse Large B-Cell Lymphomas (DLBCL) have a prognostic significance on survival. CD10 confers a favourable outcome, whilst the expression of Bcl-2, CD5 or MUM-1 appears to have an aggressive clinical course. DLBCL can be categorised Germinal Centre (GC) phenotype (CD10 +ve, Bcl-6 +/-ve, MUM-1 +/-ve) or Activated B-cell phenotype (ABC) (CD10 -ve, Bcl-6 -/+, MUM1+). We studied 29 cases (14 gastric and 15 bowel) of primary gastrointestinal DLBCL. Immunohistochemistry detecting CD20, CD79a, CD5, MIB1, CD30, CD10, Bcl-2, Bcl-6, CD138, p53 and MUM-1 was performed. Survival analysis was undertaken using Kaplan and Meier and Log Rank test. 13/29 cases were of GC phenotype, CD10+, MUM1-/-, bcl-6+/-, 9/15 bowel lymphomas were GC phenotype and 4/14 gastric lymphomas, this difference is statistically significant. CD5, CD138 and CD30 were only expressed in 1, 2 and 3 cases respectively. No survival difference was found between groups in this small study. This study has demonstrated that DLBCL of stomach and DLBCL of bowel appear to be phenotypically distinct with gastric lymphomas predominantly showing a GC phenotype and bowel lymphomas an ABC phenotype. The phenotype did not appear to affect survival in this small study.

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Case Report of Plasmablastic Lymphoma

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We present the case of a 32 year old gentleman with a paravertebral mass and skin nodules, with a background history of immunosuppressive therapy for Crohn's disease.

An MRI, performed following a complaint of back pain, revealed a paravertebral soft tissue mass at L4. The subsequent biopsy showed clusters of cells with plasmacytic differentiation. These were strongly positive for the plasma cell markers VS38C and CD138. A diagnosis of a plasma cell tumour was made, and the patient was treated with radiotherapy.

Three months later, skin nodules developed on the patient's scalp, elbows and groin. Histology showed skin infiltrated by tumour cells with plasmablastic features. Immunohistochemical stains and in-situ hybridization for EBV by EBER probe showed neoplastic cells positive for CD3, CD138 and EBV and negative for CD20, CD79a and VS38C. Plasmablastic lymphoma was diagnosed.

Plasmablastic lymphoma is an aggressive rare variant of diffuse large B cell lymphoma. It is often CD20 and CD45 negative, but expresses plasma cell markers VS38C and CD138. Plasmablastic lymphomas have typically been reported with HIV infection, often presenting in the oral cavity.

This case reports this rare EBV-driven high grade lymphoma in a patient taking immunosuppressive medication for Crohn's disease.

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A case of Kikuchi's disease with a cytogenetic abnormality.

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A 20-year-old female presented with painless axillary lymphadenopathy, weight loss and anorexia. On examination she appeared clinically well but had two firm, mobile, non-tender lumps measuring 1.0 cm in the roof of her right axilla. No breast masses or evidence of infection in areas drained by the axillary nodes were detected. Supraclavicular/cervical lymphadenopathy and extranodal involvement were absent. Blood biochemistry and haematology were within normal range. Fine needle aspiration cytology showed reactive lymphoid hyperplasia only. A review at 5 weeks detected an increase in the lymphadenopathy and a lymph node biopsy was undertaken.

Histological evaluation of the lymph node biopsy revealed features typical of Kikuchi's disease. Molecular studies were carried out because of the possibility of a malignant lymphoma; polymerase chain reaction showed polyclonal patterns of immunoglobulin heavy chain and TCR-gamma genes. However, cytogenetic analysis revealed an abnormal clone of lymphocytes carrying a (2:16) translocation in fresh lymph node tissue. Examination of a peripheral blood sample showed a normal female karyotype ruling out the possibility of a constitutional translocation. Despite the presence of this abnormal clone of lymphocytes, the patient has remained well with no episodes of recurrent disease for 2 years.

This case report illustrates that Kikuchi's disease can be associated with chromosomal abnormalities and great care should be taken when diagnosing malignant lymphoma on the basis of cytogenetics when the lymph node morphology might also suggest Kikuchi's disease.

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Clinicopathological Correlation In Infantile / Juvenile Fibromatosis And Infantile Fibrosarcoma

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Introduction: Infantile fibrosarcoma (IF) and fibromatosis (FIB) are locally invasive spindle cell neoplasms of childhood which often lead to problems of clinical management due to their site and effects of local recurrence. Diagnosis of IF has been facilitated by recognition of a characteristic ETV6-NTRK3 fusion transcript. The histopathological appearance of FIB may vary from cellular to collagenous, the relationship to behaviour remaining unknown. **Methods:** Retrospective review was carried out to identify children with IF or FIB; histological features were reviewed with outcome.

Results: 30 children were identified, 24 with FIB, six with IF. Mean age at presentation was 2.8 and 0.1 years respectively. 4/6 IF were in the extremities whereas 15/24 FIB were in the head and neck region. 20/24 FIB had surgery with 50% recurring locally and 42% receiving chemotherapy. Three patients were found to have Gardner's syndrome. Of the six patients with IF, five had chemotherapy, 50% requiring surgery with a recurrence rate of 33%. Overall around 80% of patients in both groups had complications including nerve palsies and contractures. Histopathological variants of FIB did not predict behaviour.

Discussion: IF and FIB are distinct entities occurring in different paediatric populations but which pose similar management problems. Our findings support the recent WHO classification which separates IF and other specific fibroblastic conditions but classifies all other FIB lesions as 'desmoid type or aggressive fibromatoses', with no separate categories for infantile or juvenile fibromatosis, all of which behave in a similar manner and may be associated with underlying syndromic disorders.

P75

Histopathological Predictors of Long-term Outcome of Children with Idiopathic Focal Segmental Glomerulosclerosis

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Introduction: Idiopathic focal segmental glomerulosclerosis (FSGS) is a common cause of paediatric nephrotic syndrome, which generally carries a poor long term prognosis, although there is heterogeneity in the natural history. There are no universally accepted histological criteria to reliably predict outcome.

Methods: Clinical and histological data of children with renal biopsies showing primary FSGS from a single centre with follow up more than 10 years were reviewed. The histopathological features of the initial diagnostic biopsies were reviewed by two independent paediatric histopathologists and these findings were examined in relation to renal outcome.

Results: There were 66 patients, 38 males and 28 females, aged 0.4-14.1 years (mean 6.4) at diagnosis. Number of glomeruli per biopsy ranged from 7-68 (mean 37). Focal tubular atrophy was present at first biopsy in 50 (76%) capsular adhesions in 35 (53%), focal global sclerosis in 27 (41%), glomerular tip lesions in 8 (12%) and mesangial expansion in 31 (47%). Stable remission was induced in 35 patients (responders). Presence of mesangial IgM and global sclerosis were not different between groups. The presence of tubular atrophy, capsular adhesions and sclerotic lesions in >20% of glomeruli were more common in non-responders, (LR for response 0.65, 0.63 and 0.51 respectively), whereas mesangial expansion and tip-lesions were more common in responders. (LR for response 2.1 and >7 respectively).

Conclusion: In idiopathic childhood FSGS, tubular atrophy or capsular adhesions are associated with progression whereas tip-lesions and mesangial expansion are associated with increased likelihood of response to therapy.

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Nephrogenic Rests Or Reactive Embryonal Hyperplasia In Paediatric Nephrectomy Specimens With Chronic Obstructive Or Reflux Nephropathy

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Introduction: Nephrogenic rests are associated with an increased risk of Wilms' tumour. We have noted several cases of obstructive or reflux nephropathy in childhood, which has no reported increased risk of Wilms' tumour development, with areas resembling nephrogenic rests.

Methods: Search of a paediatric histopathology database was performed to identify all nephrectomy specimens for which the indication or diagnosis was chronic obstructive or reflux nephropathy. Cases with nephrogenic rest-type structures were identified.

Results: 313 specimens were identified from 1997-2005, in which four, 1.3% (95% CI 0.3-3.2%) demonstrated areas morphologically resembling nephrogenic rests. In all, the lesions were in a peripheral subcapsular location, sometimes in scarred areas, sometimes in the adjacent cortical parenchyma. No lesions exhibited the classical 'wedge-shaped' architecture of perilobar nephrogenic rests seen in association with Wilms' tumour, and all were composed of proliferating tubular epithelial type-structures only. In no cases were any blastemal areas identified.

Discussion: Focal reactive change (embryonal hyperplasia of Bowman's epithelium) has been reported in patients on long-term dialysis. We propose that in some children with chronic obstructive or reflux nephropathy, there is either persistence of embryonal-type structures, or reversion to this phenotype as a manifestation of disordered renal development, which is unlikely to be associated with an increased risk for development of Wilms' tumour. An alternative terminology, such as peripheral embryonal tubular metaplasia, may be more appropriate.

P76

Cutaneous Graft Versus Host Disease-Like Histopathological Features Following Gene Therapy

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Introduction: Cutaneous graft versus host disease (GVHD) is a recognised complication of bone marrow transplantation, with well described histopathological features. Gene therapy is increasingly being developed as a potential curative treatment for a range of congenital disorders. We present two cases with clinical and histopathological features identical to cutaneous GVHD following gene therapy.

Cases: Two children, both infants, underwent gene therapy for severe combined immunodeficiency syndrome (SCID); the procedures appeared uneventful. Several weeks following gene therapy both patients developed a cutaneous rash, which clinically mimicked GVHD. Skin biopsies were carried out, and in both cases, similar features were identified. There was a lichenoid inflammatory reaction with focal exocytosis, basal cell vacuolation, patchy keratinocyte apoptosis and necrosis, and a superficial dermal inflammatory infiltrate, including numerous eosinophils in one case. The histopathological features were indistinguishable from those seen in GVHD following bone marrow transplantation.

Discussion: We have described a cutaneous reaction occurring in patients following gene therapy, which both clinically and histopathologically appeared indistinguishable from GVHD. Since these patients had not been transplanted with donor lymphocytes, it is presumed that the features represent an autoimmune-type reaction stimulated either by the viral vector used or as a response to reconstitution of the host immune system following partially successful gene therapy.

P78

Left Heart Failure Due To Myocardial Infarction In Two Neonates With Anatomically Normal Coronary Arteries

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Introduction: Acute myocardial infarction (MI) in neonates has been previously described, but there are few published data on its incidence. We report two cases of acute MI of unknown aetiology, in neonates with structurally normal hearts and anatomically normal coronary arteries (CAs), both infants presenting with acute left ventricular failure.

Cases: Both infants were born at term with good Apgar scores. Infant One developed cardiogenic shock at 10 minutes of age due to extensive transmural infarction of the left ventricle. There were no structural cardiac abnormalities, and detailed postmortem macroscopic and histological examination of the CAs showed no evidence of obstruction or vasculitis. Infant Two developed worsening tachypnoea at 12 hours of age, clinical investigations and subsequent necropsy findings confirming massive MI affecting both ventricles. There was a small secundum-type atrial septal defect but no significant cardiac anomalies, and the CAs were entirely normal.

Discussion: Causes of neonatal MI include 1) congenital heart disease (CHD), with or without anomalous origins of the CAs, 2) perinatal asphyxia, and 3) obstructive coronary arterial lesions. In perinatal asphyxia, and CHD with normal CAs, the infarcts are often localised to papillary muscles and subendocardial areas, in contrast to the regional, transmural pattern of infarction seen in CA occlusion. Despite numerous reports of acute neonatal MI in the literature, the relative frequency of its various causes remains unclear. Although we were unable to identify an aetiology for the massive infarction in our cases, acute MI remains an important, if rare, differential diagnosis in any newborn infant presenting with acute cardiogenic shock.

P79

Umbilical Artery Aneurysm of the Umbilical Cord Presenting As Third Trimester Intrauterine Fetal Death

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Introduction:

Umbilical artery aneurysm (UAA) is an extremely rare lesion, which may be associated with trisomy 18. We report a case of umbilical artery aneurysm with normal karyotype.

Clinical Details:

A 27-year-old mother presented with intrauterine fetal death at 32 weeks' gestation. Antenatal ultrasound scans had demonstrated a two-vessel umbilical cord with a large aneurysmal dilatation of the umbilical artery, as well as severe IUGR with oligohydramnios. A fetal blood sample had shown a normal male karyotype.

Pathological Findings:

Postmortem examination revealed a growth restricted fetus with Potter facies, in keeping with the effects of oligohydramnios, and hypospadias. There was a single left-sided umbilical artery, and the umbilical cord showed a large 2.5cm-diameter arterial aneurysm containing adherent thrombus, 2cm from its insertion into the placental disc. Microscopic examination showed thinning of the tunica media with intimal fibrosis, dystrophic calcification and adherent organising mural thrombus. PCR analysis of 100 chorionic villus trophoblast cells showed no evidence of trisomy 18 mosaicism.

Discussion:

To our knowledge, there are only six reported cases of UAA in the English literature. Of these, four were associated with trisomy 18; of the remaining two cases, one infant also displayed a two-vessel cord. The latter two infants were both stillborn, but neither showed evidence of IUGR. It is speculated that the aneurysm causes fetal hypoxia and intrauterine fetal death by compression of the umbilical vein. The cause of such aneurysms remains unclear.

P81

Histomorphometric Correlation of Ultrasound Findings in Partial and Complete Hydatidiform Moles in Early Pregnancy

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Introduction: The majority of partial (PHM) and complete (CHM) hydatidiform moles are diagnosed in early pregnancy. About half are identified as molar on ultrasonographic examination prior to evacuation. It is uncertain whether unsuspected cases represent a different molar phenotype or are simply sonographer dependant.

Methods: 54 molar pregnancies were randomly selected from the files in whom results of an ultrasound examination had been carried out prior to evacuation. In each case, the mean cross-sectional diameter of the largest 10 villi were recorded. Maximum mean villus diameters were compared between gestational age groups (<14 wks and ≥14 wks), and ultrasound detection groups (detected (d) and not detected (nd)), using Mann-Whitney test.

Results: The mean maximum villus diameter was significantly smaller in both PHM<14wks and CHM<14 wks compared to PHM<14wks and CHM<14wks (P<0.001 and P<0.0001 respectively). There was no significant difference in maximum villus diameter between PHM<14 wks and CHM<14 wks (P=0.44) or between PHM≥14wks and PHMnd≥14 wks (P=0.88).

Discussion: In general, average diameter of the largest, most hydropic villi are significantly greater in cases of PHM and CHM detected by ultrasound examination before 14 wks compared to those not detected by sonographic examination, whereas after 14 wks, there is no difference. These findings indicate that, although sonographer expertise could increase ultrasound detection rates slightly for PHM and CHM, there are a proportion of cases in early pregnancy which demonstrate minimal hydropic change and are unlikely to be suspected prior to histopathological examination of products of conception.

P80

RAT AND RABBIT PLACENTAL P-GLYCOPROTEIN EXPRESSION – A TOOL FOR IN-UTERO HUMAN RISK ASSESSMENT

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As part of the human risk assessment, Juvenile Toxicology is becoming of increasing importance, with an emphasis on in-utero effects. The ATP-dependent p-glycoprotein (P-gp), which belongs to the MultiDrug Resistance (MDR) class of proteins, is well recognised as a membrane transport protein. It is of particular interest because of known human polymorphisms and its role in drug transport. Lankas et al (1998) demonstrated the functional significance of placental P-gp in knockout mice, showing that the foetuses from homozygous dams were 100% susceptible to chemically-induced cleft palate. Conversely the incidence was 0% in the wild-type and intermediate (50%) in foetuses from heterozygous dams. In an effort to study the gestational expression of P-gp, a preliminary evaluation of normal tissues (pre-parturient uterus, foetuses and placenta) from 10 Han/Wistar rats and 10 New Zealand White rabbits was carried out. Immunohistochemistry was performed using anti-human P-gp on fixed tissues (formalin and Fekete's). Rat frozen brain tissue was used as a positive control (known staining of vascular endothelium). There was positive staining of the uterine endometrium, foetal membranes and placental labyrinth in both the rat and rabbit. Western blots from snap frozen uterine and placental tissue revealed bands (using P-gp AB) at 120 and 150 kd consistent with the native and glycosylated protein. This work will be used as a basis for further time-course studies of the developing foetus, as a model for Human exposure.

P82

Ultrastructural features Of Gaucher's Disease Treated with Enzyme Replacement Therapy Presenting as Mesenteric Mass Lesions

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Introduction: The classical ultrastructural features of Gaucher's disease include large numbers of intracytoplasmic, membrane bound lysosomal inclusions containing characteristic tubular structures within an electron-lucent background, representing the PAS positive Gaucher cells seen on light microscopy. Following enzyme replacement therapy, many of the manifestations of the condition are ameliorated, but persistent mesenteric lymphadenopathy has been reported, the ultrastructural features of which have not been previously described.

Cases: Two children, aged three and eight respectively, both presented with persistent abdominal lymphadenopathy whilst receiving enzyme replacement therapy for Gaucher's disease. Needle core biopsies were carried out, which demonstrated collections of macrophages with eosinophilic cytoplasm and only scattered storage type cells on light microscopy. PAS staining was negative in one case and only focally positive in the other. Electron microscopic examination however confirmed the cells to represent macrophages, within the cytoplasm of which were scattered abnormal lysosomes containing occasional twisted tubular structures of the type reported in classical Gaucher's disease.

Discussion: Enzyme replacement therapy in Gaucher's disease reduces accumulation of the metabolic products at many sites but for reasons that remain uncertain, abdominal lymphadenopathy due to abnormal collections of macrophages may occur. These do not form granulomas or collections of classical Gaucher cells, but appear to represent an unusual response to a relatively small amount of storage material.

P83

Primary Thoracic Myxoid Variant of Extrarenal Rhabdoid Tumor In Childhood

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Introduction: Primary extra renal rhabdoid tumours (RT) are now recognised as a specific entity in paediatric oncological pathology practice. We present an unusual case of a small myxoid variant of a thoracic RT in an infant and highlight the importance of recent developments in the diagnosis of these tumours.

Case: An eight month old child presented with a short history of cough and shortness of breath. Imaging demonstrated a large mass occupying the majority of the thoracic cavity on the right side. A percutaneous needle biopsy of the mass was carried out which showed fragments of tissue composed of malignant tumor with a predominant 'small ovoid cell' phenotype, and extensive myxoid change, with small nests and islands of tumour cells against a mucoid / myxoid stroma. Occasional cells demonstrated open vesicular nuclei and prominent nucleoli, and rare cells exhibited eosinophilic cytoplasmic inclusions.

Immunohistochemical staining revealed focal strong cytoplasmic positivity for cytokeratin, focal strong paranuclear cytoplasmic vimentin positivity and IN1 staining showed normal nuclear positivity in control tissues but was negative in tumor cell nuclei. Electron microscopy demonstrated characteristic paranuclear whorls of intermediate filaments confirming the diagnosis of extrarenal malignant RT.

Discussion: The diagnosis of malignant rhabdoid tumour may be difficult, particularly in cases, such as the present one, with a predominant small-cell myxoid phenotype. The characteristic expression patterns of cytokeratin and vimentin provide strong clues to the diagnosis and the use of IN1 antibody makes definitive diagnosis possible even on needle core biopsies.

P84

Electron Microscopical Examination of Chorionic Villus Samples for Prenatal Diagnosis of Storage Disorders

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Introduction: Storage disorders affect structural macromolecules or cause progressive accumulation of metabolites. These 'large molecule' diseases may show ultrastructural features in chorionic villus samples (CVS) manifesting as membrane-bound vacuoles in endothelial cells, fibroblasts and trophoblast.

Methods: Details of CVS (n=111) undertaken for electron microscopy (EM) for suspected storage disorders at Great Ormond Street Hospital (GOSH) over 17 years (1988 – 2005) were collated. In several laboratories, including GOSH, it has been practice to confirm prenatal diagnosis by morphology, alongside enzymic or molecular analysis.

Results: There were 31 positive diagnoses, 77 cases showed no evidence of storage disease; 3 were inadequate for assessment. Diagnoses included: glycogen storage disease type 2 (Pompe) (2 cases), gangliosidosis type 1 (GM1) (4 cases), Niemann-Pick type A disease (2 cases), Wolman disease (3), mucopolysaccharidosis (MPS) (8 cases) including 2 cases of Hurler (MPS1), mucopolipidosis (1 case), Batten disease (ceroid lipofuscinosis) (7 cases), sialic acid storage disease (1 case), storage disease, not specified (3 cases).

Discussion: Storage disorders are rare and the diagnosis may be difficult, but EM, in conjunction with enzymic and molecular analysis, helps provide a definitive diagnosis. In this large series (n=111) of high-risk cases, the detection rate was considerable, one-third of CVS cases (n=31) showing ultrastructural features of storage diseases, in many cases demonstrating characteristic ultrastructural findings, enabling prenatal counselling and management in early pregnancy of these complex disorders.

P85

Sudden Unexpected Death in Early Childhood due to Saccular Aneurysm-related Subarachnoid Haemorrhage

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Introduction: Saccular (berry) aneurysms are rare in childhood and most reported cases occur in adult/adolescent patients, however they are an important cause of primary subarachnoid hemorrhage (SAH) and should be considered in cases of sudden unexpected death (SUD) in childhood.

Case: Postmortem examination of a 16-month old child who died suddenly and unexpectedly in bed showed a ruptured giant saccular aneurysm (>2.5cm diameter) of the left middle cerebral artery with intraventricular haemorrhage and adherent blood clot over the basal surface of the brain. Histological examination demonstrated dilatation and marked intimal thickening of the aneurysmal vessel compared to an adjacent segment showing normal arterial structure. Staining with elastic van Gieson (EVG) showed deficiency of the internal elastic lamina in the aneurysmal segment.

Discussion: Focal muscular dysplasia (FMD) predisposing to saccular aneurysm is well described in genetic syndromes affecting structural vascular components but the case described here had no other syndromic features. SAH is rare in childhood, around 125 cases having been reported in the recent literature (1999-2005), the majority being secondary to underlying tumour or following trauma. In only one previous case could the underlying cause be demonstrated to be a saccular aneurysm resulting from FMD. Childhood SUD due to primary SAH resulting from a ruptured saccular aneurysm is rare but should be considered as a possible natural cause of death. This is the youngest patient thus far affected.

P86

The Immunophenotyping Of Primary Diffuse Large B-Cell Lymphomas Of The Central Nervous System

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Studies have identified that antigens expressed in nodal Diffuse Large B-Cell Lymphomas (DLBCL) have a prognostic significance on survival. CD10 confers a favourable outcome, whilst the expression of Bcl2, CD5 or MUM1 appears to have an aggressive clinical course. DLBCL can be categorised Germinal Centre (GC) phenotype (CD10+, Bcl6 +/-, MUM1+/-) or Activated B-Cell (ABC) phenotype (CD10 -, Bcl6 +/-, MUM1+).

We studied 31 cases of primary CNS DLBCL and 9 cases of nodal DLBCL involving the CNS. Immunohistochemistry detecting CD20, CD79a, MIB1, CD5, CD10, Bcl2, Bcl6, and MUM1 was performed as well as CD30, p53, and CD138.

28 of 31 were of ABC phenotype, (CD10-, MUM1+, bcl6-/-) whilst 3/31 were of GC phenotype (CD10+ but also MUM1+). 27/31 expressed p53, 4/31 expressed focal CD30, 3/31 expressed CD5 but none expressed CD138.

8 of 9 secondary DLBCL were also of ABC phenotype. All stained positive for p53, 3/9 expressed focal CD30 but all were negative for CD5 and CD138. In this series of primary CNS DLBCL, the majority are of the more aggressive ABC type. The ABC phenotype of these lymphomas is compatible with the aggressive clinical course of CNS DLBCL.

P87

Subcellular functional specificity of dynein-dynactin complex subunits – normal distribution and disturbances in neurodegenerative disease

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Background

Cytoplasmic dynein and its activating complex dynactin drive the retrograde transport of material necessary for the survival and proper functioning of neurons. Disruption of the dynein-dynactin complex by either over-expression of the dynamitin subunit or by mutations within individual dynein/dynactin subunits can cause motor neuron degeneration.

Objectives

To examine the distribution of the dynein complex subunits in the CNS, in order to test the hypothesis that this may be altered in Alzheimer (AD) and other neurodegenerative disorders

Methods:

Three dynactin subunits (p150, dynamitin p50 and p62) and two subunits of the conventional cytoplasmic dynein (dynein heavy chain and dynein intermediate chain) were localised in the central nervous system of human post-mortem sections from MND, Alzheimer's disease, and patients with no neurological disease. Tissue from mice bearing dynein mutations and wild type controls were also studied. Immunohistochemical studies of formalin fixed paraffin embedded tissue sections were performed, validated by multiple antibodies and western blotting. Cell culture studies of rat DRG neurons were also studied.

Results:

Unexpectedly, co-ordinated distribution of dynein-dynactin complex subunits was not evident, even in normal tissues, with heavy chain generalised staining seen, but synaptic staining seen with p50. Axons were stained with p150. There was no observable difference in the general cellular and structural localisation of complex subunits between dynein mutant and wild type mice, or in MND cases compared to the Alzheimer's disease and control sections. However, the localisation of certain complex subunits, but not others, to pathological structures, including inclusions in MND (p62), plaques (p50), granulovacuolar degeneration (p150) and neurofibrillary tangles in Alzheimer's disease (p62) suggest a role in pathological features of neurodegeneration.

Conclusion

The results suggest that different subunits of the dynein-dynactin complex may have different roles in subcellular function, and that primary events that disturb the function of individual components may have differential primary effects on subcellular structures.

P89

Audit of Clinical Effectiveness of frozen section at the time of revision of total joint replacement.

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Introduction:

Infection of joint tissues at the time of joint replacement is a major cause of morbidity and mortality in orthopaedic patients. Frozen sections (FS) would appear to be ideal in giving rapid information about whether a joint is infected or not at the time of revision. However, FSs are a labour intensive investigation. The following study was undertaken to assess the effectiveness of FS in uncovering joint infection, the threshold of particular surgeons leading them to send specimens for FSs and the degree to which orthopaedic surgeons used the results of the FS to guide further management of the patient.

Method:

The results of FS, H&E stained paraffin sections, cultures and pre-operative blood indices for 53 revisions of total joint replacements (46 hips, 6 knees and 1 ankle) were assessed. A FS was judged positive if there were more than 5 neutrophils in 5 high power fields. Hospital notes were also examined in order to assess whether surgeons acknowledged receipt of FS results and whether this influenced subsequent management.

Results:

8/53 patients had positive FSs; of those, 6 were confirmed on H&E. 2 were false positives. 2/53 were positive on microbiological culture. However, tissue was submitted to microbiology in 21% of cases. Interestingly, 3/6 patients who underwent FS during review of TKR had positive FS but only 3/46 for hip revision surgery were positive. In 4/8 surgical records, the positive records were recorded in the operation notes. On analysis, pre-operative inflammatory parameters were not requested in 16/53 cases. They were positive in 20 patients and negative in 17. 5/20 with positive pre-op inflammatory markers and 2/17 with negative markers had positive frozen sections.

Conclusion:

What has proven especially interesting in our study is the pick up rate for infection in knee replacement was 50%, compared to 7% in hips. We are now assessing the reasons for this difference by sending a questionnaire to the orthopaedic consultants. We are now assessing how the frozen section has affected treatment.

P88

The Pathology of Impaired Remyelination in Multiple Sclerosis

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Multiple sclerosis patients often show at autopsy both remyelinated plaques and sharply demarcated, demyelinated plaques. Why remyelination fails in some lesions but not others is unknown. This study utilized tissue from patients with early multiple sclerosis to investigate the timing and nature of changes associated with failed remyelination early in the course of the disease. Paraffin sections from ten cases were stained for myelin and axons, and for the oligodendrocyte markers CNP and MOG. 'Normal' remyelination was observed in a proportion of lesions in most cases with a clinical duration of more than a few months. The same cases also had lesions in which regenerative activity was clearly abnormal. The predominant abnormality exhibited by different lesions included a) the presence of differentiated oligodendrocytes in the absence of new myelin; b) clumped (?multinucleated) oligodendrocytes in the absence of new myelin; c) astrocyte / oligodendrocyte interaction (emperipolesis) in the absence of new myelin; d) malformed (nodular) myelin sheaths; e) active demyelination of remyelinated lesion ('second hit' lesions). Importantly, the pattern of active demyelination appeared to be the same in all newly forming lesions. Remyelination failure begins early in the course of MS, and occurs following the reappearance of differentiated oligodendrocytes in recently demyelinated tissue.

P90

Determination of P₅₃ expression in basal cell carcinomas and adjacent normal epidermis from photoexposed areas of head and neck regions

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Background and Objective:

P₅₃ mutations are common in a variety of human tumours. This protein serves as molecular policeman that prevents the propagation of genetically damaged cells. Paradoxical findings have been reported about the frequency of P₅₃ mutation in basal cell carcinoma, which point out the necessity of an exact study on this subject. The intensity of P₅₃ expression would also be a helpful key to make a more exact prognosis.

Methods:

This descriptive – analytical study was performed retrospectively over a 5-year period on 150 basal cell carcinoma cases in the pathology department of Alzahra hospital in Isfahan.

Proper quality paraffin blocks were chosen to be stained immunohistochemically for P₅₃ through immunoperoxidase method. The intensity of immunoreactivity was graded. Age of the patients was recorded as well.

Result:

Positive P₅₃ immunoreactivity was observed 123 basal cell carcinoma tissue (82%) and in 117 adjacent normal epidermal tissues (78%) (P=0.38).

Frequency of severe immunoreactivity in tumoral tissue and adjacent normal epidermis were 46% and 32% respectively (P=0.046).

The mean age of P₅₃ expression was 66.2 for tumoural tissue and 66.1 for normal epidermis. The mean age of those who do not express P₅₃ was 52.6 and 55.9 for the tumoural and normal epidermis respectively (P<0.001).

Conclusion:

No significant difference was detected between P₅₃ immunoreactivity in tumoural tissue and adjacent normal epidermis. Intensity of P₅₃ immunoreactivity is greater in tumoural specimens. Comparison of mean ages shows a significant difference between P₅₃ expressing and non-P₅₃ expressing groups.

P91

Herovici's Stain Vs Picrosirius Polarization In Cutaneous Histology

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Only two collagen stains are thought to differentiate between types I and III without immunohistochemistry. Picrosirius-polarization is increasingly used in many different settings. Herovici's stain is much less popular. These two are directly compared for the first time. We aim to establish the advantages and disadvantages of each.

Formalin-fixed, paraffin-embedded samples of human skin (n=10) and rat cutaneous wound (n=4) were stained with a modified Herovici's or picrosirius-polarization techniques.

With Herovici's stain type I collagen shows as thick red fibres located mainly in the reticular dermis. Type III shows as thin, blue fibres concentrated in the papillary dermis and healing wound. Nuclei stain dark purple and cytoplasm stains greenish-yellow.

In picrosirius-polarization type I collagen shows as thick, yellow/red, strongly birefringent fibres and type III shows as thin, greenish, weakly birefringent fibres. Colour variability is seen and differentiation of types I and III is not as impressive where they co-exist, as in the dermis. Relations to adjacent structures are not clear using polarizing microscopy.

Using Herovici's: type I and III fibres are easily distinguishable. Additional nuclear and cytoplasmic staining allows much greater clarity. Consequently it is a superior tool in the study of collagen biopathology in health and disease.

P94

Basal Cell Carcinoma Arising In a Basaloid Follicular Hamartoma Syndrome: The Importance of Good Clinical Information

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A 91 year old female presented to the GP with a lesion on her upper lip. It was thought clinically to be a basal cell carcinoma and the patient was referred.

When seen by the plastic surgeons she was noted to have multiple "sebaceous cysts" around her nose bilaterally. The left sided cysts and basal cell carcinoma were excised with direct closure.

Histology showed a predominantly nodular basal cell carcinoma surrounded by multiple basaloid proliferations with the histological appearance of a basaloid follicular hamartoma (BFH).

At a later date, the histology was reviewed together with the notes and previously unseen photographs of the lesion raising the probability of multiple trichoepitheliomas rather than a BFH.

Now we know that molecular development of BFH involves the same sonic hedgehog pathway as basal cell carcinoma, unlike trichoepitheliomas, the correct diagnosis of these lesions may be more necessary than was originally thought.

P93

Case report: A benign apocrine tumour showing features of a syringocystadenoma papilliferum and apocrine cystadenoma

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Syringocystadenoma papilliferum is an uncommon benign skin tumour, usually occurring on the scalp and forehead. We report a case of a benign apocrine adenoma showing mixed features of a syringocystadenoma papilliferum and apocrine cystadenoma, drawing parallels with other genital lesions showing similar sweat glands features and relating these to the concept of mammary-like anogenital sweat glands

P95

METASTATIC CALCINOSIS CUTIS PRESENTING AS BILATERAL VULVAL CYSTS

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BACKGROUND: Calcinosis cutis may be classified as metastatic, dystrophic and idiopathic based on the pathogenesis. In metastatic calcinosis cutis, calcium salts are deposited in normal tissue due to a defect in calcium and phosphate metabolism. Metastatic calcification often present as skin nodules mainly at the vicinity of large joints. Isolated vulval calcinosis is unusual. We present a case of metastatic calcinosis cutis manifesting as multiple vulval cysts.

CASE REPORT: A 36 year old lady on chronic dialysis presented with multiple vulval cysts involving both labia minora. The clinical diagnosis was epidermoid cysts. Excision biopsy showed large deposits of calcium within the superficial dermis. There was focal histiocytic response and transepidermal elimination. Serum levels of inorganic phosphates and PTH were markedly elevated. A diagnosis of vulval metastatic calcinosis cutis was made.

CONCLUSION: Metastatic calcification results from secondary hyperparathyroidism. The vulva is an unusual site for cutaneous calcification and this is the first reported case of metastatic calcinosis cutis presenting as vulval swellings. One needs to consider the possibility of metastatic calcinosis cutis in all dialysis patients presenting with skin nodules even in unusual sites.

P96

Expression of Endoglin and ALK1 in skin suggests a role for these receptors in normal skin function and skin tumourigenesis

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The Activin Receptor Like Kinase 1 (ALK1) and endoglin are members of the transforming growth factor β (TGF- β) receptor family that are known to be important for endothelial cell function. We have carried out an immunohistochemical to examine the expression of these proteins in normal skin and during wound healing.

We have shown that ALK1 expression can be found throughout normal skin including skin appendages such as hair follicles, apocrine and eccrine ducts and sebaceous glands. Unsurprisingly, ALK1 is also expressed in endothelial cells and fibroblasts in granulation tissue during wound healing. Endoglin expression was found to be more variable, being expressed in a proportion of the normal skin sections examined, present either throughout the epidermis, or only in the outer half. In wound healing and scarring, endoglin was also seen in a proportion of cases and in all new blood vessels within the granulation tissue. In the light of these results we decided to examine the expression of these proteins in skin tumours. ALK1 expression was seen in basal cell carcinomas and a squamous cell carcinoma, however endoglin expression was absent. These data are the first to show expression of ALK1 in human skin, and suggest that ALK1 and endoglin play an important part in determining normal keratinocyte response to TGF- β . ALK1 expression in skin tumours may also influence tumour response to TGF- β and thus provides a possible target for therapeutic intervention. Further studies are required.

P98

The Implementation and Application of Path Tracking in Virtual Microscopy

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Virtual microscopy refers to the high resolution digital scanning of entire glass slides and the generation of a digital virtual slide (VS) of the sample which can be viewed on-line. A VS file can be large sometimes exceeding 1Gb. There remains the need to develop software for the manipulation and analysis of these large images. This specifically relates to the use of VS media for education, training and quality assurance. We have developed a software platform that allows paths to be tracked and recorded during the on-line assessment of a VS. Software for this application was developed in Visual C++ for the viewing and navigation of a VS and developed as an application wrapping the Aperio ViewPort OCX. Functionality was added to record the field of view position and magnification status with time-based information. This allowed the real-time recording of all actions taken by the user, storage to a user database and the continuous playback of this path. Path recording is initiated as soon as the VS is opened and terminated when the user closes the VS. The path is saved in XML format, and records the user and VS identifier. The path can also be digitally mapped, superimposed on top of the VS and subsequently analysed for statistical features quantitatively characterising the path taken.

The ability to record paths has a number of key applications in virtual microscopy. In education and training, it allows expert based assessments of slides to be recorded and provides an important backbone for the creation of educational materials. In quality assurance programmes, it provides the capability to examine how individuals assess slides, determining their coverage and focus within a given sample. It also provides a tool for decision analysis by allowing us to explore how individuals view slides and how this impacts on diagnostic ability.

P97

Automated Identification of Benign and Malignant Urothelial Cells in Urine Cytology by Spectral Imaging

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Distinction between benign, malignant and atypical cells in cytology can be difficult and screening is labour intensive and time consuming, especially for urine cytology, and there is therefore interest in automated systems for cytological diagnosis. We used spectral imaging to determine the spectral profile of benign, malignant and atypical cells of undetermined significance (ACUS) in routinely stained urine cytology specimens. A Nuance multispectral imaging system was used to collect image stacks representative of the image intensities at each pixel at 10nm wavelength intervals across the visible spectrum, from which spectral profiles were calculated, for benign and malignant nuclei in urothelial cells, in training cases. These two profiles were then applied to test cases, which included samples with benign (n=10), malignant (n=10) and atypical cells (n=20). Image stacks were collected for each of the test cases and spectral analysis, using a maximum likelihood method, used to determine, on an objective and automated basis, whether the spectrum for any given cell in the test cases corresponded to that of a benign or malignant cell. All benign and malignant cases in the test samples were correctly assigned, whilst the cells in the ACUS cases had a spectral profile closer to that of the malignant than the benign cells, and we are now in the process of subdividing the ACUS cases into those of reactive or neoplastic origin.

The results demonstrate the ability of an automated, objective spectral imaging method to distinguish benign and malignant urothelial cells, and to identify atypical cells. This opens the possibility of an automated system for cytological analysis, and, since the method relies on spectral distinction alone, it may be generically applicable to other diagnostic scenarios.

Abstracts

Oral

O1

Chromosome Translocations on 8p in Breast Cancers

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Chromosome translocations, as important oncogenic events, have long been ignored in epithelial cancers, however several lines of evidence now suggest that some are recurrent and target specific genes. Many carcinomas show chromosome translocations with breakpoints on 8p, distal 8p is often lost with the break frequently occurring in the region of 8p11-8p21.

We have previously identified a recurrent 8p12 translocation breakpoint in the *NRG-1* gene, the first consistent chromosome breakpoint described in a common epithelial cancer¹. A second region of recurrent breakage was identified between *NRG1* and *FGFR1*². Recently we have described an amplicon adjacent to *FGFR1* encompassing the genes: *FLJ14299*, *BRF2* and *SPFH2*³.

We describe here the comprehensive analysis of 8p rearrangements in 48 cancer cell lines performed using array-CGH on an 'in-house' array with tiling path coverage of 8p12. Thirty of the cell lines have breaks within 8p12 and show a bewildering variety of rearrangements with no apparent common theme: loss of distal 8p including *NRG1*; translocation, fusion and amplification of *NRG1*; a variety of inversions, deletions and duplications in between *NRG1* and *FGFR1*; amplification of the *FGFR1* region; amplification of the region distal to and excluding *FGFR1*.

We conclude that the rearrangements that occur in the 8p12 region in cancer cell lines are extremely complex. As yet we do not have a satisfactory hypothesis to explain this observation, but it seems unlikely that a single gene is the target for all these rearrangements.

¹Huang et al., (2004). *Cancer Research*. 64(19):6840-4; ²Courtay-Cahen et al., (2000). *Genomics*. 66:15-25; ³Garcia et al., (2005). *Oncogene*. 24(33):5235-45

O2

Basal phenotype provides the strongest prognostic factor in a subgroup of breast cancer

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Breast cancer is now recognised to be a heterogeneous disease with different histological types and grades showing different outcomes and different patterns of genetic derangement. Current routine management of breast cancer relies on the established prognostic factors particularly tumour grade and lymph node (LN) status. Although basal phenotype (BP) showed an inverse association with survival, previous studies showed conflicting results for different subgroups of breast cancer. We have examined 1944 cases of invasive breast carcinoma with a long-term follow-up to examine the prognostic significance of BP in the different subgroups. We found that BP as defined by expression of CK5/6 and/or CK14 in $\geq 10\%$ of tumour cells (347 cases, 18.6% of our series), is associated with poor prognosis and shorter outcome in terms of shorter overall survival and disease free interval in the whole series as well as in both LN negative and LN positive groups. However, when we stratified the cases into different grades, we found that BP has a prognostic value in grade 3 tumours (894 cases, 46% of the whole series) but not in grade 1 or 2. In a subgroup comprised LN negative, grade 3 tumours (30% of cases), BP was the only prognostic marker identified in our series compared to other markers (Nottingham Prognostic Index, vascular invasion, age, menopausal status, ER, p53, *cerbB-2*, EGFR, E-cadherin or P-cadherin). These results demonstrate that BP is the most significant prognostic factor in grade 3 tumours and provide robust data that it can provide prognostic information in this large subgroup of breast cancer stronger than any other well established marker. We recommend routine staining of breast cancer with basal markers particularly in grade 3 tumours.

O3

High Frequency of Co-existence of Columnar Cell Lesions, Lobular Neoplasia, and Low Grade DCIS with Invasive Tubular Carcinoma and Invasive Lobular Carcinoma

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Aim To determine the frequency of association between putative precursor lesions involved in development of some special types of breast carcinoma. Method: Three pathologists reviewed 127 successive low grade breast tumor cases, comprising pure tubular carcinoma (TC, G1; n=54), tubular carcinoma mixed type (n=10), invasive lobular (ILC) classic type (n=56) and tubulolobular carcinoma (n=7). The presence of invasive and pre-invasive lesions including columnar cell lesions (CCL), usual epithelial hyperplasia (UEH), ductal carcinoma insitu (DCIS), and lobular neoplasia (LN) was determined.

Results: Tubular carcinoma-pure: 96% patients had CCLs with the majority showing columnar cell hyperplasia with atypia. DCIS was present in 91% patients. Co-localization of CCL, DCIS and TC was seen in 83% patients, all displaying the same cytoplasm-nuclear morphology. LN was seen in 15%. Tubular carcinoma-mixed: Co-existence of CCL, DCIS and TC was seen in 80%. LN was seen in 60%.

ILC: 91% cases showed LN. CCL and DCIS were seen in 52% and 41% cases, respectively.

Conclusion: Our findings support the hypothesis that CCLs are associated with pure and mixed forms of tubular carcinoma, and that LN is involved in ILC development. Co-existence of both LN and CCLs with invasive tubular and with lobular carcinoma was common suggesting that these may represent a family of precursor, insitu and invasive neoplastic lesions of the breast.

O4

Inflammation and prognosis in invasive carcinoma of the breast

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The prognostic significance of inflammation in invasive carcinoma of the breast is controversial. Previous studies have produced conflicting results. Cells necessary for a cell-mediated immune response are often present, but there is evidence that their function is impaired. Inflammatory cells may also stimulate tumour growth by release of digestive enzymes or angiogenic factors. 1599 patients aged less than 71 years with operable invasive carcinomas, diagnosed from 1974 to 1989, with median follow up 9.4 years were studied. No patient received adjuvant systemic treatment. An overall assessment of the intensity of lympho-histiocytic inflammation was made on haematoxylin and eosin sections by one observer. Inflammation was associated with higher grade, ductal and medullary type, tumour size and inversely with patient age. On univariate analysis patients with tumours with marked or moderate inflammation had a better survival than patients with tumours with absent or mild inflammation ($P = 0.04$). On multivariate analysis survival was associated with inflammation (relative risk 0.61, $P = 0.0002$) in addition to lymph node stage, histological grade, tumour size, vascular invasion and tumour type; survival was not related to patient age or oestrogen receptor status. This study suggests that the anti-tumour effects of inflammation predominate over the pro-tumour effects.

O5

Survivin is an independent predictor of poor outcome in breast cancer

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Clinico-pathological factors can predict outcome for patients with breast cancer, but even within the good and poor prognostic groups that these identify there are differences in behaviour and in the benefits of different types of therapy. Apoptosis is an important mechanism by which many therapeutic agents work, so alterations in apoptotic machinery in breast cancers are important. The aim of this study was to evaluate the relevance of p53, bcl-2 (anti-apoptotic), bax (pro-apoptotic), Survivin and XIAP (inhibitors of apoptosis) in a poor prognostic group.

Immunohistochemistry for p53, phosphorylated p53, bcl-2, bax, Survivin, XIAP, proliferation (MIB1) and apoptosis (M30) was performed on 165 cases, all having died from breast cancer (duration of survival 12-127 months, median 38; 73% T2/3, 74% node positive, 75% grade 3, 51% ER positive). 18 received neoadjuvant therapy, the rest adjuvant.

In univariate analysis grade, proliferation, phosphorylated p53, bcl-2, ER and Survivin related to duration of survival. On multivariate analysis grade (p=0.001) and Survivin (p=0.005) were independent prognostic factors.

Presence of Survivin correlated with shorter duration of survival, particularly for the neoadjuvant and adjuvant chemo-and hormonal therapy groups.

Survivin as an inhibitor of apoptosis could be of value for identifying patients who will have a poor response and outcome.

O7

An Overlapping Homozygous Deletion At 1p36.22-23 In Glioblastomas Identified By Chromosome 1 Tile Path Array-CGH

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Deletions of chromosome 1 are the most frequent genetic abnormalities among oligodendroglial tumours. However, the incidence and significance of chromosome 1 abnormalities is not known among astrocytic tumours, the commonest brain tumour in adults. We constructed a chromosome 1 tile path array-CGH and examined 108 adult astrocytic tumours (9 diffuse astrocytomas (A), 22 anaplastic astrocytomas (AA), 77 glioblastomas (GB)). The array consists of 2222 overlapping BAC/PAC clones that cover 98% of euchromatic regions of chromosome 1 as well as 538 BAC clones distributed on all autosomes with average interval of 5.2 Mb. We identified 1p deletions in 53 tumours (2 A, 9 AA, 42 GB). Loss of all of 1p, a hallmark of oligodendroglial tumours, was found only in 7 tumours. However, smaller deletions involving 1p36.22-23 were found in 45 tumours. Nine GBs were found to have overlapping homozygous deletions in this region. Amplification was identified in 11 tumours (1 AA, 10 GB). Some of the amplified regions also overlapped. We conclude that 1p deletions in astrocytic tumours are common and the pattern of deletion is distinct from that of oligodendroglomas. Our results strongly indicate that chromosome 1 harbours novel oncogenes and tumour suppressor genes for astrocytic tumours. (198 words)

O6

Interferon- β Promotes Rapid Episomal Loss and the Emergence of Latent Integrants in Cervical Keratinocytes Infected with Human Papillomavirus Type 16

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While infection with high-risk human papillomavirus (HRHPV) is known to cause malignancy in the anogenital tract, mechanisms of neoplastic progression remain poorly understood. Integration of HRHPV into host DNA is an important step, as it leads to overexpression of the viral oncogenes E6/E7, provided there is loss of the viral transcriptional repressor E2. In a related study (see Pett-MR abstract), we used the W12 model of cervical neoplastic progression to show that selection of keratinocytes containing integrated HPV16 correlates closely with loss of E2-encoding episomes and activation of antiviral response genes that are inducible by type I interferon (IFN). Here, we show that exogenous IFN- β causes very rapid loss of episomes from W12, associated with the emergence of cells bearing previously latent integrants. Episome clearance resulted in reduced growth in IFN- β -containing medium, while the emergent integrants showed accelerated growth. We conclude that integrated HRHPV DNA can exist in naturally infected cells for long periods with no selective growth advantage until loss of episomes occurs, with depletion of E2 and de-repression of E6/E7. A revision of the current model of HRHPV-related carcinogenesis is required. Of direct clinical relevance, treating HRHPV lesions by inducing an IFN response may cause lesion progression.

O8

Application of array CGH on archival formalin fixed paraffin embedded tissues

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Array CGH (aCGH) is increasingly used for investigation of chromosomal gains and losses in disease, particularly cancers. It remains to be established whether aCGH can be reliably applied to archival formalin-fixed paraffin-embedded (FFPE) tissues and to small numbers of cells microdissected from a defined histological lesion.

DNA samples purified from nine pairs of matched fresh and FFPE glioblastoma xenograft tissues were subjected to aCGH analysis using an in-house 1MB genomic array. DNA quality was assessed by amplification of genes of variable sized fragments.

3 FFPE tumours amplified DNA fragments ≥ 400 bp in size and showed high quality reproducible aCGH data. In contrast, the remaining FFPE tumours failed to show amplification of fragment ≥ 400 bp in size. Formalin fixation up to 96 hours and storage of FFPE tissue up to 15 years did not appear to have a major effect on aCGH analysis, while presence of necrosis dramatically increased the background. Furthermore, as few as 2,000 cells microdissected from FFPE tissues could be successfully used for aCGH analysis when whole genome amplification was incorporated.

In conclusion, microdissection and whole genome application allows aCGH analysis of a small number of cells microdissected from a defined histological lesion, such as carcinoma *in situ*.

O9

Characterisation of CpG Island Methylation in a Colorectal Cancer Cell Line by a Novel Array-based Method

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DNA methylation plays an important role in the regulation of gene transcription and is strongly implicated in cancer development. The identification of abnormal patterns of methylation requires a practical and reliable high-throughput method for identifying CpG methylation. We describe here a method called Microarray-based Methylation Assessment of Single Samples (MMASS) for identifying genome-wide CpG island methylation, which directly compares methylated to unmethylated DNA sequences within a single sample after digestion using methylation sensitive enzymes. We have used a CpG island array, based on a previously published CpG island library. We used bioinformatics methods to characterize the full sequences of approximately 7000 clones. The average size of the characterized CpG islands in this library is ~531 bp and, of the sequences analyzed thus far, ~1000 clones consist of 100% repeat elements. We used the annotated CpG island array to compare a rational design method for choice of methylation enzyme and enrichment by subtraction for methylated sequences against previously published protocols. The optimized MMASS method had greater statistical power to detect hyper- and hypomethylation in single samples when compared to existing methods. Methylation status of a group of representative CpG islands was validated by COBRA which showed concordant results. Study of the colorectal cancer cell line HCT116 confirmed the methylation status of known hypermethylated CpG islands and also detected novel methylated CpG islands within this cell line.

O11

The VDR Promoter Polymorphism A-1012G affects T-cell Response in Malignant Melanoma and Psoriasis Through GATA-3 Signalling

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We recently reported a novel polymorphism, A-1012G, within the major promoter region of the vitamin D receptor (VDR) gene. We have shown that the A allele of A-1012G is associated with occurrence and increased metastatic risk in malignant melanoma (MM) but with improved treatment response in psoriasis.

Computer modelling showed that A-1012G genotype altered a putative binding site for the transcription factor GATA-3. Using gel-shift assays, we have shown that the A allele of A-1012G binds GATA-3 more strongly than the G allele. GATA-3 promotes the polarisation of T-helper cells to Th2 and vitamin D treatment has also been shown to favour a Th2 response, reducing the Th1 response. Therefore, as VDR signalling is important during T-cell differentiation, A-1012G is likely to modulate T-helper activity, with the A allele associated with a reduced Th1 response. *In vitro* assays support this hypothesis. This mechanism explains the reported associations of A-1012G genotype and disease. In MM, a reduced Th1 response will lead to weaker immunosurveillance so the A allele of A-1012G is linked with increased occurrence and metastasis. In an association study of 230 cases of MM we have shown that the A allele is linked with metastasis independent of Breslow depth. Therefore this VDR promoter genotype combined with Breslow depth is potentially a more accurate prognostic marker for MM than current measures. In psoriasis, an overactive Th1 response is detrimental such that the A allele is beneficial in reducing inflammation and increasing the affect of vitamin D treatment on lesional T-cells. A-1012G is likely to show associations with other diseases where T-cell activity is an important factor.

O10

GLYCOSYLATION AND GOLGI apparatus STRUCTURAL INTEGRITY ARE essential IN TARGETING GLUT1 TO MEMBRANES OF COLORECTAL CANCER CELLS

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Background: GLUT-1 is a high affinity glucose transporter up-regulated at cancer cell membranes. Factors involved in targeting Glut1 to membranes are not understood in colorectal cancer.

Methods: We examined the role of N- and O-linked glycans and Golgi apparatus structural integrity in targeting Glut1 to cell membranes. Using five colorectal cell lines, we inhibited N-glycosylation with tunicamycin, O-linked glycan synthesis using benzyl-N-acetylgalactosamine and disrupted the Golgi transport system using monensin. Using these drugs, cells were treated singly and in combination for 24 hours. Cell proliferation rates were assessed using a crystal violet assay. Glut 1 immunofluorescence and confocal microscopy were used to evaluate the localisation of Glut1 before and after treatment.

Results: Following treatment, rates of cellular proliferation were reduced in all cell lines. Growth inhibition with monensin was significantly higher compared to other treatments (all p values <0.02). Immunofluorescence and confocal analyses localised Glut1 to membranes of untreated cells. Treatments significantly altered cell morphology and localisation of Glut1 to a diffuse cytoplasmic and/or perinuclear expression pattern.

Conclusion: N- and O-linked Glut1 glycosylation and intact Golgi apparatus structure are essential for colorectal cancer cell proliferation and intracellular trafficking of Glut1 to cell membranes where it functions as a glucose transporter.

O12

Comparison Of Wnt5a Expression With Alterations Of p16 And B-raf In Cutaneous Melanoma Progression

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There is no effective therapy for metastatic cutaneous melanoma (CM); hence prognosis is poor. Molecular alterations during CM progression may yield therapeutic targets, but remain poorly understood. Preliminary studies suggest that expression of Wnt5a is important in CM progression. Our aim was to characterise Wnt5a expression in CM and compare this with changes of p16 and B-raf, in the context of Clark's progression model.

Wnt5a and p16 expression was determined in 173 melanocytic tumour samples via immunohistochemistry. Radial growth phase (RGP), vertical growth phase (VGP) and metastatic CM samples were examined for B-raf mutation. Trend analysis showed increasing Wnt5a expression with progression (p=0.001), while p16 expression was reduced (p<0.001). B-raf mutation was frequently a pre-metastatic event that usually correlated with the matched metastasis. Alterations of Wnt5a, p16 and B-raf appear to be non-random events and may be incorporated in a progression model of CM. There were significant changes in expression between different phases of the progression model. Differential expression between RGP tumours and RGP-like areas surrounding VGP tumours (RGPA) was shown. Researchers regard RGP and RGPA as equivalent in CM progression models, however, our results suggest they are different and that RGPA progression data should be interpreted with caution.

O13

Angiogenesis and Lymphangiogenesis in Testicular Germ Cell Tumours (TGCT)

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Introduction: TGCT readily metastasize to the lymphatics but there is little in the literature about the role of lymphangiogenesis. We aimed to study novel lymphatic markers in TGCT.

Methods: 34 cases of stage I TGCT and 10 controls were studied using archival paraffin-embedded material. Immunohistochemistry was performed for CD31 and the lymphatic-specific markers podoplanin, flt-4 and LYVE-1. Staining was assessed directly in both the tumour and the periphery of the tumour.

Results: Intratumoral counts for CD31, podoplanin, flt-4 and LYVE-1 were significantly higher as compared with controls ($p=0.0033$, <0.0001 , <0.0001 , <0.0001). Similarly, the tumour periphery counts were also significantly higher for each respective marker as compared to controls ($p=0.0003$, <0.0001 , <0.0001 , 0.0011). CD31 and podoplanin counts were significantly higher in the tumour periphery than intratumorally ($p=0.024$, 0.0057).

Discussion: Increased microvessel density (MVD) and lymphatic vessel density (LVD) suggest a role for angiogenesis and lymphangiogenesis in tumour biology of TGCT. Intratumoral lymphatics have been demonstrated again suggesting their possible role in lymphangiogenesis. Our findings also support the hypothesis that intratumoral lymphatics are present and may have a possible role in lymphangiogenesis. Furthermore, increased MVD and LVD peripherally may represent an 'expanding front' of tumour with neolymphangiogenesis occurring.

O15

DISAPPEARING LYMPH NODES. ONE EXPLANATION FOR THE FALL IN NUMBER WITH INCREASING AGE..

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INTRODUCTION: Increasing patient age correlates with reduced lymph node yield from colorectal cancer (CRC) specimens (ACPGI and NYCRS audits). Lower numbers of lymph nodes reduce the frequency of Dukes C cases and thus reduced administration of adjuvant therapy and loss of the associated benefits. As age increases it is known that T cell counts remain stable whilst B cell counts reduce with age (Maclennan et al 1994 Infections in Elderly Patients Edward Arnold p1) but the impact of this on size of node in CRC is unknown. The cause of the reduced yield could be a fall in number with age or a reduced size leading to a smaller harvest by the pathologist. This question was investigated.

METHOD: The areas of 1776 lymph nodes were measured on digital files using the aperio scanning system on 112 cases from the MRC Classic trial. Median age 71.2 (32-94). Node count for each case was noted and area of lymph node calculated by two methods physical measurement and an alogram pixel count
RESULTS: The lymph node count per case ranged from 1-53 with a median value of 13.2. Lymph nodes were measured with an area from 0.1mm² to 146.8mm² with a median value of 3.5mm². Both methods were highly correlated ($r = 0.99$ $p < 0.001$) with the algorithm being quicker to apply. When age is analyzed by lymph node area and lymph node count the reduction of number of nodes found to increasing age is confirmed ($r = -0.06$ $p = 0.56$). Lymph node area also reduces with increasing age ($r = -0.49$ $p = 0.1$) and average lymph node size to age stronger still ($r = -0.83$ $p = 0.041$). The relationship of reduction of lymph node area to increasing age correlates better than with node count.

CONCLUSIONS: The prior findings of a reducing number of lymph nodes found with age have been confirmed in a randomized study. We also demonstrate by two independent methods a strong association between age and lymph node size with older patients having a reduced cross sectional area. Thus it seems likely that reduced size of lymph nodes contribute towards the lower lymph node count in the elderly but further studies are required of series with smaller and larger numbers of median lymph nodes found to define the exact contribution of the reducing size to the reduction in number that occurs with age.

O14

Global Gene Expression Analysis of Paediatric Malignant Germ Cell Tumours Reveals Common Patterns of Transcription and Expression Signatures of Different Histological Types

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We are using a microarray approach to study the gene expression characteristics of childhood malignant germ cell tumours (MGCTs). We aim to determine whether these clinically and histopathologically heterogeneous tumours share fundamental genetic abnormalities and whether distinct gene expression profiles associate with particular clinical and/or pathological features. To date, RNA from 32 childhood MGCTs has been hybridised to the Affymetrix U133A genechip and analysed using GeneSpring software. The samples analysed represent 12 germinomas (3 within teratomas), 19 yolk sac tumours (8 within teratomas) and 1 embryonal carcinoma. Interestingly, we have observed substantial homology in gene expression between these heterogeneous tumours. Supervised clustering against normal gonad controls reveals potential new biomarkers of this tumour group. Unsupervised clustering divides the tumours by histology, producing cancer signatures, with only a limited number of genes (as few as 133 using ANOVA, Welch's t-test, $p < 0.01$ & Bonferroni multiple testing correction) discriminating between germinomas and non-germinomatous tumours. Importantly for internal validation, one of these discriminatory mRNAs is alpha-fetoprotein. Global gene expression data will be compared with the available clinical information in this ongoing project, with the ultimate aims of developing improved methods of diagnosis, better risk stratification for treatment schedules and novel therapeutic targets.

O16

MONOCLONAL CONVERSION IN HUMAN GASTRIC GLANDS: INSIGHTS INTO STEM CELL AND CLONAL ARCHITECTURE

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Studies on the intestine of animals and man have confirmed, beyond reasonable doubt, that the structural units, the crypts, are clonally-derived. The unit found in the stomach, the gastric gland, especially in the human, remains an enigma. Some animal reports show clonal-derivation, whereas others show that a minority of glands remain polyclonal, suggesting the possibility of multiple, possibly independent stem cells. In the human, results are also mixed. We have used mutations in the mitochondrial cytochrome c oxidase subunit 1 gene (COXsu1), as a clonal marker (J. Clin. Invest. 2003;112(9)), detecting COXsu1 in conjunction with a nuclear-encoded oxidative enzyme, succinate dehydrogenase (SDH), by double enzyme histochemistry. We have observed body-type gastric glands which are partially filled with mutated cells, and others glands where all the cells in the gland are mutated (COXsu1-, SDH+). These suggest that a stem cell bearing this mutation expands to fill the entire gastric gland. Early observations suggest that all cell lineages in these mutated body-type glands are involved in this process. Moreover, the mutated gland then appears to spread in the mucosa by gland fission.

Several conclusions are made possible by these observations:

1. Gastric glands undergo a process of monoclonal conversion, where the gland becomes gradually replaced by mutated cells;
2. Human gastric glands are maintained by a number of stem cells;
3. Each body-type gland is derived ultimately from a single stem cell which gives rise to all the contained lineages, and are therefore multi-potential;
4. These mutated glands then appear to expand by gland fission, even in the adult stomach.

O17

Circumferential surgical margins in rectum and right colon in the MRC CLASICC trial. 3 year disease free survival and local recurrence.

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Involvement of the circumferential resection margin (CRM+) in the rectum increases local recurrence and reduces survival. The MRC CLASICC trial investigated involvement of the CRM in both the rectum and the right colon in 794 patients. The quality of pathology is unique for a colorectal cancer trial as pathologists were trained, filled in a detailed proforma and over 90% of material was centrally reviewed on a duplicate set of slides. We report the 3 year disease free survival and local recurrence data on the centrally reviewed cases only.

Results

Rectum: 35/274 (12.7%) were CRM+. This strongly predicted both survival (table 1 3-year DFS difference 41.2% p=0.0001) and local recurrence (CRM +ve 22.8% vs CRM-ve 8.7% p=0.007).

1 Circumferential margin positive rectum?	3-year DFS estimate	Standard error	95% CI lower limit	95% CI upper limit
yes	27.1	8.6	10.2	43.9
no	68.2	3.1	62.1	74.4
2 Circumferential margin positive right colon?	3-year DFS estimate	Standard error	95% CI lower limit	95% CI upper limit
yes	56.3	12.5	31.7	80.9
no	62.1	4.6	53.1	71.0

Right sided cancers: 17/140 (12%) showed CRM involvement but this did not reach statistical significance at 3 years for survival (table 1 p=0.4) or local recurrence (CRM +ve 13.5% vs CRM-ve 9.2% p=0.5).

Laparoscopic vs open surgery. In exploratory sub-group analyses, there was no evidence of a difference in survival or local recurrence for margin status and operation type.

Conclusion: With modern surgical techniques CRM status is a very important predictor of outcome in rectal cancer. There is no evidence in this small sub-group at 3 years that CRM status is important in right sided colonic cancer.

O19

Sequence Analysis of the Immunoglobulin Heavy Chain Genes of Tumour Cells from Thyroid Follicular Lymphoma

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Extra-nodal follicular lymphomas, like MALT lymphomas, may arise within MALT formed as a result of inflammation, and transformed cells may remain responsive to antigen. The rearranged immunoglobulin heavy chain (IgH) genes of tumour clones from 6 cases of thyroid follicular lymphoma were cloned and sequenced. Antigen selection of the tumour cells was revealed in all cases by either biased accumulation of replacement mutations in the CDR regions compared to the framework, or acquisition of novel N-glycosylation sites. No biased VH, D and JH segment usage was found to show response to the same antigen. Intra-clonal variations that indicated ongoing somatic mutation were seen in five cases. Sequence analysis did not reveal any significant differences between the IgH genes of primary follicular lymphoma cases and cases known to have disseminated to the thyroid from elsewhere. The degree of somatic hypermutation

THEt was similar to that reported for other cases of extra-nodal follicular lymphoma. The level of somatic hypermutation, presence of ongoing mutation, bias in the accumulation of replacement mutations in the CDR regions and acquisition of novel glycosylation sites is comparable to that of nodal follicular lymphomas described in the literature.

O18

Quantitative Proteomic Analysis Shows Downregulation of MAPK Pathway in Colon Cancer

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Background: The Mitogenic Activated Protein Kinase (MAPK) pathway is considered a key pathway in colonic carcinogenesis and a candidate area for inhibitory therapy. The expression of several key proteins in the MAPK pathway were therefore included in proteomic profiling of colon cancer using reverse phase protein microarrays (RPPMs). This approach to quantify protein signaling can determine protein activation status using antibodies specific for post-translational modifications such as phosphorylation.

Methods: Stroma and epithelium from cancer as well as uninvolved mucosa from 36 colon cancer resections were isolated via laser capture microdissection. RPPMs were constructed by printing dilution curves of lysates from microdissected tissue onto nitrocellulose-coated glass slides. Slides were probed with multiple antibodies including p38, phospho (p)-p38, Erk, p-Erk, MEK and p-MEK. Antibodies were validated by Western blot. Data were analyzed with MicroVigeneTM software.

Results: Total Erk was similar and total p38 increased by 30% in cancer compared with uninvolved mucosa (p=0.0025 and p=0.081 in epithelium and stroma respectively). In contrast p-p38 was decreased by 50% in cancer compared with uninvolved mucosa (p<0.0001 and p=0.0023, epithelium and stroma) and p-Erk by 60% (p=0.0003 and p=0.0023, epithelium and stroma). Also, p-p38 and p-Erk expression in carcinomas decreased with higher Dukes' stage.

Conclusion: Total p38 and Erk expression were unchanged or even slightly increased in cancer. However, their activated (phosphorylated) forms were substantially decreased which could not have been predicted from translational studies. This may have implications for therapy targeted at inhibiting MAPK signalling in colon cancer.

O20

Chlamydia psittaci is Variably Associated with Ocular MALT Lymphoma in Different Geographical Regions

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Infectious agent plays an important role in the development of MALT lymphoma, as exemplified by H. pylori infection in gastric MALT lymphoma. Studies from Italy showed evidence of Chlamydia psittaci infection in 80% of ocular lymphomas. To understand ocular MALT lymphoma aetiology, we assessed the presence of C. trachomatis, C. pneumoniae, C. psittaci, herpes simplex virus type 1 and 2 (HSV1, HSV2), and adenovirus type 8 and 19 (ADV8, ADV19), commonly associated with chronic eye diseases, by PCR in 92 ocular MALT lymphomas and 10 ocular DLBCLs from 6 geographical regions, and 21 other non-Hodgkin lymphomas and 39 conjunctival biopsies without lymphoma as controls. C. psittaci was found at variable frequencies in ocular MALT lymphomas from different regions: high for Germany (47%), Italy/Bologna (33%) and China/Canton (20%), but low for the UK (10%), Italy/Ancona (0%), and China/Hainan-Shanghai (0%), which did not significantly differ from controls (other lymphoma 9.5%, no lymphoma 10.2%). Interestingly, China/Canton DLBCL showed high C. psittaci incidence (33.3%), as observed for MALT lymphomas from the same region. No correlation was observed between ocular MALT lymphoma and C. pneumoniae, C. trachomatis, HSV1, HSV2, ADV8 and ADV 19. C. psittaci was thus preferentially but geographically variably associated with ocular MALT lymphoma.

O21

Immunophenotyping And Clonality Analysis In The Diagnosis Of Refractory Coeliac Disease

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Background: A proportion of coeliac disease patients who don't improve after a gluten-free diet may develop refractory CD (RCD), but clinical-pathological diagnosis is often difficult. Although abnormal immunophenotype (cytCD3⁺CD8⁺) and monoclonality are associated with RCD, their specificity and sensitivity in diagnosis of RCD remain to be evaluated.

Design: Consecutive biopsies were retrieved from 11 CD, 6 suspected RCD, 11 RCD and 2 enteropathy-type T-cell lymphoma (ETL). Histology reviewed and IEL phenotype determined by CD3/CD8 double-immunostaining. T-cell clonality determined by PCR on whole tissue sections and/or microdissected samples. In one ETL, clone specific PCR was performed.

Results: All CD cases were polyclonal with a normal phenotype. 5 suspected RCD cases had an abnormal immunophenotype; 2 persistently monoclonal, 1 transiently monoclonal, 2 polyclonal. 4 RCD cases had an abnormal immunophenotype, 2 persistently monoclonal. 7 RCD cases showed normal immunophenotype; 2 persistently monoclonal, 1 transiently monoclonal. In 1 ETL, clonal T-cells were detected in tumour free duodenal biopsies 4 years before the lymphoma diagnosis. Clonal T-cells were detected both in IEL and lamina propria in 4 RCD cases and 1 ETL.

Conclusions: Abnormal immunophenotype (cytCD3⁺CD8⁺) and monoclonality are not a feature of classic CD, but are associated with 36% and 45% of RCD respectively, 64% of cases showing either abnormality. Combined immunophenotypic and clonality analysis would maximise RCD diagnosis and identify CD cases at risk of progression.

O23

Renal regeneration following acute tubular necrosis utilises cells of bone marrow origin, but is unaffected by EPO

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Erythropoietin (EPO) is a multifunctional cytokine with a significant role in regenerating the kidney in certain models. We tested the hypothesis that EPO acts in part by increasing the number of bone marrow-derived tubular cells (BMDTCs).

Thirty six female mice were transplanted with male whole bone marrow (2x10⁶ cells after 10 Gy irradiation); half were given HgCl₂ (3 mg/kg b.w. i.p.) to induce acute tubular necrosis; half received EPO (up to 5 doses at 1000 IU/kg b.w. s.c. over 2 weeks).

Tubular injury scores (assessed by point counting) were high 3d after Hg but had recovered partially at 14d irrespective of EPO, in line with blood urea nitrogen levels (6.4-fold and 5.1-fold control values at 3d and 14d respectively). Y-chromosome in situ hybridization was combined with lectin histochemistry to identify proximal and distal tubule epithelium, and the abundance of BMDTCs in each location was determined. Renal damage increased the abundance of BMDTCs in the proximal tubules to 3.9% at 3d and 4.0% at 14d from a baseline of 1.3% (P<0.05) and in the distal tubules from 3.0% at 3d and 2.8% at 14d from a baseline of 1.1% (P<0.05).

EPO treatments increased the haematocrit (39.2 to 47.7%; p<0.05), but did not affect the tissue injury score, BUN, the abundance of BMDTCs.

Tritiated thymidine was used to label cells in S-phase, and autoradiography was combined with Y and lectin stains on single sections to assess the S-phase fraction of indigenous tubular epithelial cells and BMDTCs. The majority of cells in S-phase, and thus contributing to regeneration, were of indigenous origin, nevertheless 1 in 15 were of bone marrow origin and this was not affected by EPO.

O22

Human macrophages kill human mesangial cells by Fas-L-mediated apoptosis when triggered by antibody via CD16

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Glomerulonephritis may be triggered by antibody deposits that activate macrophages to promote tissue damage. Macrophages induce apoptosis of vascular smooth muscle cells and mesangial cells is potentially relevant to glomerulonephritis. Therefore, studies of macrophage-induced apoptosis were extended to antibody-activated macrophages. That is, we studied antibody dependent cellular cytotoxicity (ADCC). We studied cell death in direct coculture of macrophages and target cells. For corroboration of results, we studied biochemical and microscopic measurements, soluble and immobilised immunoglobulins and vascular smooth muscle cells (VSMCs) and mesangial cells as targets. First, we used anti-Thy1.1 antibody to coat mesangial cells. Human peripheral blood macrophages provoked antibody-dependent killing of mesangial cells, and U937 macrophages provoked as much killing as blood-derived macrophages. Macrophage-induced death was apoptotic as assessed by nuclear morphology, electron microscopy, annexin-V, activated caspase-3 and hypodiploid DNA. Killing was inhibited by antagonistic antibodies to Fas-L and to the low affinity antibody receptor CD16 (Fc-γ-RIII) but not to CD64 (Fc-γ-R1). Immobilised polyclonal IgG promoted macrophage-mediated apoptosis of human mesangial cells, also inhibited by anti-CD16 antibodies. Immobilised polyclonal IgG promoted macrophage-mediated apoptosis of human VSMCs, also inhibited by anti-CD16 antibodies. Finally, incubation with immune complexes upregulated macrophage surface Fas-L. In conclusion, antibody-dependent killing of human mesangial cells by human macrophages is via Fas-L and CD16.

O24

Complement C1q plays a bidirectional role in early atherosclerotic lesions in LDL receptor knock-out mice

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Objective: We explored the role of the classical complement pathway in atherogenesis using C1q deficient mice (*C1qa*^{-/-}) intercrossed with low density lipoprotein receptor knock-out mice (*LDLR*^{-/-}). **Methods and results:** In *C1qa*^{-/-}*LDLR*^{-/-} mice fed standard chow for 12 weeks, aortic root lesion area (p < 0.03), apoptotic cells (p < 0.015) and macrophages (p < 0.04), were increased compared to *LDLR*^{-/-} mice. In contrast, *C1qa*^{-/-}*LDLR*^{-/-} mice fed a high cholesterol chow diet for 12 weeks had reduced aortic root lesion area compared to *LDLR*^{-/-} (p < 0.01). Differences in lesion area between *LDLR*^{-/-} and *C1qa*^{-/-}*LDLR*^{-/-} were not observed at 40-44 weeks. **Conclusions:** This is the first direct demonstration of a role for the classical pathway in atherogenesis. The greater early lesion size in *C1qa*^{-/-}*LDLR*^{-/-} mice after 12 weeks on a standard chow diet is consistent with the emerging homeostatic protective role for C1q in the disposal of debris such as dying cells. On the other hand, reduced lesion development in *C1qa*^{-/-}*LDLR*^{-/-} mice fed a high cholesterol diet suggests that an increased atherogenic load results in classical complement pathway activation being deleterious, perhaps by overwhelming local downstream complement pathway inhibitors. These data emphasize the importance of studying the effects of gene deletions on atherosclerosis across a range of dietary conditions. **Summary:** Using *C1qa*^{-/-}*LDLR*^{-/-} receptor double knock-out mice we show that the classical complement pathway is protective in early atherosclerotic lesions in mice.

O25

Immunohistochemical expression of VEGF_{xxx}b predicts metastasis in primary melanoma.

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Prediction of prognosis of malignant melanoma relies upon assessment of Breslow thickness, but 10-15% of patients with thin tumours go on to develop metastasis.

VEGF_{xxx}b is a family isoforms of VEGF-A formed from alternative mRNA splicing, and has been shown to be downregulated in renal cell cancer. Expression of VEGF_{xxx}b in primary melanoma was assessed with a VEGF_{xxx}b specific antibody (56/1). Paired 6µm sections of primary melanoma were stained with 56/1 or control IgG at 4µg/ml. Following haematoxylin counterstaining sections were analysed by 3 assessors (blinded to the metastatic outcome) scoring horizontal and vertical growth phases, and surrounding normal skin for intensity of staining (0-4). Normal epidermis stained significantly in all samples, and intensity was no different in samples that had subsequently metastasised (MM) (1.6±0.39) compared to those that had not (2.03±0.27, NM), but significantly weaker expression was seen in MM in the vertical (1.7±0.24 NM, 0.48±0.26 MM, p<0.05) and horizontal (1.5±0.25NM, 0.37±0.21MM, p<0.05) growth phases than in NM. There was no correlation between thickness and staining intensity. These results suggest that VEGF_{xxx}b expression is downregulated in metastatic but not non metastatic melanoma, and may be a useful aid in identification of patients likely to metastasise, irrespective of tumour thickness.

O27

Communication Of Autopsy Findings: The Role Of A Pathology Follow-Up Clinic After Autopsy

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Introduction: It is suggested to bereaved relatives that they discuss autopsy findings with their general practitioner or the consultant in charge of the deceased. There are data and local experience to suggest that this is not optimal. A pathology follow-up clinic has been established, which provides relatives with an opportunity to meet with a pathologist in order to discuss the examination and the significance of the findings.

Objectives: To determine:

1. if relatives benefit from the information in autopsy reports.
2. the need for post-autopsy explanatory consultations.
3. the adequacy of information provision to meet the needs of bereaved relatives.
4. the need, benefit, or role for a meeting with a pathologist.

Method: The study involved self completion postal questionnaires incorporating fixed choice answers. 100 general practitioners, 90 hospital consultants, 57 pathologists, 91 bereaved relatives were consulted. Questionnaires were sent to all relatives who had attended the 'Follow-up Clinic'.

Results: 56.5% of bereaved relatives would have wanted to discuss the post mortem report with a doctor. 83.6% of doctors thought that bereaved relatives benefit from the post mortem report. 44.6% of all doctors thought that bereaved relatives would benefit from meeting a pathologist. If offered the opportunity to attend the 'Pathology Follow-up Clinic', 37.5% of bereaved relatives would have wished to attend.

Conclusions: There is a need and role for a Pathologist run post-autopsy clinic. We suggest this should be a secondary service with access either via referral from Coroners, G.P.'s, hospital consultants or at bereaved relatives' request.

O26

Prognostic scoring in patients with melanoma after adjuvant Isolated Limb Perfusion (ilp)

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Introduction

The clinical course in melanoma is extremely variable. The aim of our study is to assess the individualized prognosis scoring system proposed by Cochran et al in patients with Limb melanoma after adjuvant ILP

Methods

All patients in a University Hospital with nonulcerated limb melanoma who had adjuvant ILP over 10 years (1986-95) were included. Details were prospectively recorded from the national database including follow-up and death. All were risk scored as proposed by Cochran et al to yield an individual survival probability at 3, 5 and 10 years

Results

85 patients had adjuvant ILP for nonulcerated limb melanoma. 14 died (O) within 10 years. The Cochran score predicted (E) 20 deaths within 10 years (O/E ratio 0.7, p=0.13). The O/E ratios in the 0-3, 3-5 and 5-10 year intervals were 8/7.4, 5/6.0 and 1/6.5 respectively (p=0.14), showing a tendency for late deaths to be overpredicted. When patients were grouped by predicted 10-year mortality (<20%, 20-40%, >40%) the overprediction was found to occur mainly in the highest risk group (O/E ratios 6/5.9, 6/8.4 and 2/5.6 respectively, p=0.10, Hosmer-Lemeshow test).

Conclusion

Cochran scoring accurately predicted 3 and 5 year survival in patients who received adjuvant ILP for limb melanoma. It overpredicted deaths in 5-10 year group and in patients with a probability of survival of less than 60%.

O28

Diagnostic Accuracy of Internet-based Virtual Microscopy – A randomised controlled trial.

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Recent advances in "virtual microscopy" raise the possibility of diagnostic pathologists examining digitised images rather than using microscopes. Such images may be viewed over the internet. Diagnostic accuracy must not be compromised by this new approach; this has not yet been adequately tested. The UK National Renal Pathology EQA Scheme runs two parallel circulations of glass slides. In July 2005, at a point where each current circulation had been seen by half the participants, the slides were digitised and made available over the internet using the Aperio slide-scanning system. Each 'virtual' circulation was offered to those participants who had not at that stage seen the glass slides. Variations in diagnostic terminology make comparisons of diagnostic accuracy complex, but the EQA scores achieved using each system were not significantly different. This suggests that the diagnostic accuracy of the two methods is equivalent.

However, some participants were unable to submit 'virtual' diagnoses because of technical problems and there were numerous complaints about the increased time taken to assess virtual slides.

It would appear that with current technology, virtual microscopy permits the reliable generation of accurate diagnoses, but it is not yet sufficiently rapid and user friendly to replace light microscopes in routine work.

Abstracts

Speakers

S1

Colorectal cancer: basic science & clinical applications

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Colorectal carcinogenesis involves transit from normal mucosa via adenomas to carcinomas. The transitions are associated with characteristic genetic changes, such as alterations to *APC* (>80%), the DNA mismatch repair (MMR) genes (~15%), *K-ras* (45%), *SMAD4/2* on chromosome 18, and *p53* (>60%) amongst others. Mutation of *APC* (which can be inherited in familial adenomatous polyposis) or loss of *APC* is seen in adenomas and this represents the major pathway of adenoma formation. Progression to carcinoma is often associated with chromosomal instability and acquired genetic and epigenetic alterations. A second pathway involves the transition of hyperplastic polyps to serrated adenomas then to carcinomas, with evidence of associated microsatellite instability (MSI) due to deficiency of MMR. This accounts for the susceptibility to cancer formation in colorectum, endometrium and other sites in Hereditary Non-Polyposis Colorectal Cancer (HNPCC) patients with germline *MSH2* or *MLH1* mutations. Around 15% sporadic colorectal cancers also show MSI largely due to *MLH1* promoter methylation. Of all human cancers, perhaps the most is known of the molecular biology of colorectal cancer. It is therefore surprising that molecular techniques currently add little to the clinical pathological assessment of colorectal cancer. We are still primarily dependent on good macroscopic technique and routine morphological assessment. Increasingly we will be expected to undertake specialised techniques, perhaps immunohistochemical assessment of MMR or EGFR status, and we can expect diagnostic pathology to increasingly incorporate these and other molecular techniques.

S3

The Role of the Tumor Microenvironment in Breast Cancer

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Increasing evidence suggests that changes in the cellular microenvironment contribute to tumorigenesis, but the molecular basis of these alterations is not well defined. We characterized molecular alterations that occur during breast tumor progression using various genomic technologies including SAGE (Serial Analysis of Gene Expression) for gene expression profiling, SNP (Single Nucleotide Polymorphism) arrays and array CGH (Comprehensive Genomic Hybridization) for analyzing genetic changes, and MSDK (Methylation Specific Digital Karyotyping) for the characterization of DNA methylation patterns. In addition to analyzing the tumor epithelial cells we also characterized all major cell types that compose normal breast tissue and *in situ* and invasive breast carcinomas. Using these approaches we determined that gene expression and epigenetic alterations occur in all cell types during breast tumor progression, while clonally selected genetic changes are restricted to tumor epithelial cells. A significant fraction of abnormally expressed genes encode secreted proteins and receptors implicating a role for abnormal autocrine/paracrine signaling in breast tumorigenesis. Based on these findings we suggest that interactions among epithelial and stromal cells play a role in tumorigenesis and targeting these interactions may be exploited for cancer therapy and prevention.

S2

New perspectives on neoplasia and the RNA world

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Key tenets of modern biology are the central place of protein in cell regulation and the flow of genetic information from DNA to RNA to protein. However, it is becoming increasingly apparent that genomes are much more complex than hitherto thought with remarkably complex regulatory systems. The notion that the fraction of the genome involved in coding protein is all that matters is increasingly being questioned as the roles of non-coding RNA (ncRNA) in cellular systems becomes recognised. The RNA world, including microRNA (miRNA), small inhibitory RNA (siRNA) and other RNA species, are now recognised as being crucial for the regulation of chromatin structure, gene expression, mRNA processing and splicing, mRNA stability and translational control. Furthermore such ncRNA systems may be perturbed in disease states and most notably in neoplasia, including in haematological malignancies. Here the burgeoning evidence for a role of miRNA in neoplasia and other diseases is reviewed and the importance of understanding the RNA world emphasised. Finally the enormous significance of alternative splicing is discussed with reference to our own research on septin biology.

S4

Precursor lesions in the breast

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It is well established that ductal carcinoma in situ (DCIS) behaves as a true precursor lesion in the breast. It is, however, also clear that this is a heterogeneous spectrum of disease with differing biology, genetics and clinical behaviour.

There is emerging evidence that, in at least some cases, lobular in situ neoplasia (atypical lobular hyperplasia and lobular carcinoma in situ) and atypical columnar cell lesions may also behave as precursors of breast cancer. The biology and diagnosis of these lesions will be discussed.

S5

Therapeutic exploitation of the DNA repair defects in BRCA mutant tumours

{P} A Ashworth

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About one in nine women in the Western world develop cancer of the breast and at least 5% of these cases are thought to result from a hereditary predisposition to the disease. Two breast cancer susceptibility (*BRCA*) genes have been identified and mutations in these genes account for most families with four or more cases of breast cancer diagnosed before the age of 60. Women who inherit loss-of-function mutations in either of these genes have an up to 85% risk of breast cancer by age 70. As well as breast cancer, carriers of mutations in *BRCA1* and *BRCA2* are at elevated risk of cancer of the ovary, prostate and pancreas. The genes are thought to be tumour suppressor genes as the wild-type allele of the gene is observed to be lost in tumours of heterozygous carriers. Both *BRCA1* and *BRCA2* have significant roles in the maintenance of genome integrity via roles in the repair of DNA damage via homologous recombination. The specific DNA repair defect in *BRCA*-mutant cells provides opportunities for novel therapeutic approaches based on selective inhibition of functionally interacting repair pathways. Progress towards developing these 'synthetic lethal' approaches will be discussed.

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S6

Tumour specific integrins in cancer invasion: functional effects and targeting possibilities

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The active migration of tumour cells, out of their tissue of origin and into adjacent tissues of different types, which constitutes cancer invasion necessitates the involvement of adhesion receptors of the integrin family. Our group has focused on the epithelial-specific $\alpha\beta6$ heterodimer because it is undetectable on normal adult tissues but is highly upregulated on a range of carcinomas. Often detected at the leading edge of many of these cancers we have shown that $\alpha\beta6$ can promote invasive activity, through the upregulation of members of the MMP family of degradative enzymes, and that downregulation, by RNAi, results in diminished invasion. Using the yeast 2-hybrid assay to identify proteins which interact with the cytoplasmic tail of the $\beta6$ sub-unit we have identified an intracellular protein which regulates integrin internalisation and shown that this process is a fundamental component of the invasive phenotype.

However $\alpha\beta6$ is not only of interest because of its functional role in cancer invasion. Expressed at the cell surface of neoplastic cells, and being highly upregulated compared to normal tissue, the $\alpha\beta6$ molecule offers potential as a tumour-specific target that can be used both for imaging and therapy. Using NMR structural data, which has shown the necessity for an α -helix c-terminal to the RGD motif, we have developed potent peptide antagonists of $\alpha\beta6$ (20 mers) and used them to image $\alpha\beta6$ -positive tumours in animals. Our results indicate the feasibility of this type of approach as a means of targeting invasive, aggressive tumours.

S7

Investigation of signalling pathways in human endothelial cells using Bayesian Gene Regulatory Networks

{P} C G Print

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Endothelial cells assist with the co-ordination of angiogenesis and vascular regression, and are gate-keepers of inflammation. To understand the molecular signals that underlie these functions, we have used siRNA to inactivate 355 signalling molecules and transcription factors in endothelial cells. We then used gene arrays to examine the effects of these inactivations, and to catalogue the changes that endothelial cells undergo over time when they are treated with drugs or exposed to stressful or inflammatory conditions. In collaboration with mathematicians, we have used this data to infer Bayesian endothelial cell gene regulatory networks. Gene regulatory networks are best described as circuit diagrams showing putative cause and effect relationships between signalling molecules and putative regulatory 'hubs' from which numerous downstream genes are regulated. This work has provided interesting insights into endothelial cell biology, in particular into the response of endothelial cells to inflammation, stress and the anti-hyperlipidaemia drug fenofibrate

Abstract S8 is shown overleaf

Role of vascular receptor tyrosine kinases during angiogenesis, tumor progression, and metastasis

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Three classes of receptor tyrosine kinases, the VEGF receptors, the Tie-2 receptor, and the Eph receptors have been identified as critical, rate limiting signaling molecules for developmental and tumor angiogenesis. The VEGF/VEGFR system is hierarchically high and controls early events of angiogenesis induction and capillary sprouting associated with the growth of blood vessels (VEGF-A, -B) and lymphatic vessels (VEGF-C, -D). Correspondingly, VEGF is involved in the progression of almost all solid human tumors. VEGF neutralizing therapies (Bevacizumab [Avastin]) have received clinical approval for the first line therapy of colorectal cancers in combination with chemotherapy in 2004 and have proved effective for other tumors including lung tumors and mammary tumors as well. A number of small molecular weight VEGFR inhibitors are in advanced clinical trials and are expected to receive clinical approval in 2006/7. The Angiopoietin/Tie2 (Ang-1 and Ang-2) system controls later steps of the angiogenic cascade regulating vessel maturation, endothelial cell survival, and vessel quiescence. Translational exploitation of the Ang/Tie system is still in its early stages and follows similar avenues as VEGF/VEGFR inhibitory strategies with Angiopoietin neutralizing antibodies (Ang-2 trap) and small molecular weight Tie2 receptor inhibitors. The latest identified class of vascular receptor tyrosine kinases, the Eph receptors, was originally identified as neuronal path finding molecules that controls the outgrowth of axons by interacting with their membrane bound ephrin ligands (A class ephrins = GPI anchored; B class ephrins = transmembrane molecules). Surprisingly, genetic loss-of-function experiments revealed that EphB/ephrinB interactions exert critical, rate-limiting functions during vascular assembly and arteriovenous differentiation. As such, the receptor EphB4 and its ligand ephrinB2 have been identified as the first arteriovenously expressed receptor-ligand system with EphB4 being preferentially expressed by venous endothelial cells (EC) and ephrinB2 being expressed by arterial EC. A growing list of Eph receptors and ephrin ligands has also been identified in tumors. Eph/ephrin expression by tumor cells (TC) points towards a role of the Eph/ephrin system in controlling malignant cell interactions with the host microenvironment that control tumor progression and metastatic dissemination. We have performed functional experiments in an experimental A375 melanoma model which indicated that perturbation of bi-directional EphB4/ephrinB2 signaling by overexpression of dominant-negatively acting soluble monomeric EphB4 (sEphB4) inhibits tumor growth. This dramatic growth inhibitory effect was observed despite the fact that sEphB4 exerted only mild effects on TC and EC. TC proliferation and soft agar colony formation were not affected. Instead, sEphB4 overexpressing A375 tumor cells had altered adhesive properties as evidenced by their perturbed ability to organize in three dimensional spheroids. Likewise, total microvessel density in sEphB4 overexpressing tumors was only moderately reduced. Yet, the combined targeting of the tumor and the vascular compartment could massively inhibit tumor progression even if sEphB4 elicited only subtle effects on TC and EC. These findings suggested that bi-directional EphB/ephrinB signaling may regulate multiple cell-cell interactions between the TC compartment and the stroma compartment. We therefore hypothesized that EphB/ephrinB interactions control not just EC-EC and TC-TC interactions, but also TC-EC interactions that may play a role during tumor progression and metastatic TC dissemination. We consequently, established sensitive luciferase based metastatic cell tracing techniques and studied the trafficking of WT and EphB4 expressing A375 cells in the circulation. Expression of EphB4 by TC altered the cells' distribution pattern in the circulation and affected site-specific metastasis. Collectively, the data show that bi-directional EphB/ephrinB interactions control multiple cell-cell interactions that control angiogenesis and tumor cell progression including metastatic dissemination.