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P1

METHOD TO DETERMINE INTERNALIZATION OF MONOCLONAL ANTIBODY BR96 INTO TUMOUR CELLS

Mr Amir Iranpour, Prof Bo Baldetorp, Prof Hans-Olov Sjogren, Prof Sven-Erik Strand, Prof Jan Tennvall
Institute of Clinical Medicin , Department of Oncology, Lund University, Sweden
Intitute of Experimental Medical Research, Department of Tumour Immunology, Lund University, Sweden
Institute of Clinical Medicin, Department of Radioation Physics, Lund University, Sweden

Amir.Iranpour@onk.lu.se

Introduction. Earlier studies on human breast cancer cell line H3396, using confocal microscopy (CM) indicate that the mAb BR96 internalizes when bound to the Lewis Y antigen. Since, flow cytometry (FCM) enables qualitative measurements on thousands of individual cell, this technique was investigated for analysis of internalization.
Material and Methods: A rat colon-tumour cell line H1D2-WT, expressing Lewis Y antigen was used. BR96 and anti-MHC-I (negative control) antibodies were both conjugated with fluorescein isothiocyanate (FITC). Corresponding anti-IgG antibody was conjugated with phycoerythrin (PE) and used for detection of non-internalized antibody at the cell surface. FCM analysis was performed after 0, 1, 2, and 4 hours of incubation with the FITC conjugated mAbs and subsequent binding of anti-IgG mAb to all cells. Proportion of antibody remaining at the cell surface at various time points was analyzed as the ratio of PE/FITC.
Results. Proportion of anti-BR96 antibody remaining at the cell surface declined after 1-2h, whereas anti-MHC-I antibody remained constant. These findings are consistent with an internalization of anti-BR96 antibody within 1-2h. After 4 hours a significant loss in FITC fluorescence intensity was detected indicating degradation of BR96.
Conclusions. Present data suggest that qualitative FCM may be useful for demonstrating antibody internalization. We have indications of the existence of subpopulations with different degrees of internalization. Next step will be to quantify the degree of internalization by FCM, CM and electron microscopy autoradiography (EMARG).

P2

SCREENING METHOD OF SHORT TERM IN VITRO ASSAY FOR ANTI-TUMOR PROMOTERS

Ms Harukuni Tokuda, Dr Fumio Enjo, Dr Takao Konoshima, Dr Midori Takasaki, Ms Ayako Kumagai, Ms Hiroyuki Kushida, Dr Hoyoku Nishino
Dept. Mol. Biochem., Kyoto Prefectural University of Medicine, Kyoto, Japan
Dept. Pharm., Chiba Science University, Chiba, Japan
Dept. Eng., Kansai University, Osaka, Japan

htokuda@koto.kpu-m.ac.jp

The application of a new screening procedure which utilizes the synergistic effect of short-chain fatty acids and tumor promoting diterpene esters enabled rapid and easy detection of tumor promoters and anti-tumor promoters using human lymphoblastoid Raji cell. Several tumor promoters are able to activate the Epstein-Barr virus early antigen (EBV-EA) induction in both latently and productively infected cells, Raji cells. The EBV activating effect of tumor promoters can be synergistically enhanced through protein kinase c pathway if they are administred together with n-butyric acid. N-butyric acid is itself a potent activator of infected virus in Raji cell when used at higher concentrations. Interestingly, the EBV-EA activation induced by 12-O-tetradecanoylphorbol-13-acetate(TPA), can be diminished by the addition of chemopreventive agents, ascorbic acid, curcumin and herbal plant, Tabebuia avellanedae including naphthoquinones to the culture medium. These samples are an inhibitor of tumor promotion, chemopreventive agents. In this present study, we tried to arrange the screening and detection of chemopreventive agents.

P3

MUTATION ANALYSIS OF THE HIF-1α OXYGEN DEPENDENT DEGRADATION DOMAIN IN INVASIVE BREAST CANCER

Marije M. Vleugel, Astrid E. Greijer, Elsken Van der Wall, Paul J. Van Diest
Department of Pathology and Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
Division of Internal Medicine and Dermatology, University Medical Center Utrecht, Utrecht, The Netherlands
Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands

p.j.vandiest@azu.nl

Purpose. Hypoxia Inducible Factor-1 (HIF-1) is an important transcription factor that stimulates tumour growth and metastases via several pathways. Activation of HIF-1 depends on the presence of its a-subunit. Hypoxia increases HIF-1α levels by inhibiting prolyl-hydroxylase mediated hydroxylation and thereby preventing proteosome degradation. However, various other mechanisms might contribute to HIF-1α expression such as mutation of the oxygen dependent domain (ODD), which prevents binding of prolyl-hydroxylases. Therefore, the presence of ODD mutations was evaluated as a possible explanation for diffuse HIF-1α protein expression often seen in invasive breast cancer.
Methods. From a group of 200 primary breast cancers, 24 strong diffusely HIF-1α positives were identified by HIF-1α immunohistochemistry. DNA from these tumours was extracted from microdissected paraffin material and, after nested PCR, sequence analysis was performed to detect hif-1α ODD mutations. Additionally, five perinecrotically HIF-1α positive breast cancers were analyzed as controls.
Results. All 24 diffuse and perinecrotic HIF-1α positive breast cancers showed wildtype DNA sequences in the ODD domain.
Conclusions. No mutations seem to occur in the ODD of HIF-1α; in HIF-1α overexpressing invasive breast cancer, which rules ODD mutations out as a possible explanation for the diffuse HIF-1α expression pattern often seen in this cancer.

P4

PKB (AKT) ACTIVITY IS IMPORTANT FOR HIF-1α STABILIZATION IN EARLY RESPONSE TO HYPOXIA

E.H. Gort, T.L.P. Derks van der Ven, E. van der Wall, P.J. van Diest, A Shvarts
Department of Pathology, University Medical Center Utrecht, The Netherlands
Department of Oncology , University Medical Center Utrecht, The Netherlands

Introduction. Oxygen deprivation is one of the hallmarks of solid tumors and induces a broad cellular response. Cells adapt differentially to early hypoxia compared to prolonged hypoxia. Early response serves cell survival, whereas late response rather concerns cell cycle arrest and apoptosis. The Hypoxia Inducible Factor 1 alpha (HIF-1α) gene is a key regulator of early as well as late hypoxic responses. HIF-1α is regulated by an oxygen sensing system involving the E3-ligase Von Hippel-Lindau protein (pVHL). Under normoxic circumstances pVHL binds to HIF-1α in an oxygen-dependent fashion and HIF-1α is degraded in the proteosome. Under hypoxia HIF-1α is not bound by pVHL and HIF-1α becomes stabilized. Next to regulation by pVHL, HIF-1α is also regulated by oncogenic signaling involving proteins like Protein Kinase B (PKB or AKT) and Mitogen Activated Protein Kinase (MAPK).
Materials and Methods and Results. We show in a number of cell types that stabilization of HIF-1α after a short hypoxic period (1-6 hours) is largely dependent on PKB activation. After longer periods of hypoxia HIF-1α protein levels are restored compared to hypoxic response in cells with activated PKB. The impaired HIF-1α stabilization was due to decreased protein synthesis of HIF-1α and not to protein degradation. Contribution of PKB activity to the expression of HIF-1α target genes after short hypoxic stimuli is being analyzed by quantitative RT-PCR.
Conclusion. We conclude that PKB might be especially important for early hypoxic response. Effects of PKB signaling on HIF-1α activity might not be necessary in tumors with prolonged hypoxia, although we cannot exclude its contribution to the late hypoxic response.

P5

HIGH FREQUENCY OF HIF-1α OVEREXPRESSION IN BRCA1 GERMLINE MUTATION RELATED INVASIVE BREAST CANCER

Petra van der Groep, Alwin Bouter, Fred H. Menko, Elsken van der Wall, Paul J. van Diest
Department of Pathology, University Medical Center Utrecht, The Netherlands
Department of Internal Medicine, University Medical Center Utrecht, The Netherlands
Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands

Recent studies have revealed that BRCA1 germline mutation related breast cancer bears a basal epithelial phenotype (CK5+/EGFR+/ER-/PR-/ErbB2-) with frequent areas of necrosis. Increased levels of HIF-1α have been found in cells around areas of necrosis in sporadic breast cancer. The aim of this study was therefore to examine the expression of HIF-1α in BRCA related breast cancers in relation to necrosis to determine if HIF-1α expression fits within the BRCA1 basal phenotype.
Thirty two invasive hereditary breast cancers were stained by immunohistochemistry for HIF-1α, regarding only cells with completely and darkly stained were regarded as positive, as well as for EGFR, ER, PR, and ErbB2 (paraffin sections). Presence of necrosis was noted.
19/32 (59%) cases showed necrosis. 29/32 (91%) of cases showed HIF-1α overexpression. HIF-1α correlated negatively with ER, PR, and ErbB2, and positively with EGFR.
In the subgroup of proven BRCA1 germline mutation carriers cases, 17/18 (94%) showed HIF-1α overexpression, and the BRCA2 mutated case was HIF-1α negative.
We conclude that the BRCA1 germline mutation related breast cancers show a high frequency of HIF-1α overexpression, largely related to presence of necrosis. In view of the positive associations between HIF-1α overexpression and EGFR and the negative associations between HIF-1α overexpression and ER, PR and ErbB2, HIF-1α overexpression seems to further characterize the basal phenotype of BRCA1 germine mutation related invasive breast cancer.

P6

COMPREHENSIVE EXPRESSION PROFILING OF THE HUMAN SEPTIN GENE FAMILY

SE Hilary Russell, Ken Jung, Ken J Hillan, Peter A Hall
Centre for Cancer Research and Cell Biology, Queen's University Belfast, City Hospital, Lisburn Road, Belfast BT9 7AB, UK
Genentech Inc, 1 DNA Way, South San Francisco. CA 94080, USA

The septins are a family of conserved GTP-binding proteins thought to oligomerise and act as a regulatable scaffold for recruitment of other proteins. A key feature of the 12 mammalian septins is the existence of multiple splice variants. Septins are involved in diverse processes including vesicle trafficking, apoptosis, infection and remodelling of the cytoskeleton. Recent reports link septins to disease processes including neoplasia and neurodegenerative disorders. We have undertaken a comprehensive study of septin gene expression by DNA microarray methods in 7579 samples of normal, diseased and tumour human tissues. We show that septins are expressed in all tissue types but some show high expression in lymphoid (SEPT1, 6, 9 and 12) or brain tissues (SEPT2, 3, 4, 5, 7, 8 and 11). The analysis indicates that although some septin isoforms may be highly expressed in brain, other splice variants are not. For example, SEPT8_V2 and V1, 1* and 3 are highly expressed in brain and cluster with SEPT2, 3, 4, 5, 7 and 11. However, a probe set specific for SEPT8_V1 with low brain expression, clusters away from this set. Similarly SEPT4 has lymphoid and non-lymphoid forms, SEPT2 has lymphoid and CNS forms and SEPT6 and SEPT9 are elevated in lymphoid tissues but both have forms that cluster away from the lymphoid forms. Perturbation of septin expression was widespread in disease and tumours of the various tissues examined, particularly for conditions of the CNS were alterations in all 12 septin genes were identified. This analysis provides the first description of global septin expression patterns and provides a comprehensive catalogue of septins in health and disease. This is a key step in understanding the role of septins in physiological and pathological states and provides insight into the complexity of septin biology.

P7

THE SEPTIN BINDING PROTEIN ANILLIN IS OVER-EXPRESSED IN DIVERSE HUMAN TUMOURS

PA Hall, S McDade, C Todd, M Dattani, H Grabsch, KJ Hillan, SEH Russell
Centre for Cancer Research and Cell Biology, Queen's University Belfast, City Hospital, Lisburn Road, Belfast BT9 7AB, UK
Dept of Histopathology, Leeds Teaching Hospitals NHS Trust
Academic Unit of Pathology, University of Leeds, UK
Genentech Inc, 1 DNA Way, South San Francisco, CA 94080 USA

Anillin is an actin binding protein that can form associations with septins, and has been shown to be a component of the cytokinetic ring. We assessed the anillin expression in 7579 human tissue samples and cell lines by DNA microarray analysis. The Affymetrix 222608_at probe set provided a comprehensive analysis of anillin expression and demonstrated its presence in all tissues. The median level of anillin expression was higher in tumours than normal tissues (median fold increase 2.58, 95% confidence intervals 2.19 to 5.68, $p<0.0001$) except in CNS were anillin levels were lower in tumours. These data were confirmed in an independent data set of gastric and colorectal cancer samples where anillin levels were 4.5 fold higher ($P<0.0001$) than in normal control tissues. Anillin expression was analysed during tumour progression in breast, ovarian, kidney, colorectal, hepatic, lung, endometrial and pancreatic tumours and in all tissues there was progressive increase in anillin expression from normal to benign to malignant to metastatic disease. We compared anillin with Ki67 expression using the Affymetrix 212020_s_at probe set and found a clear linear relationship between anillin and Ki67 mRNA expression (Spearmann $r \sim 0.65$ to 0.75, $p<0.0001$) except in the CNS where $r = -0.11$ ($p<0.0001$). We used a sensitive RT-PCR strategy to demonstrate that anillin mRNA is expressed in cell lines, thus validating the microarray data. We generated an anti-anillin serum and found nuclear anillin immunoreactivity to be widespread in tissues with altered patterns of expression in tumours. Anillin is thus over-expressed in diverse common human tumours and may have potential as a novel biomarker. These data also provide insight into non proliferation associated activities of anillin in tissues such as the brain where anillin levels are high in the absence of proliferation.

P8

MULTIMODALITY CHARACTERISATION OF SEPT 6 GENE EXPRESSION IN CELL LINES AND TISSUES: THE DISCOVERY OF A NUCLEAR FORM

CB Todd, M Dattani, SS McDade, KJ Hillan, PA Hall, SEH Russell
Centre for Cancer Research & Cell Biology, Queens University Belfast, University Floor, Tower Block, Belfast City Hospital, Belfast BT9 7AB
Academic Unit of Pathology, University of Leeds
Genentech Inc, 1 DNA Way, South San Francisco, CA 94080 USA

Septins are an evolutionarily conserved family of GTPases implicated in a variety of processes including cytokinesis, membrane transport and fusion, exocytosis, and apoptosis. Identified as a fusion partner of MLL in acute myeloid leukaemias, Septin 6 (SEPT6) has also been implicated in the development of neoplasia. However, only a limited analysis of the expression of SEPT6 at the transcript and protein level exists. We have characterised the genomic architecture of the SEPT6 locus and found 5 distinct SEPT6 mRNA species (v1, v2, v3, v4 and v4*) produced by alternative splicing of exons 10, 11 and 12. SEPT6 v4 and v4* are distinct transcripts encoding the same polypeptide (as is seen in other human septins). We developed an RT-PCR strategy to define the SEPT6 transcript profile of cell lines and normal human tissues and correlated these data with Affymetrix array data on 7579 human tissue samples and cell lines. In addition we generated polyclonal sera to SEPT6 peptides and characterised these by Western blotting of cell lysates and expressed SEPT6 constructs. We have thus been able to provide a multimodality comprehensive characterisation of human SEPT6 mRNA and protein expression. We find that SEPT6 transcript profile varies in different cell lines and is highest in Jurkat cells. The microarray data similarly show that SEPT6 mRNA is widely expressed but highest in lymphoid compartments. The immunohistological data similarly indicate variable widespread expression with highest levels in lymphoid cells. We find SEPT6 expression in the cytoplasm and occasionally in the nucleus. Immunofluorescence studies of cultured cells similarly shows both nuclear and cytosolic localisation. These studies provide a comprehensive characterization of the SEPT6 gene and its mRNA and protein expression in vitro and in vivo and demonstrate the existence of previously unrecognised nuclear forms of SEPT6. Further studies are aimed at functional characterisation of SEPT6.

P9

IS SEPT9 A P53 REGULATED GENE?

C Moloney, PL Hyland, SS McDade, CB Todd, PA Hall, SEH Russell
Centre for Cancer Research & Cell Biology, Queens University Belfast, University Floor, Tower Block, Belfast City Hospital, Belfast BT9 7AB

Septin 9 [SEPT9] is an evolutionarily conserved GTPase and several lines of evidence suggest a role for derangement of SEPT9 expression and function in the development of neoplasia. This role is complicated by the fact that SEPT9 undergoes extensive alternative splicing and has at least 18 splice variants. Previously we have shown that SEPT9 is not mutated but undergoes altered patterns of expression. In particular with the ratio of SEPT9_v4 and SEPT9_v4* change in neoplasia. Furthermore, SEPT9_v4 and SEPT9_v4* transcripts appear to be translated at different efficiencies in vitro. The regulation of SEPT9 transcription is not yet elucidated. As part of our studies in this area bioinformatics analysis of SEPT9 upstream regulatory regions show the existence of numerous potential p53 binding sites within particularly in the upstream region of SEPT9_v4*. To investigate the potential involvement of p53 in the regulation of SEPT9 protein expression, p53 null and wild-type cells were treated with adriamycin and the levels of p53, p21waf1 and SEPT9 protein were examined by Western analysis. Following treatment with 1.5 μM adriamycin, the level of SEPT9_v4 protein were shown to decrease as p53 levels increased with time. Time course treatments of p53 null and wild-type cells with adriamycin at intervals from 0-6 hours also showed the same result. These results suggest that p53 may be a transcriptional repressor of SEPT9_v4 protein. We are currently defining the changes in SEPT9 mRNA by quantitative RT-PCR analysis and aim to test the function of the putative p53 responsive elements in the SEPT9 regulatory regions. It may be that the enhanced levels of SEPT9 seen in neoplasia are a consequence of loss of function of p53.

P10

DETECTION OF CK-19 MRNA-POSITIVE CELLS IN THE PERIPHERAL BLOOD OF BREAST CANCER PATIENTS WITH HISTOLOGICALLY AND IMMUNOHISTOCHEMICALLY NEGATIVE AXILLARY LYMPH NODES

Dr E.N. Stathopoulos, Dr E. Sanidas, Dr M. Kafousi, Dr D. Mavroudis, Dr J. Askoxylakis, Dr V. Bozionelou, Dr M. Perraki, Dr D. Tsiftsis, Dr V. Georgoulias
Department of Pathology, University General Hospital of Heraklion, Greece
Department of Surgical Oncology, University General Hospital of Heraklion, Greece
Department of Medical Oncology, University General Hospital of Heraklion, Greece

georgoul@med.uoc.gr

Purpose. To investigate the incidence of direct hematogenous spread of cancer cells in patients with early stage breast cancer by studying the presence of occult tumor CK-19 mRNA+ cells in the peripheral blood in relation to the status of sentinel (SLNs) and axillary lymph nodes (ALNs).
Materials and Methods. SLNs and ALNs from 111 patients with operable stage I-II breast adenocarcinoma were evaluated for the presence of tumor cells by H&E staining and, if negative, by immunohistochemistry (IHC) using an anti-cytokeratin-19 (CK-19) antibody. Peripheral blood was also analyzed for the presence of CK-19 mRNA+ cells by nested RT-PCR, before the initiation of adjuvant treatment and in CΚ-19mRNA+ patients following the completion of adjuvant chemotherapy and hormonal treatment.
Results. After both H&E staining and IHC analysis, 29 (26%) patients were axillary lymph node negative (N0). In 78(70%) patients H&E staining and in 4(3.6%) IHC analysis revealed tumors cells and were considered as axillary lymph node positive (N+). Peripheral blood CK-19 mRNA+ cells were detected in nine (31%) out of 29 N0 and in 31 (38%) out of 82 N+ patients (p=0.5) before any adjuvant treatment. Adjuvant chemotherapy and hormone treatment resulted in the disappearance of the CK-19 mRNA+ cells in all N0 patients and in 15 out of 31 N+ patients. After a median follow up of 40 months, all the N0 CK-19mRNA+ patients were relapse-free whereas four (13%) N+ CK-19mRNA+ patients have relapsed.
Conclusions. Direct hematogenous dissemination of occult tumor cells may occur in a substantial proportion of patients with early stage breast cancer. The prognostic implication of the detection of these cells requires long follow-up periods and further studies.

P11

DIFFERENT GENOMIC PROFILES IN GASTRIC CANCERS OF YOUNG AND OLD PATIENTS

Miss T.E. Buffart, Dr B. Carvalho, Mr E. Hopmans, Mr V. Brehm, Dr E. Klein Kranenbarg, Miss B.M. Visser, Dr B. Ylstra, Prof C.J.H. Van de Velde, Prof G.A. Meijer
Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
Dept. Surgery, Leiden University Medical Center, Leiden, The Netherlands
Micro-array Core Facility, VU University Medical Center, Amsterdam, The Netherlands

T.Buffart@vumc.nl

Introduction. Gastric cancer in young patients has been associated with a more aggressive behavior and poorer prognosis, compared to older patients. We recently demonstrated that patterns of DNA copy number alterations in gastric cancer correlated with clinical outcome (Weiss et al., Oncogene 2003; 22:1872-1879). The present study aimed to compare such copy number alterations between gastric cancers of young (<50 years) and old (³ 70 years) patients with high resolution at a whole genome scale.
Methods. DNA derived from 46 paraffin embedded gastric cancer tissue samples of 17 patients <50 years (median 43 (21-49)) and 29 patients ≥70 years (median 75 (70-83)) were analyzed by genome wide array-CGH using a BAC array of 5000 clones printed in triplicate. Patterns of chromosomal aberrations were analyzed by K-means cluster analysis of the normalized log2 tumor to normal fluorescence ratios of all (but the sex chromosomes derived) clones using TMEV software (www.tigr.org/software). Cluster membership was correlated to age.
Results. K-means cluster analysis of the array-CGH results revealed two clusters with different genomic profiles that correlated significantly with age (p=0.003). Since gastric cancer cases from cluster one, with mainly young patients, showed a significantly higher incidence of lymph node metastasis compared to cluster two, with mainly elderly gastric cancer patients (P=0.04), a sub-group analysis was performed with lymph node positive cases only (young: n=12; elderly: n=20). Again, K-means cluster analysis yielded two clusters of tumors with different genomic profiles, where cluster membership correlated significantly with age (P=0.008).
Conclusion. Gastric cancers of young and old patients have different genomic profiles, and this is independent of lymph node status. This may well reflect different pathogenic mechanisms of the disease.

P12

DNA POLYMERASE EPSILON MUTATIONS IN COLORECTAL CANCER

Dr Yrjo Collan, Dr Qi Zhou, Dr Kati Talvinen, Dr Jari Sundström
Department of Pathology, University of Turku, Turku, Finland

yrjo.collan@utu.fi

Introduction. DNA polymerases are important in DNA synthesis and consequently active in cancer tissue. These molecules are often complicated in structure and may contain several subunits. The functions of these subunits may vary, and in addition to DNA synthesis proper, proof reading, and assistance in DNA repair are important. There is some evidence that cancer tissue may show abnormalities in DNA polymerases which may be expressed at protein, RNA, and genomic DNA level. We studied the small 55 kDa subunit of DNA polymerase epsilon in association with colorectal cancer.
Methods. We had 17 colorectal cancer DNA samples. In addition 8 control samples, isolated from the nearby normal mucosa, were evaluated. 19 different sets of intronic primers, designed to amplify the entire coding region of the 55 kDa subunit of human polymerase epsilon gene, were used in PCR amplification, using 35 cycle runs. Evidence of mutations were looked for by single strand conformational polymorphism (SSCP). Sequencing followed when abnormal patterns were detected. Both procedures were carried out in ALFexpress II DNA Sequencer, and the results compared with available data on the DNA sequence of polymerase epsilon.
Results. As in our earlier study on breast cancer, primer pair 137/138 showed an AATT deletion in intron 18. This was found in 3 cancer samples, and in one corresponding control sample. Because the mutation was found in the intronic area, the mutation could not change the structure of the protein product. A G-A transition in intron 14 was also found. In two cancer samples there was a mutated triplet in exon 7, which was also found in one corresponding control. Single triplet transitions could be found three locations in the reading frames. Several of these changes proposed amino acid changes in the translated protein. Potential single base polymorphisms were found in introns 6 and 13.

Conclusions. Our study suggests that different types of cancer may contain cancer specific changes in the gene of human polomerase epsilon. These changes can take place in intronic parts of the gene, but also exonic sequences showed changes suggesting amino acid changes in the protein product.

P13

A STUDY OF THE EXPERIENCE OF CACHEXIA IN PATIENTS WITH CANCER AND THEIR SIGNIFICANT OTHERS

Miss Joanne Reid, Prof Hugh McKenna, Dr Donna Fitzsimons, Dr Tanya McCance
University of Ulster, Belfast City Hospital, NIPEC

j.reid1@ulst.ac.uk

Introduction. This study is undertaken as a PhD study funded by the R&D office of the DHPSS of Northern Ireland. Cancer cachexia is operationally defined, within this study as, the involuntary weight loss of more than 10% (Ottery, 1995; Brown et al, 2001) of pre-morbid body weight (Fainsinger, 2004) within the last six months (Ottery, 1995; European Institute for Oncological and Immunological Research, 2002) resulting from tumour induced metabolic alterations (MacDonald et al, 2003; Younes & Noguchi, 2000; Tisdale, 1997).
Research aim. To explore the lived experience of cachexia in patients with cancer and their significant others.
Research approach. A qualitative phenomenological research approach is being employed within this study, specifically Heideggerian phenomenology. This focuses on hermeneutics, which can be described as the interpretation of meaning. A narrative methodology is being used within this interpretative approach that focuses on participants telling their stories. The compatibility of using narratives within an interpretive approach has also been supported within current literature.
Methodology. The method of data collection is the unstructured interview. Data analysis is a two-staged process, combining the use of narrative analysis and the in-depth analysis of discrete narratives. Given the specified aim and method of this study, purposive sampling is the most appropriate sampling strategy. The study is being conducted within the Regional Cancer Centre for Northern Ireland located at the Belfast City Hospital. Permission to undertake the study has been gained from the Ethics Committee of Northern Ireland and the Research Governance Framework within the hospital trust whilst the University of Ulster act as sponsor.
Results & Conclusion. The pilot study is completed and the main study is currently underway. Preliminary results reflect the biological, psychological and social dimensions of the experience of cachexia in patients with cancer and their significant others.

P14

THE MOLECULAR BASIS OF REGIONAL PREDISPOSITION TO BLADDER CARCINOGENESIS

Dr Sharon Doherty, Prof Stephanie McKeown, Dr Jesus Lopez, Dr Ian Walsh, Prof Valerie McKelvey-Martin
University of Ulster, School of Biomedical Sciences, Cancer & Aging Group, Co Londonderry, UK
University of Southern Queensland, Department of Mathematics and Computing, Toowoomba, Queensland, Australia
Belfast City Hospital, Urology, Belfast, UK

sc.doherty@ulster.ac.uk

Introduction. Clinical studies have established that over 70% of primary tumours arise in the area around the ureteric orific(Melicow, 1974; Page et al, 1978). In this study microarray technology was employed to provide a genomic approach to explore the molecular mechanisms leading to this phenomenon and help elucidate genes whose differential expression may influence primary tumour development.
Methods. Normal mucosa was obtained from the ureteric orifices (UO) and the dome (D) of the bladder of 33 male patients (26-81 years age) who had no evidence of malignancy on cystoscopy. Total RNA was isolated from each individual sample using Qiagen Mini-kit. The UO and dome samples were then pooled, the mRNAs labelled with Cy5 and Cy3 and these probes hybridized to the gene chip (UniGEM 2.0, Incyte Genomics Inc.). Confirmation of the microarray results was obtained using RT-PCR on the original pooled mRNA samples for 4 (Cdc25B, TK1, PKM, Seladin-1) up-regulated genes and one (PDGFra) down-regulated gene.
Results and Conclusion. The cDNA microarray analysis revealed differential expression of 312 genes. Further analysis of five genes by RT-PCR supported the reliability of the micorarray analysis. While Cdc25B, TK1, PKM, and PDGFra have all been documented in various cancers little is known regarding Seladin-1's role

in this disease. This study of two anatomically distinct areas of normal human bladder may provide clues as to the predisposition to tumour development observed in the UO as compared to the dome. Finally it may also provide clues to predisposition to carcinogenesis in other epithelial tissues.

P15

A PREDICTIVE RISK-ASSESSMENT MODEL FOR DIET-RELATED CHEMICAL MIXTURES USING METALLOTHIONEIN CRYPT-RESTRICTED IMMUNOPOSITIVITY INDICES (MTCRII)

Dr Eilish Donnelly, Dr Margaret Hoper, Ms Helen Bardwell, Dr Geraldine Thomas, Prof Dillwyn Williams, Mr Paul Crowe, Prof David Phillips, Dr Stephen McCullough, Dr Glenn McCluggage, Prof Charles Campbell
Surgery, School of Medicine, Queen's University Belfast
Strangeways Research Laborstories, University of Cambridge
Section of Molecular Carcinogenesis, Institute of Cancer Research, Surre Anatomy, School of Medicine, Queen's University Belfast
Pathology, School of Medicine, Queen's University Belfast

e.donnelly@qub.ac.uk

Introduction. Humans are frequently exposed to mixtures of harmful dietary or lifestyle chemicals that may be implicated in colorectal cancer (CRC). Assessment of cancer risk is hampered by the lack of a mechanistically based predictive model. We test the hypothesis that metallothionein crypt-restricted immunopositivity indices (MTCRII) provide the scientific basis for a risk assessment model that is predictive of early CRC formation.
Methods. N-methyl N-nitrosourea (MNU) and undegraded lambda carrageenan (λCgN) were administered as model diet related chemicals to female Balb/c mice. Effects of each agent, given alone or within a MNU/ λCgN mixture, were investigated upon MTCRII and aberrant crypt foci (ACF) in murine colon. MTCRII may be detected within 6 weeks of chemical exposure and ACF develop at 20 weeks. The predictive power of MTCRII was tested against later ACF formation.
Results. MNU alone induced dose-dependent increases in MTCRII ($p<0.01$) while λCgN alone had no significant effects. Treatment by MNU/ λCgN mixtures induced significantly greater MTCRII ($p<0.01$), greater number ($p<0.001$) and size ($p<0.01$) of ACF than MNU alone. Linear correlations were observed between MTCRII and number ($r=0.732$; $p<0.01$) and size ($r=0.84$; $p<0.01$) of ACF.
Conclusion. MTCRII may detect harmful interactive effects of chemicals within a diet-related mixture and are predictive of later ACF formation in murine colon. MTCRII may provide the scientific basis for a robust colon tumour risk assessment model, suitable for testing of chemical mixtures.

P16

CYTOCHROME P450 1B1 PROTEIN EXPRESSION IN RAT OESOPHAGEAL TUMOURIGENESIS

Miss Andrea Knipe, Dr Marie McIlory, Dr Pramod Bonde, Dr Carlos Menzies, Dr Chrisitine Swarbrick, Dr Tracy Robson, Prof David Hirst, Prof Stephanie McKeown, Dr Jim McGuigan, Dr Charles Campbell
University of Ulster, Coleraine, UK
Department of Surgery, Queen's University Belfast, UK
Department of Pathology, Queen's University Belfast, UK
Department of Pharmacy, Queen's University, Belfast, UK
Royal Victoria Hospital, Belfast, UK

AH.Knipe@ulster.ac.uk

Introduction. Cytochrome P450 1B1 (CYP1B1) protein is expressed in tumours but not in normal tissues. CYP1B1 activates carcinogens, including dimethylbenz[a]anthracene. It also hydroxylates 17b-estradiol to 4-hydroxyestradiol, a more potent carcinogen than estrogen. Epidemiological evidence suggests production of 4-hydroxyestradiol by CYP1B1 may be responsible for the initiation or progression of cancers in humans. To investigate if CYP1B1 could contribute to carcinogenesis, CYP1B1 protein expression was examined in the morphologically distinct stages of oesophageal carcinoma development.
Methods. Sprague-Dawley rats received surgery to induce gastric or duodenal reflux. Oesophageal sections were stained for CYP1B1 using a modified amplified fluorescein tyramide protocol.
Results. Normal oesophageal structures showed no immunoreactivity for CYP1B1. All hyperplastic epithelium showed moderate or strong cytoplasmic

CYP1B1 immunoreactivity. CYP1B1 immunoreactivity was absent from squamous dysplasia and Barrett's oesophagus. CYP1B1 immunoreactivity was observed in one of four dysplastic Barrett's oesophagus lesions and 87.5% of squamous cell carcinomas (SCC) in situ. An association between CYP1B1 expression in the lamina propria and the presence of inflammation was also observed.
Conclusions. These results support the evidence that CYP1B1 protein is tumour specific. It was absent from normal rat oesophageal tissues and present in SCC in situ, the earliest form of SCC. Absence of CYP1B1 from squamous dysplasia suggests CYP1B1, or the mechanisms which lead to its protein expression, may be linked to the progression from high-grade dysplasia to carcinoma in situ. The aim of this study was determine if CYP1B1 protein was expressed during oesophageal tumourigenesis. We have shown CYP1B1 is expressed in hyperplasia and SCC in situ, where increased production of the mutagen 4-hydroxyestradiol by CYP1B1 may provide a mechanism for tumour initiation and progression. In addition we have shown the first evidence that inflammatory infiltrate induces CYP1B1 protein expression which merits further investigation.

P17

PARAMETERS DERVIED FROM THE FAST FOURIER TRANSFORM ARE PREDICITIVE FOR THE RECURRENCE OF BASAL CELL CARCINOMA

Prof Konradin Metze, Dr Valcinir Bedin, Dr Randall L. Adam, Prof Maria L Cintra, Prof Elemir M de Souza, Prof Neucimar J Leite.
Department of Pathology, Faculty of Medicine, State University of Campinas, Brazil
Department of Internal Medicine , Faculty of Medicine , State University of Campinas, Brazil
Institute of Computing, State University of Campinas, Brazil

kmetze@fcm.unicamp.br

Introduction. Basal cell carcinomas usually follow a rather benign clinical course, some however, recur after treatment causing local tissue destruction. The aim of our study was to investigate whether texture analysis based on the Fast Fourier Transform of the nuclei in routinely stained histologic sections could be helpful to identify patients with an increased risk for recurrence.
Material and Methods. Our study was based on 98 patients with surgically excised basal cell carcinomas and a follow-up of at least 60 months. Digitalized images of 100 nuclei captured at the invasion front of routinely HE-stained paraffin sections were analyzed. All nuclei were rotated in a standardized manner. A special algorithm was used in order to diminsh the concentric (Airy) rings at the nuclear edges in the frequency domain. We calculated the inertia for each 20-degree sector of the FFT image.
Results. In univariate Cox-analyses the most important prognostic factor was the difference of the inertia values of the two sectors neighbouring the longest chord of the nucleus. Increasing differences indicated less homogeneity of the nuclear chromatin, and this was related to an increased probability of tumor recurrence. This variable remained as an independent prognostic parameter in an multivariate Cox-regression when tested together with the two well known prognostic factors "histologic tumor type" and "nearest distance to the resection margin".
Conclusions. Texture analysis by FFT is suitable to detect subtle chromatin changes predictive for the recurrence in routine histologic preparations of basal cell carcinoma. Basal cell carcinomas at risk for recurrence are characterized by a more inhomogeneous chromatin texture in HE sections.

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P18

TEXTURE ANALYSIS OF AGNOR STAINED NUCLEI IN LUNG CANCER

Prof Konradin Metze, Mrs Ana C S Piaza, Mr Adilson A Piaza, Dr Randall L Adam, Prof Neucimar J Leite
Department of Pathology, Faculty of Medicine, State University of Campinas, Brazil
Institute of Computing , State University of Campinas, Brazil

kmetze@fcm.unicamp.br

Introduction. The prognostic relevance of of silver-stained nucleolar organizer region (AgNOR) proteins has been documented for many neoplasias.Their use for diagnostic purposes, however, is still under debate. According to the cell type, the AgNOR configurations may vary considerably. The aim of our study was to investigate whether texture analysis of AgNOR stained

nuclei could differentiate between two types of lung cancer, adenocarcinoma and squamous cell carcinoma comparing the approximate entropy method with other types of texture analysis.
Material and Methods. We studied paraffin sections from 20 patients with adenocarcinoma and 14 patients with squamous cell carcinoma of the lung .AgNOR staining was performed according to the guidelines of the Committee on AgNOR Quantification. We acquired gray-scale transformed digitalized images of 100 AgNOR-stained nuclei per patient. Pincus' approximate entropy (ApE) measures information on the complexity of both deterministic and random processes by calculating the probability that runs of patterns that are close to each other will remain close in the next incremental comparisons. For the one-dimensional ApE analysis 2D images were transformed into 1D signals by peel-off-scanning. For the bidimensional ApE-analysis we used gliding boxes. Furthermore fractal dimensions, Shannon´s entropy , integrated opital density and granulometric features based on h-basin analysis were calculated. Linear discriminant analysis was used in order to test the diagnostic value of the different variables.
Results. Bi-dimensional ApE was the most powerful discriminative variable, classifying correctly 70,6% of the cases, followed by the entropies of the original images or their morphologic residues. Fractal dimensions or mean optical density were not able to discriminate between the two entities.
Conclusions. Although a complete separation between the two entities was not possible, bidimensional approximate entropy was the most powerful tool for the characterization of the AgNOR configurations.

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P19

MICROARRAY-CGH ANALYSIS OF GASTRIC ADENOMAS: COMPARISON BETWEEN INTESTINAL-TYPE AND PYLORIC GLAND ADENOMAS

Dr B. Carvalho, Mr T. Mons, Miss T.E. Buffart, Dr R.M. Reis, Dr M. Vieth, Prof M. Stolte, Miss C. Moutinho, Dr B. Ylstra, Prof F. Carneiro, Prof G.A. Meijer
Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
Life and Health Sciences Research Institute, Health Sciences School, University of Minho, Portugal
Klinikum Bayreuth, Bayreuth, Germany
Institute of Pathology and Molecular Immunology of University of Porto - IPATIMUP, Porto, Portugal
Microarray core facility, VU University Medical Center, Amsterdam, The Netherlands

b.carvalho@vumc.nl

Introduction. Chromosomal instability (CIN) is the most prevalent type of genomic instability in gastric tumours, but its role in malignant transformation of the gastric mucosa is still obscure.
The purpose of the present study was to apply microarray-CGH to analyse DNA copy number changes in gastric adenomas. By comparing the aberrations observed in the adenomas with the ones described in carcinomas we aimed to clarify which alterations contribute to the progression from adenoma to carcinoma. Furthermore, intestinal-type and pyloric gland adenomas, were analysed in order to clarify if these entities represent distinct genetic pathways of gastric carcinogenesis.
Material and methods. We analysed 29 gastric adenomas, including 12 intestinal-type adenomas and 17 pyloric gland adenomas, by microarray-CGH using a full genome coverage array with an average resolution of approximately 1 Mb.
Results. In all the cases analysed the most consistent copy number changes observed were gains of chromosomes 8, 9q, 11q and 20 and losses of chromosomes 5q, 6, 10 and 13. The observed gains of chromosome 11q, 20q and losses of chromosome 5q were consistent to what was previously known in adenomas. Gains of 8q and 20q as well as losses of 5q, 6q and 13q are known to occur frequently in gastric carcinomas pinpointing for an early role of genes mapping at these regions in gastric cancer development.
Losses of 13q were only found in the intestinal-type of adenomas and gains of 20q were only found in the pyloric gland adenomas. These differences were statistically significant.
Conclusion. We observed that both types of adenomas contain chromosomal aberrations that are known to be present in carcinomas. These alterations likely represent early events in gastric carcinogenesis. Moreover, pyloric gland adenomas show a significant 20q gain, which is known to be associated with a more aggressive clinical behaviour.

P20

HISTONE DEACETYLASE INHIBITION INDUCES ALTERED GENE EXPRESSION PROFILES IN PROSTATE CANCER

J.A. Orr, D. McKenna, V. McKelvey-Martin, PW. Hamilton
Queen's University Belfast, Department of Pathology, Royal Victoria Hospital
University of Ulster, Centre of Molecular Biology, Coleraine

orr_jenny@hotmail.com

Introduction. Chromatin reorganisation associated with reversible epigenetic modifications is considered responsible for changes in gene expression observed during prostate cancer progression. Histone acetylation is an important epigenetic modification promoting an 'open' chromatin configuration and transcriptional activation. Trichostatin A (TSA), an inhibitor of HDAC activity, has been associated with a global hyperacetylation that may lead to the disruption of chromatin phenotype and the transcription of a selection of genes that inhibit tumour growth. We have already shown that at concentrations of >50ng/ml, TSA induces apoptosis and G2M cell cycle arrest in these cell lines. However, even at low doses of TSA, which have no obvious impact on the cell cycle or apoptosis (sub-lethal doses), subtle changes in chromatin phenotype are induced. The aim of this study was to establish the underlying alterations in gene expression in normal prostate cell line (PNT1A) and lymph node metastatic cell line (LNCaP) induced by Trichostatin A.
Materials and Methods. Following treatment of PNT1A and LNCaP cell lines with sublethal (12ng/ml) and lethal (100ng/ml) doses of TSA, total cellular RNA was isolated from cell samples. cDNA was synthesised from extracted RNA, and control and test samples were differentially labelled with fluorescent Cy3 and Cy5 dyes in situ for direct comparison. Dye swaps were incorporated into the study design to ensure precise interpretation of expression profiles. Hybridisation of appropriately labelled cDNA to MWG Human 30K array A containing 9,984 oligonucleotide probes, was performed under optimum binding conditions. Scanning and analysis of the microarray slide was completed using GenePix and Acuity (Axon Instruments) software.
Results. Gene expression was clearly disrupted in both PNT1A and LNCaP in response to TSA induced hyperacetylation. A significant trend for up-regulation in gene expression was identified in PNT1A following high concentrations of TSA. This includes the increased expression of cell cycle genes such as a 2.27 fold increase in cyclin g2, as well as a selection of genes related to the onset of apoptosis. This is likely to be associated with increased apoptosis and cell cycle arrest seen at these concentrations. Interestingly, altered gene expression was also evident in cells treated with sublethal doses of TSA, where no cytotoxic effects of the treatment are visible. Several functional gene groups were found to be down-regulated across both cell lines in response to TSA, including a range of general transcription factors, along with genes involved in the MAP Kinase pathway. A consistent up-regulation can also be reported in both a subset of homeobox genes, and cytochrome c oxidase genes. Also important is the observation that the Kallikrein (prostate specific antigen) gene has been found to be down-regulated in both normal prostate and metastatic cell lines in response to TSA treatment.
Conclusions. Histone hyperacetylation and subsequent chromatin redistribution are associated with the disruption of gene expression profiles in prostate cancer. Disruption in gene expression occurs in the absence of cytotoxic effects which may be due to subtle chromatin phenotypic changes observed at sublethal doses of TSA, and may therefore be indicative of those genes directly or indirectly regulated by histone acetylation.

P21

ANTI-METASTATIC EFFECTS OF OLIVE OIL PHENOLICS IN COLON CANCER CELL LINE

Mrs Yumi Z.H-Y. Hashim, Dr Chris I.R. Gill, Dr Hugh McGlynn, Dr Maurizio Servili, Prof Ian R. Rowland
Northern Ireland Centre for Food and Health (NICHE), University of Ulster (Coleraine)
Dipartmento di Scienze deli Alimento, Sezione di Tecnologie e Biotecnologie degli Alimenti, Via S. Costanzo, 06126 Perugia, Italy

hashim-y@ulster.ac.uk

Introduction. Colorectal cancer (CRC), the second most common fatal malignancy in the Western world is associated with poor prognosis. The metastatic spread of tumour is the most important factor influencing cancer patients' survival. The low incidence of cancer in the Mediterranean area as compared to the Western countries was the impetus for research on effects of constituents olive oil towards cancer, in particular phenolic components. The aim of the present work was to study the anti-metastatic effect of olive oil phenolics on a colon

cancer cell line, since this important stage of tumour development has received little attention.
Materials and Methods. Matrigel Invasion Assay, cell viability and cell attachment assays were performed using HT115 adenocarcinoma cell line with different doses of olive oil phenolics extract and hydroxytyrosol (DHPEA). The experiments were carried out in duplicate and the results were means from three independent experiments analyzed using SPSS software.
Results. Olive oil phenolics extracts (OOPs) significantly inhibited HT115 invasion at 25, 50, 75 and 100 g/ml. The OOPs range tested had no effect on cell viability, but affected the ability of cells to adhere to the surface of the culture flasks. DHPEA also showed inhibition on HT115 cells invasion at over the range of concentration tested (10 – 60M). Inhibition of cell attachment was also observed.
Conclusion. OOPs and its phenolics compound, DHPEA, showed inhibited HT115 invasion. Future work would include comparison of other phenolics in the olive oil as well as to study the underlying molecular mechanism of the phenolics towards the invasion of metastatic cells.

P22

GENE COPY NUMBER CHANGES IN 56 LARYNX AND PHARYNX SQUAMOUS CELL CARCINOMAS AND THEIR CORRESPONDING METASTASES

Dr Mario Hermsen, Dr Marta Alonso Guervós, Dr José Luis Llorente Pendas, Dr César Alvarez Marcos, Dr Carlos Suárez Nieto
Department of Otolaringology, Hospital Central de Asturias, Oviedo, Spain
Department of Otolaringology, Valle del Nalón Hospital, Oviedo, Spain

mhermsen@hca.es

Introduction. Long-term exhibition of the epithelium to factors such as tobacco and alcohol induces morphologic changes, ranging from pre-neoplastic hyperplasia and dysplasia to carcinoma in situ and invasive squamous cell carcinoma (SCC). This process is accompanied by the accumulation of genetic abnormalities. Gene copy number gains and losses in 56 primary larynx/pharynx SCC and 26 metastases were investigated with multiplex ligation probe amplification (MLPA). The aim was to find gene copy number changes that may predict the presence of metastases and worse prognosis.
Methods. All patients were mine workers with a history of tobacco and alcohol use. Nine tumours were stage I, 13 stage II, 9 stage III, and 25 stage IV. Twenty-six cases were lymph node positive. The MLPA probe mixture was set up to detect copy number changes of 42 genes that according to the literature may play a role in the development and progression of head and neck SCC.
Results. Preliminary data analysis showed frequent gain and amplification of cyclin D1 and EMS1 at 11q13, in 30 primary tumors cases. Fifteen out of thirty cases occurred in patients with lymph node metastasis. In addition, loss of CDKN 2A or 2B at 9p21 occurred in 29 cases, fifteen of which in patients with lymph node metastasis. Amplification of cERBB2 (17q12) was seen in 5 cases, and p53 (17p13) deletions in 6 cases. Also these alterations seemed unrelated to metastasis.
Conclusion. The above six genes do not appear to be associated with lymph node metastasis, and can therefore not be used as prognosticators in invasive larynx and pharynx SCC. We will continue the data analysis of the primary tumors and are finalizing the experiments on the 26 corresponding metastases.

P23

RAMAN MICROSCOPY FOR THE CHEMOMETRIC ANALYSIS OF TUMOUR CELLS

A. Taleb, J. Diamond, J. J. McGarvey, J. R. Beattie, C. Toland, P. W. Hamilton
Biomedical Imaging and Informatics Research Group, Queen's University Belfast, UK
School of Chemistry & Clinical Raman Microscopy Group, Queen's University Belfast, UK

a.taleb@qub.ac.uk

Introduction. Raman spectroscopy involves the inelastic scattering of light by a material sample, leading to the generation of spectral signatures, characteristic of the chemical constituents of the sample. Confocal Raman microscopy confers the additional advantage of chemometric analysis at the cellular level. We have used the term "CytoRaman imaging". CytoRaman imaging has the potential to provide the cell biologist or pathologist with a methodology for the analysis and quantitative chemical assessment of tissue and cell samples.
Material and Methods. The aim of this work was to validate CytoRaman imaging in discriminating two well defined prostatic cell lines based on their chemometric

characteristics. The two cell lines were PNT1A (immortalised normal prostate cell line) and LNCaP (malignant cell line derived from prostate metastases). These cultured cells were fixed in ethanol, and washed twice in double distilled water, and cytospun onto quartz slides. The spectra from 15 LNCaP and 14 PNT1A cell type were acquired, covering the spectral region from 735 to 1765 cm-1 at 633 nm laser wavelength using a confocal Raman microscope (JY Labram HR). On average, 40 spectra per cell were acquired, with a step of 1.6 um in X and Y. The confocal aperature was set to 100um and a 600 g/mm grating was used, to optimise spatial and spectral resolution.

Results. The comparison of spectra from the two cell lines shows subtle but repeatable differences. The ANOVA test shows significant differences in a range of relative spectral bands ($P < 0.01$). The ratio of some spectral band areas about 1670cm-1, 1614cm-1, 1575cm-1, 1549cm-1, 1483cm-1, 1231-1255cm-1, 813cm-1, 785cm-1, 762cm-1, 714cm-1 and 669 cm-1 (can be variously attributed to beta sheet and alpha helical conformations of protein, DNA/RNA bases and backbone, tyrosine side chain and tryptophan) with respect to their adjacent lower bands were selected for discrimination between the two cell lines. The spectra from the cell line samples were divided into a training and test set. Discriminant analysis was performed on the training set of the bands defined above, and used to predict the group membership of the test set of cell spectra. Each individual spectrum was assigned to the closest group, and the cells classified depending on where the majority of its spectra fell. This revealed that the benign and malignant cell types could be classified with 96% accuracy using the generated chemometric spectra.

Conclusion. This study has shown that Raman microscopy can be used to probe the chemometric characteristics of individual cells and demonstrate subtle differences in underlying pathobiology. This could have potential applications in the identification of cell markers in diagnostic pathology that are not visible to the naked eye and also to probe for changes in cell biochemistry without the use of chromogenic, fluorescent or bioluminescent markers.

P24

THE USE OF RAMAN MICROSCOPY TO DIFFERENTIATE NORMAL AND MALIGNANT LUNG TISSUE SECTIONS IN LUNG CANCER

Dr Nicholas Magee, Dr Rene Beattie, Dr John McGarvey, Dr Madeleine Ennis, Dr Peter Hamilton, Dr Stuart Elborn
Respiratory Research Group, Queen's University Belfast
School of Chemistry, Queen's University Belfast
Department of Pathology, Queen's University Belfast

nick@nickmagee.com

Introduction. Early diagnosis and treatment of lung cancer are key aims in the management of this condition, the most common fatal neoplasm in Northern Ireland, accounting for 10% of all cancers here and for which there is currently no effective screening method for the early diagnosis. Several studies have recently supported the use of Raman microscopy to differentiate malignant from normal tissue. There is therefore the potential to develop this technique as a screening tool for lung cancer.

Methods. The aim of our study was to investigate if Raman microscopy can differentiate malignant from normal tissue taken from lung resections of patients with lung cancer. Both tumour and normal tissue were sliced into 5 micron sections, placed on quartz microscope slides and fixed with 99% ethanol. The samples were investigated with a Jobin Yvon LabRam HR Raman microscope using 785 nm excitation.

Results. We have shown a significant difference between normal and malignant spectra and also between adenocarcinoma and squamous carcinoma cell types. The intensity of the 1450 cm-1 band was similar in both the normal and the malignant tissue. However the intensity of the 1662 cm-1 band in both malignant cell types was higher than that in the normal tissue. Previous studies4,5 have used a ratio of intensities at 1455 and 1655 cm-1 to classify tumour versus normal tissue in the brain, breast, colon and cervix. The squamous cell carcinoma samples also demonstrated a marked increase in 1590 cm-1 and 785 cm-1 bands, regions containing contributions from DNA, consistent with the histological increase in nuclear/cytoplasmic ratio in malignant tissue.

Conclusion. This preliminary study has shown that Raman microscopy can differentiate malignant from normal lung tissue.

P25

QUANTITATIVE BIOIMAGING OF CHROMATIN ORGANISATION, EPIGENETIC BIOMARKERS AND CHROMATIN REMODELING ENZYMES AND RESPONSE TO TREATMENT IN PROSTATE CANCER PATIENTS

M Mohamed, R Montironi, R Nairn, G McCusker, O Sharaf, P Greif, P Maxwell, J Diamond, M Young, R Meddings, PW Hamilton
Biomedical Imaging and Informatics Group, Centre for Cancer Research and Cell Biology, QUB
Institute of Pathological Anatomy and Histopathology, University of Ancona, Italy
Department of Pathology and Urology, Ayrshire & Arran Acute Hospitals
Department of Urology, Craigavon Area hospital

Introduction. In prostate adenocarcinoma, localized disease is managed by either radical surgery or radical radiotherapy and metastatic disease is managed by androgen deprivation therapy (HDT). However, some patients may not respond to HDT or else a period of initial response may progress to androgen independent disease. Numerous studies have tried to identify reliable prognostic markers for prostate cancer progression from tumour tissue samples, including morphological, immunohistochemical and molecular markers. While epigenetic mechanisms and chromatin remodelling enzymes are thought to play a major role in prostate cancer progression, little work has been carried out to explore their role as predictive markers for response to therapy. The aim of this study was to assess the role of digital chromatin phenotype, epigenetic profile and remodelling markers in predicting the clinical response of prostate cancer patients to HDT treatments.
Materials and Methods. A tissue microarray (TMA) was constructed from TURP paraffin embedded specimens of 38 patients prior to treatment. Based on 10 year follow-up of prostatic specific antigen (PSA) levels after treatment, patients were divided into (i) HDT responsive (8 patients, 13 cores) and (ii) HDT poorly responsive (23 patients, 38 cores). Following TMA construction, nuclear and Gleason grading were defined on each core by an experienced uro-pathologist. Using high resolution image analysis, chromatin phonotype was assessed on H&E stained tissue sections. Global nuclear epigenetic characteristics and chromatin remodelling were assessed using quantitative immunohistochemistry of acetylated histone3 lysine 9 (AcH3K9), 5'-methylcytidine (5MeC), ISW1 (Snf2h & Snf2l) and BRG1 (SWI2/SNF2) antibodies.
Results. The results showed firstly that conventional tumour grading was of little value in identifying HDT responsive patients. Statistical analysis showed significant differences in staining intensities and nuclear organization in all of the measured markers between HDT responsive and poorly responsive patients ($p<0.01$). For chromatin phenotype, 5'-MeC, & BRG1, significantly higher staining intensity was seen in HDT poorly responsive patients demonstrating increased chromatin density and associated global hypermethylation in these patients. In contrast, AcH3K9 & ISWI ATPase expression were significantly reduced in HDT poorly responsive patients. Multivariate discriminant analysis and the definition of classification score based on a small number chromatin, epigenetic and remodelling markers was able to predict HDT responsive patients with up to 80% accuracy. The addition of Gleason and nuclear grades did not enhance the classification results.
Conclusion. Quantitative imaging of chromatin phenotype, epigenetic biomarkers and chromatin remodeling enzymes has been shown to be of promising value in predicting pre-treatment response to HDT in prostatic adenocarcinoma patients. It also highlights that higher level chromatin topology appears to have functional role in prostate cancer development and therapy. Further work is needed to explore this in a larger independent set of cases and to understand the underlying biology of epigenetics and chromatin remodeling in prostate cancer.

P26

THE EFFECT OF MITOMYCIN C (MMC) ON BCL-2/ RAS PROTEIN EXPRESSION IN T24 BLADDER CELL LINE USING FLOW CYTOMETRY

Osama Sharaf, Perry Maxwell, Ken Arthur, Kate Williamson
Uro-oncology Group, QUB Department of Pathology, Grosvenor Road, Belfast 12 6BN
Department of Pathology, Royal Hospitals Trust

Introduction. Aggressive behaviour in bladder cancer is associated with over expression of both the anti-apoptotic bcl-2 protein and the signal transducer ras. The T24 bladder cell line has aggressive behaviour and high expression of ras and bcl-2. As a prelude to investigations of ras siRNA/MMC synergy in T24 cells,

the ras and bcl-2 protein expression response together with the apoptotic response were characterised 4h and 24h after incubation with low and high MMC concentrations.
Material And Methods. T24 cells exposed to high MMC concentrations (0, 50, 100, 200, 400 and 800µg/ml) for 1h were assessed for apoptosis, ras and bcl-2 protein expression 4h after treatment was initiated and similarly those exposed to MMC at low concentrations (0, 1, 3, 10, 30 and 100 µg/ml) were assessed after 24h. The effects on bcl-2 and ras protein expression in T24 cells were quantitated using the EPICS ELITE flow cytometer after immunostaining with either Pan-ras IgG2 antibody (Code No. op40-100UG, Calbiochem, USA) or bcl-2 IgG1 (Code No. M 0887, DAKO, USA), and goat anti-mouse secondary antibody (Code No. R 0480, DAKO, USA). Corresponding isotype controls were included for each time point and concentration. The percentage of positively staining cells (PPSC) and mean channel fluorescence (MCF) were determined using Immuno-4 software (Coulter Corporation, Florida, USA).
Results. Four hours after high dose of MMC, T24 cells were resistant to apoptosis, ras levels of protein expression increased in a dose dependent manner and slight increases were observed in bcl-2 protein expression . Twenty four hours after low doses an apoptosis dose response was evident , ras protein expression was consistent across all concentrations and the number of cells staining positively for bcl-2 exhibited a dose dependent response.
Conclusion. This study highlights the complexities of protein expression changes induced by chemotherapy which need to be considered in study designs investigating siRNA effects on drug modulations. The T24 bladder cell line is a suitable model for investigation of the synergistic effects of ras siRNA/MMC on apoptosis induction 24 h after low dose MMC incubations.

P27

DUB-3 EXPRESSION REGULATES SIGNALLING THROUGH THE RAS/MEK/ERK PATHWAY

Dr James Burrows, Mr Michael McGrattan,
Prof Jim Johnston
Centre for Cancer Research and Cell Biology, Queen's Universit, Belfast

J.Burrows@qub.ac.uk

Introduction. The DUB/USP17 family of deubiquitinating enzymes were first identified in mice as cytokine inducible immediate early genes that play a role in the control of cell proliferation. Recently we have identified a number of human family members and shown that one of these, DUB-3, is induced in response to IL-4 and IL-6.
Methods. To examine the role of DUB-3 expression in intracellular signalling we used Ba/F3 cells which express DUB-3 from a Tet-Off construct. We used these cells to examine the activation of various intracellular signalling molecules using phosphospecific antibodies both in the presence and absence of DUB-3 expression.
Results. Here we report that DUB-3 can block proliferation in both Ba/F3 and NIH3T3 cells. In addition, we demonstrate that DUB-3 regulates signalling through the Ras/MEK/ERK pathway. In particular we show that expression of DUB-3 blunts ERK and MEK activation. In addition we have observed the appearance of a slower migrating form of Ras in the presence of DUB-3 expression suggesting that it acts to disrupt the processing of Ras. Finally we have examined the affect of DUB-3 expression upon Ras processing and determined that it can act to modulate the turnover of RCE1, an enzyme which acts to proteolytically cleave Ras during its processing.
Conclusion. These observations suggest that DUB-3 can play a role in the regulation of proliferation through its modulation of the Ras/MEK/ERK pathway and we propose that this regulation may account for the ability of DUB-3 to block proliferation in both Ba/F3 and NIH3T3 cells.

P28

IMMUNOLOGIC PROPERTIES OF DIFFERENTIATED MESENCHYMAL STEM CELLS (MSCS)

Miss Xi Chen, Dr Marilyn Armstrong, Dr Gang Li
Department of Orthopaedic Surgery and Trauma, Queen's University of Belfast, School of Medicine, Musgrave Park Hospital, Belfast, BT9 7JB, UK
Department of Immunology, Queen's University of Belfast, School of Medicine, Musgrave Park Hospital, Belfast, BT9 7JB, UK

x.chen@qub.ac.uk

Evidences of interaction between T cells and MSCs demonstrated that MSCs possess immuno-suppressive properties. Dendrtic cells (DCs) are antigen-presenting

cells, and play an exclusive role in priming primary immune responses. The aim of this study was to examine the immunologic properties of differentiated MSCs in co-culture with DCs.
MSCs were harvested from rat long bone marrow and cultured. After passage and reaching confluence, MSCs were subjected to adipogenic, chondrogenic and osteogenic induction conditions. The growth curves of both undifferentiated and differentiated MSCs were drawn using Brdu incorporation after 22 days culture. Cytotoxic assays were performed with human peripheral blood lymphocytes (hPBLs) from healthy donors. Dunn Chamber chemotaxis analysis was carried out using human peripheral blood monocyte-derived DCs as sources of chemotactic factors.
The differentiation of MSCs under induction was confirmed: Oil red O staining of lipid vacuoles for adipogenic; Alcian Blue staining of acid glycosaminoglycans for chondrogenic and Von Kossa staining of mineralization for osteogenic. Cell proliferation was significantly reduced only in MSCs under chondrogenic induction (OD value adi=0.65, chon=0.21, osteo=0.93, MSCs=0.96). The chondrogenic MSCs had shown significant cytotoxic-inducing ability upon co-culture with hPBLs ($p=0.737$ Vs positive control), while the other groups had very low level of cytoxic effect when compared with positive controls (pMSCs=0.000, padi=0.000, posteo=0.001, respectively). For the Dunn Chamber chemotaxis test, chondrogenic MSCs migrated towards hPB-derived DCs during 10h observation time (speed = 18.30 ± 1.86 μm/ hr, $p< 0.0001$), while the other groups showed random movement.
The data indicated that among the three differentiated lineages, only chondrogenically differentiated MSCs were capable of interacting with DCs and eliciting T cell-mediated cytotoxic immune responses in vitro, whereas the adiopogenic and osteogenic MSCs still retained the immuno-suppressive properties of undifferentiated MSCs. Thus, repair of cartilage using MSCs needs careful consideration as chondrogenic differentiation of MSCs may induce immunologic responses.

P29

MODELLING CHEMOTHERAPEUTIC TREATMENTS OF COLORECTAL CANCER

Dr. SFC O'Rourke, Mrs H McAneney,
Prof. PG Johnston, Dr J Boyer
Centre for Cancer Research and Cell Biology, Queen's University Belfast
s.orourke@qub.ac.uk

In advanced colorectal cancer, 5-FU monotherapy produces response rates of only 10-15% [1]. We will mathematically model the response of a vascular tumour chemotherapeutic treatment for colorectal cancer by adopting a modelling approach similar to that of Jackson [2], which studied the spatially dependent transfer of chemotherapeutic drugs into and out of the tumour. Our model differs from [2] in that we will take into account specifically the mechanism of cell cycle behaviour based on the biochemical properties of the chemotheraupetic drugs 5-FU, and the combination of 5-FU with the drugs irinotecan and oxaliplatin [1]. Additionally, we are developing cell cycle models of vascular tumour growth based on treating the tumour mass as a spatially uniform mass which evolves at a prescribed rate. Three possible modes of growth are considered; exponential, Gompertz and logistic laws. Crucially, our model differs from that of Shochat et al's [3] cell cycle specific and non-specific models in that we are using a compartment system to model cycling, resting and resistant cells and competition effects. The results of these models are compared and contrasted with differences highlighted and discussed.

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P30

EFFECTS OF THE DUAL 5 ALPHA REDUCTASE INHIBITOR DUTASTERIDE ON APOPTOSIS IN PRIMARY CULTURES OF PROSTATE CANCER EPITHELIAL CELLS

Ms Ann Maria Mc Crohan, Dr Colm Morrissey,
Dr Connor O' Keane, Dr Niall Mulligan, Mrs Chanel Watson, Mr James Smith, Prof John M. Fitzpatrick,
Dr R. William G. Watson
Department. of Surgery, Mater Misericordiae University Hospital, Conway

Institute of Biomolecular and Biomedical Research, University College Dublin and Dublin Molecular Medicine Centre, Dublin
Department. of Histopathology, Mater Misericordiae Hospital, University College Dublin, Dublin

annmaria.mccrohan@ucd.ie

Introduction. The most potent androgens in prostate cancer (PCa) aetiology are testosterone (T), which is converted to dihydrotestosterone (DHT) by the 5 alpha-reductase type I and type II enzymes. The aim of this study was to determine if dutasteride, a dual 5 alpha-reductase inhibitor, induces apoptosis in primary cultures of prostate cancer epithelial cells.
Materials and Methods. The first step was to validate previous findings that dutasteride induces apoptosis in androgen-dependent (PwR-1E) and androgen-independent (PC-3) prostate cell lines. Primary epithelial cells were then extracted from fresh prostate tissue. Total cellular protein was extracted at the time of primary cell culture with dutasteride to confirm the phenotype by cellular morphology and expression of Cytokeratin (CK) 18 and AMACR. Apoptosis and viability were assessed by propidium iodide DNA staining and flow cytometry after 24 hours of culture with 0-10 mM dutasteride.
Results. Dutasteride induced dose-dependent apoptosis in the PwR-1E cells, but there was no significant apoptosis in the androgen receptor-negative PC-3 cells. Primary cultures were confirmed as being luminal epithelium based on morphology and CK18 expression. Analysis together (n = 16) showed that there was no significant increase in dutasteride-induced apoptosis following incubation for 24 hours. However, further analysis revealed two specific groups: responders (n = 7) and non-responders (n = 9). Their response to dutasteride was correlated with Gleason scores ($p = 0.04$) of the primary tumours as well as expression of AMACR in the cultured cells.
Conclusion. Dutasteride represents an important chemo-prevention strategy for PCa and has led to the initiation of the REDUCE study. The possible chemopreventative role of dutasteride will not be known until this trial is complete in 2008. Our study demonstrates that only specific patients respond to dutasteride and the biochemical identification of these patients has implication to the success of this therapeutic approach.

P31

SOCS2 ACCELERATES SOCS3 DEGRADATION

Dr Joanne Elliot, Miss Gillian Tannahill, Miss Anna Barry, Prof Jim Johnston
Queen's University Belfast

joanne.elliott@qub.ac.uk

Introduction. Cytokines regulate diverse biological processes through their interaction with multisubunit receptor complexes. The Suppressor of Cytokine Signalling (SOCS) proteins play a key role in regulating the strength and duration of cytokine responsiveness. SOCS are induced by a range of stimuli and act to inhibit signalling in a classical negative feedback loop. Dysregulation of these feedback pathways are associated with diseases as diverse as inflammatory bowel disease, allergy, autoimmune diseases and diabetes. The SOCS protein family consists of eight members; CIS (Cytokine inducible SH2 domain- containing protein) and SOCS1-7. Common features include a central SH2 domain and a C-terminal SOCS box. The SOCS box motif has been implicated in E3 ligase activity through its association with Elongin-BC. SOCS are thought to target associated proteins for degradation via the ubiquitin-proteasome pathway but relatively little is known regarding how their activity is regulated.
Methods. Tissue culture, transfection, Western Blotting.
Results. Here we demonstrate that SOCS1 and SOCS3 associate with Elongin-BC in vitro and that this interaction is disrupted by phosphorylation. In addition, SOCS2 and SOCS3 are shown to associate in peptide pull-down experiments and SOCS2 expression in vivo results in a marked proteasome-dependent reduction of SOCS3. This loss of SOCS3 is enhanced in the presence of Elongin-BC and dependent on an intact SOCS box. These results suggest that like SOCS1 and SOCS3, SOCS2 can also bind to Elongin-BC to form an E3 ligase complex to target associated proteins for degradation.
Conclusion. SOCS2 can enhance signalling responses by accelerating the turnover of SOCS3 via the proteasome. Since altered SOCS expression can result in a broad range of pathologies, determining how SOCS proteins are regulated will aid the development of novel therapeutic strategies for autoimmune and inflammatory diseases.

P32

GENOMIC PROFILING OF SALIVARY GLAND TUMOURS

Miss Hedy Vekony, Prof G.A. Meijer, Dr B. Ylstra, Prof I. van der Waal, Prof E. Bloemena
Department of Oral and Maxillofacial Surg/Oral Pathol, Academic Centre for Dentistry (ACTA), VUmc, Amsterdam, Netherlands
Department of Pathology, VUmc, Amsterdam, Netherlands
Microarray Core facility, Medical Faculty, VUmc, Amsterdam, Netherlands

h.vekony@vumc.nl

Introduction. Salivary gland tumours are rare neoplasms that show various morphological patterns as well as a diverse clinical behaviour. Their classification depends solely on histopathological features. The precise genetic alterations that underlie these neoplasms have been poorly characterized. A better understanding of these aberrations in salivary gland tumour development and progression could significantly improve classification and prognostication of these tumours. One high-resolution technique that detects and maps changes in copy number of DNA sequences is microarray based comparative genomic hybridization (array CGH). It provides a global overview of chromosomal gains and losses throughout the whole genome of a tumour and allows subsequent associations to be made with clinical outcome.
Patients, Materials and methods. DNA was isolated from archival formalin-fixed paraffin-embedded tissue samples from 17 primaries and 2 recurrences of adenoid cystic carcinomas (ACC). We analysed the chromosomal aberrations in these tumours by array CGH using a 5K BAC array platform. ACCs were located in the major salivary glands (parotid n=9, submandibular n=8) or elsewhere in the oral cavity (n=2). The male-female ratio was 1:2, the mean age at diagnosis was 50.6 (range 25-81) and the mean follow-up time was 69 (range 7-128) months. Nine patients (47%) developed either a recurrence or a metastasis. Seven patients (37%) died of disease.
To detect chromosomal regions that statistically significantly differ between patients with and without metastases, the Wilcoxon two-sample statistic with ties was used.
Results. In all ACCs, chromosomal aberrations were observed, with gains being far more frequent than losses. Amplifications were rare, but a hotspot was observed at chromosome 1q21-22. A gain at chromosome 18q11.2 was related to the occurrence of metastases.
Conclusions. Adenoid cystic carcinomas show frequent chromosomal aberrations with arrayCGH. A discrete region at 18q11.2 seems to be correlated to the development of metastases. Currently, the data are further analysed in relation to the clinical outcome.

P33

ANALYSIS OF ERYTHROPOIETIN RECEPTOR STRUCTURE AND SIGNALLING IN NON SMALL CELL LUNG CARCINOMA

Miss Elaine Dunlop, Dr Anita Yakkundi, Dr Marion Borland, Prof Terence Lappin
Centre for Cancer Research and Cell Biology, Queen's UniversityBelfast, Belfast, UK

e.dunlop@qub.ac.uk

Introduction. The erythropoietin receptor (EpoR) is essential for red blood cell production(1), but has also recently been found on a number of tumour types including breast cancer(2). We have identified EpoR on non small cell lung carcinoma (NSCLC) cells. As many cancer patients are given erythropoietin to combat anaemia, it is important to establish whether the EpoR expressed on the tumour cells is functional.
Methods. The structure and signalling pathways of EpoR in 3 NSCLC cell lines have been investigated using RT-PCR, sequencing and western blotting.
Results. Two NSCLC cell lines, H157 and H838, were found to express wild type, unmutated EpoR. A C to G mutation was found at base 1138 of the EpoR mRNA in the H23 cell line. This corresponded to a proline to alanine substitution at amino acid 356 of the mature EpoR protein which is in the intracellular region. Signalling results revealed low levels of phosphorylated STAT5 in unstimulated cells. These levels remained unchanged in H23 and H157 cells treated with Epo. In contrast, a dose-dependent increase in the phosphorylation of STAT5 was observed in H838 cells in response to Epo stimulation. The Akt pathway in H838 and H157 cells responds to Epo, while no response was detected in H23 cells.
Conclusion. The H23 NSCLC cell line contains a mutation within the cytoplasmic signalling domain and preliminary results indicate that Epo stimulation of these cells does not cause a change in the phosphorylation of STAT5 or Akt signalling proteins. Therefore the mutation could be affecting EpoR signalling. Although

both the H157 and H838 cell lines express wild type EpoR, our data show only a clear response to Epo stimulation in the H838 cells. Experiments are in progress to establish the role of EpoR in these cells.

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P34

C-JUN ACTIVATION IS ASSOCIATED WITH ANGIOGENESIS IN INVASIVE BREAST CANCER

M. M. Vleugel, A. E. Greijer, R. Bos, E. van der Wall, P.J. van Diest
Department of Pathology VU University Medical Center, Amsterdam, The Netherlands
Division of Internal Medicine and Dermatology
Department of Pathology, University Medical Center, Utrecht, The Netherlands

Background. c-Jun is a component of the transcription factor AP-I, which binds and activates transcription at TRE/AP-I elements. The transcriptional activity of c-Jun is regulated by phosphorylation of c-Jun at Ser63/73. Extracellular signals, including growth factors, transforming oncoproteins and UV irradiation, stimulate phosphorlylation of c-Jun at Ser 63/73 and activate c-Jun dependent transcription. Thereby, activated c-Jun potentially plays an important role in carcinogenesis and cancer progression. The aim of this study was to evaluate expression patterns of activated c-Jun in breast cancer.
Methods. Immunohistochemistry was performed on 103 cases of invasive breast cancer with an antibody recognizing activated c-Jun at Serine 73.
Results. c-Jun showed a predominantly nuclear expression at the invasive front in 38% of invasive breast cancer cases in varying percentages (1-20% of nuclei, mean 4%). Furthermore, expression of activated c-Jun was seen in the invasive front in mitotic cells of 40% of cases, sometimes however also centrally in the tumours. Occasionally, fibroblasts, endothelial and benign breast cells showed nuclear expression. Activated c-Jun expression showed positive correlations with expression of hyperphosphorylated pRb, VEGF and with microvessel density. Mitotic c-Jun expression showed positive relations with pRb, and microvessel density. These associations were largely dependent on ER expression. In ER positive cases, also a positive association between activated c-Jun and proliferation was seen.
In survival analysis, no significant prognostic value was found for nuclear, mitotic or stromal activated c-Jun expression.
Conclusion. Activated c-Jun is mainly expressed at the invasive front in invasive breast cancer where most proliferation and angiogenesis takes place. Indeed, it is often expressed in mitotic cells, and associated with angiogenesis. As earlier studies have established a functional link between activated c-Jun and angiogenesis, c-Jun/AP-1 targeting may provide new ways to block tumour angiogenesis.

P35

EFFECT OF INTERFERON-GAMMA ON CHEMOTHERAPY-INDUCED APOPTOSIS IN BREAST CANCER CELLS

Katherine M.A. Rogers, Michelle Thomas, Leeona Galligan, Patrick G. Johnston, Daniel B. Longley
Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast City Hospital, Lisburn Road, Belfast, BT9 7AB, Northern Ireland

Introduction. The DNA topoisomerase-II (topo-II) poison doxorubicin is widely used in the treatment of breast cancer. We have shown doxorubicin can potentiate STAT1 activation in a panel of breast cancer cell lines treated with interferon-gamma (IFNγ), resulting in enhanced apoptosis
Methods. MDA-435 cell viability was determined by MTT assay and the effect of dual drug treatment analysed using CalcuSyn® software. Protein expression was determined by Western blotting and caspase-8 activity measured by a spectrofluorimetric assay. Cell cycle profiles were analysed by propidium iodide staining followed by flow cytometry. Cell surface expression studies were conducted by immunofluorescence staining.
Results. We found procaspase-8 expression was enhanced in response to IFNγ. Furthermore, a 4-fold increase in levels of caspase-8 activity was observed following co-treatment with IFNγ and doxorubicin compared to treatment with each agent alone. Activation of caspase-8 correlated with an increase in PARP cleavage. Additionally, we have shown that treatment with the caspase-8 inhibitor, Z-IETD-FMK, resulted in reduced PARP cleavage in response to IFNg and doxorubicin co-treatment. Subsequently, we analysed

the cell surface expression of death receptors, Fas, DR4, DR5 and their ligands, FasL and TRAIL in response to IFNg and doxorubicin, and found that DR5 and Fas cell surface expression was significantly up-regulated in response to doxorubicin and IFNγ respectively.
Conclusion. IFNg and doxorubicin synergistically induce apoptosis in MDA-435 cells, an effect mediated by caspase-8 activation which may result from increased cell surface expression of the death receptors DR5 and Fas. Studies are ongoing to determine the relative importance of the Fas/FasL and DR5/TRAIL apoptotic pathways for mediating the synergistic interaction between IFNγ and chemotherapy in breast cancer cells.

P36

NEAR-DIPLOID AND HIGH-ANEUPLOID HUMAN SPORADIC COLORECTAL CARCINOMAS ARE STRONGLY ASSOCIATED WITH KRAS2 AND TP53 MUTATIONAL STATUS

Walter Giaretti, Elena Leonadi, Claudia Rengucci, Andrea Sciutto, Angelo Scaramuccia, Davide Malacarne, Mauro Risio, Paolo Dalla Palma, Daniele Calistri
National Institute for Cancer Research, Genoa, Italy.
Dept. of Pathology, St. Chiara Hospital, Trento, Italy.
Istituto Oncologico Romagnolo, Forlì, Italy
Unit of Pathology, Institute for Cancer Research and Treatment, Candiolo, Italy

Introduction. Previous work in human sporadic colorectal cancer (hsCRC) suggested that KRAS2 protoncogene and TP53 oncosuppressor gene mutations might contribute to chromosomal instability (CIN) and aneuploidy. In particular, it was shown that near-diploid aneuploid and high-aneuploid subpopulations might be associated by endoreduplication, while near-triploid subpopulations might be linked with a diploid-tetraploid transition. Along with these hypotheses, we investigated the relationship of KRAS2 and TP53 mutations with DNA aneuploidy in hsCRC.
Methods. Multiple samples were obtained from 210 hsCRC to provide nuclei suspensions for flowcytometric analysis of the DNA Index (DI) and isolated DNA for DNA sequencing of KRAS2. In a subgroup of 89 hsCRC, the TP53 mutation spectrum was additionally evaluated by DNA sequencing.
Results. We detected 56 DNA diploid (DI=1) and 154 aneuploid (DI ≠ 1) hsCRC; the DI aneuploid distribution was characterised by non-random peaks and subdivided in 59 near-diploid (DI ≠ 1 and DI ≤ 1.4), 39 near-triploid (1.4<DI<1.6), and 56 high-aneuploid (DI=1.6) cases. The incidence of mutations of the KRAS2 oncogene was lowest (15%) among the DNA near-triploid subpopulations and highest among the near-diploid (51%) and high-aneuploid ones (41%). The strongest significant association between KRAS2 and DI ($p<0.0005$ by the Fisher's exact test) was obtained by combining DNA diploid, near-triploid and tetraploid cases versus near-diploid/high-aneuploid ones. These DI classes correlated also with TP53 and combined KRAS2/TP53 status. In particular, absence of mutations for both genes in the DNA diploid/near-triploid/tetraploid group strongly influenced the significance of association between DI with KRAS2/TP53 status (Pearson Chi-squared $p=0.001$).
Conclusion. The present data agree with previous models of DNA aneuploidization and evolution, indicate that KRAS2 and TP53 gene mutations may cooperate to generate specific mechanisms of CIN and aneuploidy, and suggest that the newly combined classes of DNA diploidy/near-triploidy/tetraploidy versus near-diploidy/high-aneuploidy might have more efficacy than the classical diploidy/aneuploidy classification to predict clinical behaviour and therapy response.

P37

EFFECTS OF 1 ALPHA, 25-DIHYDROXYVITAMIN D3 (VITD3) AND TWO LOW-CALCEMIC VITD3 ANALOGUES ON THE WILD-TYPE AND MUTATED OSTEOPONTIN PROMOTER

Miss Mella McCann, Dr Mohamed El-Tanani, Dr Gary Posner, Prof Charles Campbell
Surgery, School of Medicine, Queen's University Belfast.
Department of Chemistry, Faculty of Arts and Sciences, Johns Hopkins University, Baltimore

mella_mccann@hotmail.com

Introduction. Osteopontin (OPN) is implicated in neoplastic transformation and colorectal cancer. Regulation of OPN transcription may be an important goal for prevention or treatment colorectal cancer. Ligand activated nuclear vitamin D receptor (nVDR) binds to a vitamin D responsive element (VDRE) on the OPN promoter, but may also have indirect effects on OPN transcription through the β-catenin/Tcf-4/Lef-1 pathway. This study tests the hypothesis that (i) vitamin D3 binds to VDREs to exert its effects (ii)

components of the β-catenin/Tcf-4/Lef-1 pathway influence ligand-nVDR activated OPN transcription.
Methods. Rama 37 benign epithelial cells were transiently transfected with an OPN promoter-reporter luciferase construct, with or without the expression vector for human (hVDR), then treated with either 1alpha, 25-dihydroxyvitamin D3 (VitD3), high affinity (Hopkins-QW-1624F2-2 hybrid deltanoid [QW]) or low affinity (BTW-1alpha,3β-2,2Me2-nor [BTW]) low calcemic VitD3 analogs. Cells were then co-transfected with a Tcf-4 expression vector. Transient transfections were then carried out with the OPN promoter after site-directed mutation of the two VDREs present in the rat OPN promoter.
Results. OPN transcription was induced by > 50 fold in excess of control by QW 1 nM vs BTW 100 nM (vs VitD3 10 nM). Co-transfection of the expression vector for Tcf-4 strongly inhibited spontaneous or VitD3 stimulated OPN transcription (e.g. OPN + hVDR + VitD3 10 nM vs OPN + hVDR + Tcf-4 + VitD3 10 nM = 112.2 vs 34.6 fold induction). OPN promoter-reporter activity was reduced by 60% (76.5 vs 30 fold induction) with one of the VDREs mutated. When both VDREs are mutated, promoter activity is reduced to background levels.
Conclusions. Noncalcemic analogs QW and BTW activate OPN transcription in proportion to nVDR binding affinity. The expression vector for Tcf-4 inhibits ligand-nVDR activated OPN promoter-reporter gene activity. VitD3 exerts its effects through two VDREs present in the OPN promoter.

P38

BRCA1 AND C-MYC ASSOCIATE TO TRANSCRIPTIONALLY REPRESS PSORIASIN

Dr Julia Gorski, Dr Richard Kennedy, Dr Jennifer Quinn, Miss Gail Stewart, Dr Colin James, Dr Paul Harkin, Dr Patrick Johnston
Queens University Belfast

j.gorski@qub.ac.uk

Introduction. Evidence is accumulating to suggest that some of the diverse functions associated with BRCA1 may relate to its ability to transcriptionally regulate key downstream target genes 1. Here we identify S100A7 (Psoriasin), S100A8 and S100A9, members of the S100A family of calcium binding proteins 2, as novel BRCA1 repressed targets.
Results. We demonstrate that functional BRCA1 is required for repression of these family members and that a BRCA1 disease associated mutation abrogates BRCA1 mediated repression of psoriasin. Furthermore we show that BRCA1 stabilizes c-Myc on the psoriasin promoter and that BRCA1-mediated repression of psoriasin is dependent on functional c-Myc and a complete c-Myc consensus sequence in the psoriasin promoter. Finally we provide evidence that mutation of BRCA1 is associated with expression of psoriasin in human breast tumour samples.
Conclusion. These findings suggest that BRCA1 represses expression of psoriasin and upregulation of the psoriasin gene may be a surrogate marker of loss of BRCA1 transcriptional function.

P39

MITOMYCIN-C (MMC) INDUCED ACTIVATION OF FAS, P53 AND AN S-PHASE CHECKPOINT IN TRANSITIONAL CELL CARCINOMA OF THE BLADDER (TCCB)

CJ Watson, HF O'Kane, P Maxwell, K Arthur, KE Williamson
Queen's University Belfast, Uro-Oncology Research Group, Department of Pathology, Northern Ireland Royal Hospitals Trust, Belfast

Introduction. The response to immune mediated therapies administered intravesically in bladder cancer is enhanced by pre-sensitisation with low doses of MMC, a drug which creates DNA interstrand crosslinks. Increased tumour cell apoptotic susceptibility could be attributable to up-regulation of Fas expression mediated through a MMC mechanism. Using the RT4 bladder cancer cell line, known to express functional Fas and wt p53, this study investigated the following after 0,1,3,10, 50, 100 and 200µg/ml MMC: (1) whether Fas protein expression increased (2) the relationships between Fas and p53 protein expression and cell cycle perturbations.
Methods. Protein expression levels were characterised 3, 6, 12 and 24 hr after MMC using flow cytometry, immunocytochemistry, western blotting and confocal microscopy. Cell cycle DNA histograms were analysed using Multicycle software. Flow cytometry mean channel fluorescence data were normalised to facilitate p53/Fas comparisons. Graphs of MMC concentration against p53 and Fas protein expression and against cell cycle phases were plotted at each time-point. Data were analysed using SPSS software for Windows.

Results. Although treatment with MMC at all concentrations increased the proportion of S-phase cells, concentrations of □50μg/ml induced significantly greater increases when compared to those induced by 100 and 200μg/ml ($p<0.01$). The position of the S-phase block (beginning mid or end) was dose dependent. Treatment with □50μg/ml triggered significant increases in p53 and Fas protein expression at 6hr and 12hr respectively ($p<0.01$). At 24hr Fas and p53; and Fas and S-phase percentages were significantly correlated ($r = 0.915$ and -0.830 respectively (Spearman's Rank)) ($p<0.01$).
Conclusions. The RT4 cell line exhibits sensitivity to induction of Fas post MMC treatment that is preceded by increased expression of p53 suggestive of p53 mediation. The threshold differential MMC response is indicative of alternative concentration dependent pathways. The RT4 cell line is an appropriate model for investigating further the role of Fas in MMC induced apoptosis.

P40

THE ROLE OF DNA METHYLATION AND DOWN-REGULATION OF THE APOPTOTIC DEATH RECEPTOR FAS IN TRANSITIONAL CELL CARCINOMA OF THE BLADDER (TCCB)

CJ Watson, HF O'Kane, P Maxwell, I Petak, KE Williamson
Queen's University Belfast, Uro-Oncology Research Group, Department of Pathology, Northern Ireland Royal Hospitals Trust, Belfast
Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest

Introduction. Loss of expression of Fas, located at chromosome 10q, is associated with a poor prognosis in TCCB. DNA hypermethylation at promoter and enhancer regions of certain genes can silence gene transcription and thus be involved in cell pathway alterations leading to cancer. The promoter region of Fas is GC rich and contains a large number of CpG dinucleotides as does a p53 responsive element found within the first Fas intron. The role of DNA methylation and reduced expression of Fas receptors in TCCB was investigated.
Methods. Before and after demethylation using 0.5-12μM 5-aza-2'-deoxycytidine (5azadC) for 72hr RT4 and HT1376 cells were examined. Fas expression and global methylation were assessed using flow cytometry and confocal microscopy. Gene specific methylation was assessed using Methylation Specific PCR (MSP) to determine the methylation status of the p53 enhancer region.
Results. RT4 cell line expressed endogenous membranous Fas which was up-regulated post treatment with 5azadC (0.5μM≤). Fas was re-expressed in approximately 30% of the previously Fas negative HT1376 cell line after 5azadC (9μM≤). Global demethylation increased as 5azadC concentration increased. MSP revealed that the p53 enhancer region within the Fas gene was unmethylated in the 2 TCCB cell lines.
Conclusions. Up-regulation/ re-expression of Fas was detected in these bladder cancer cell lines following 5azadC treatment, which would indicate a possible epigenetic control over gene transcription. However, the increase in expression could be indirect and attributed to alterations in pathways acting on the gene of interest. MSP detected no methylation at the p53 enhancer region. Therefore PCR clones containing the Fas promoter from RT4 and HT1376 bisulphite converted DNA have been generated and are currently being sequenced to determine the methylation status of 28 CpG sites of interest. The pending sequencing results should indicate if DNA methylation has a direct or an indirect role in downregulation of Fas receptors.

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ANALYSIS OF METASTATIC BLADDER CANCER USING REAL TIME IN VIVO 3D-FLUORESCENCE IMAGING

V. Koo, P Hamilton, K Williamson
Uro-oncology Research Group, QUB
Department of Bio-imaging & Informatics, RVH

Background. At presentation, about 30% bladder tumours are muscle-infiltrative and these tumours have significant risk of metastasis with poor prognosis. S100A4 has been associated with metastasis and a phenomenon called EMT, the conversion from an epithelial to mesenchymal phenotype. EMT is increasingly being considered as a threshold event the in progression of cancers. This project will establish a metastatic model of bladder cancer that can be monitored using the Xenogen IVISR200 in vivo imaging system. This system will be invaluable in pre-clinical research and in our understanding of the mechanism involved in metastatic bladder cancer.

Aims. To investigate S100A4 as a key modulator of the metastatic phenotype in transitional cell carcinoma of the bladder (TCCB) using in vivo imaging.
Proposed Research plan.
1) To validate tumour burden measurements using real time in vivo imaging in the F344/AY-27 model of bladder cancer using DsRed:

- The AY-27 syngeneic cell line will be transfected with pIRES2-DsRed2. Cells with high levels of DsRed2 expression will be selected using flow cytometry and then expanded into cell lines. Prior to inoculation into the bladders of 20 x F344 rats, the fluorescence intensity of the DsRed2 transfected cells will be analysed using flow cytometry and the IVISR200 system.
- The animal will be monitored to screen for tumours and metastases using imaging on days 3, 7, 10, 14, 17, 21, 24 and 28. The animal will be imaged at 4 different position to obtain a 3D image.
- On Day 28 the animals will be sacrificed and ex vivo tumour dimensions will be correlated with day 28 in vivo measurements. The individual organs will be fixed in cacodylate/PFA, agarose embedded and sectioned using a vibratome for fluorescence microscopy analysis.

2) To determine the effect of high S100A4 expression on tumour size, invasion and metastases using in vivo real time imaging:

- The gene encoding S100A4 amplified from pSVneoS100A4 will be cloned into multiple cloning sites of pIRES2-DsRed2.
- This resultant S100A4/DsRed2 vector will be transfected into AY-27 cell line.
- S100A4 mRNA will be quantitated using RTPCR and flow cytometry.
- Animal inoculation and imaging experiments will be undertaken as described in Objective 1. The bladder, brain, lungs, kidneys and liver will be removed, weighed and then placed into the IVIS imaging system for ex vivo image capture and the detection of micro-metastases.

3) To generate, compare and contrast simulated signatures of evolution in invasive and metastatic TCCB disease.

- Recorded images of tumours in vivo, collected in the 4 planes, will be used to estimate tumour volume using standard stereological techniques and computer modelling.
- Collaborative parallel study: Funding has been obtained for a study that will be undertaken in collaboration with University College Dublin (UCD) that will investigate the influences of the immune status of the host and the stromal component of the tumour on the EMT phenotype in both the established F344/AY-27 model and the F344/AY-27 (S100A4) model.

P42

PREDICTION OF RADIOSENSITIVITY IN HUMAN BLADDER CELL-LINES USING NUCLEAR CHROMATIN PHENOTYPE

Nor F. Rajab, Declan J. McKenna, Jim Diamond, Kate Williamson, Peter W. Hamilton, Valerie J. McKelvey-Martin

dj.mckenna@ulster.ac.uk

Nuclear chromatin organisation can provide subtle clues to the underlying patho-biology of the cell. Nuclear texture analysis is a method that quantitatively measures phenotypic changes in chromatin distribution within a cell nucleus whilst the alkaline Comet assay is a sensitive method for measuring the extent of DNA breakage in individual cells. Both these methods can provide useful information about the sensitivity of cells to DNA damaging agents, such as ionising radiation.
In the current study, the alkaline Comet assay was performed on 6 human bladder carcinoma cell-lines and 1 human urothelial cell-line exposed to γ-radiation doses from 0 – 10 Gy. Nuclear chromatin texture analysis of 40 features was then performed in the same cell-lines exposed to 0, 2 and 6 Gy to investigate if chromatin phenotype was related to radiation sensitivity.
Based on the extent of DNA damage measured by the Comet assay, we demonstrated that these 7 cell-lines exhibit different levels of radiosensitivity and can be divided into a radiosensitive group or a radioresistant group. Identification of a subset of important texture features using discriminant analysis and the definition of a classification function showed that 81.75% of all cells could be correctly identified as radiosensitive or radioresistant based on their pre-treatment chromatin phenotype. We also observed changes in chromatin texture features in the same cell-lines exposed to increasing doses of γ-radiation, indicating how chromatin organisation within the nucleus is altered by DNA damage.
We conclude that both the alkaline Comet assay and nuclear texture methodologies may prove to be valuable aids in predicting the response of tumour cells to radiotherapy.

P43

IMPLEMENTATION OF ROUTINE CONFOCAL DNA CYTOMETRY OF CLINICAL SAMPLES IN 3D

Dr Lennert Ploeger, Dr Jurryt van der Gugten, Mrs Dionne van der Giezen, Dr Jeroen Beliën, Mr Abdel Abbaker, Dr Hub Dullens, Mr Andre Huisman, Prof William Grizzle, Dr Neal Poulin, Prof Paul van Diest
Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands
Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands
Department of Pathology, University of Alabama at Birmingham,Birmingham, Alabama, USA

l.s.ploeger@lab.azu.nl

Introduction. Confocal Laser Scanning Microscopy (CLSM) provides the opportunity to perform 3D DNA content measurements on intact cells in thick histological sections. Because the technique is technically challenging and time consuming, only a small number of usually manually selected nuclei were analyzed in different studies, not allowing wide clinical evaluation. The aim of this study was to describe the conditions for accurate and fast 3-D CLSM cytometry with a minimum of user interaction to arrive at sufficient throughput for clinical application.
Materials and Methods. Nuclear DNA was stained in 14 µm thick tissue sections of normal liver and adrenal stained with either YOYO-1 iodide or TO-PRO-3 iodide. Different pre-treatment strategies were evaluated: antigen retrieval by boiling in citrate buffer (pH 6.0) followed by RNase application for 1 or 18 hours, or hydrolysis. The image stacks obtained with CLSM at microscope magnifications of ×40 or ×100 were analyzed off-line using in-house developed software for semi-automated 3-D fluorescence quantization. To avoid sectioned nuclei, the top and bottom of the stacks were identified from ZX and YZ projections. As a measure of histogram quality, the coefficient of variation (CV) of the diploid peak was assessed.
Results. The lowest CV (10.3%) was achieved with a protocol without antigen retrieval, with 1 hour RNase treatment and TO-PRO-3 iodide staining, and a final image recording at ×60 or ×100 magnifications. A sample size of 300 nuclei was generally achievable. By filtering the set of automatically segmented nuclei based on volume, size and shape, followed by interactive removal of the few remaining faulty objects, a single measurement was completely analyzed in approximately 3 hours.
Conclusions. The described methodology allows one to obtain a largely unbiased sample of nuclei in thick tissue sections using 3D DNA cytometry by confocal laser scanning microscopy within a clinically acceptable time frame, and with a CV small enough to resolve smaller near diploid stemlines. This provides a suitable method for 3-D DNA ploidy assessment of selected rare cells based on morphologic characteristics and of clinical samples that are too small to prepare adequate cell suspensions.

P44

CONFOCAL 3D DNA CYTOMETRY: ASSESSMENT OF REQUIRED COEFFICIENT OF VARIATION BY COMPUTER SIMULATION

Dr Lennert Ploeger, Dr Jeroen Beliën, Dr Neal Poulin, Prof William Grizzle, Prof Paul van Diest
Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands
Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA

l.s.ploeger@lab.azu.nl

Introduction. Confocal Laser Scanning Microscopy (CLSM) provides the opportunity to perform 3D DNA content measurements on intact cells in thick histological sections. So far, sample size has been limited by the time consuming nature of the technology. Since the power of DNA histograms to resolve different stemlines depends on both the sample size and the coefficient of variation (CV) of histogram peaks, interpretation of 3D CLSMDNA histograms might be hampered by both a small sample size and a large CV. The aim of this study was to analyze the required CV for 3D CLSM DNA histograms given a realistic sample size.
Material and Methods. By computer simulation, virtual histograms were composed for sample sizes of 20000, 10000, 5000, 1000, and 273 cells and CVs of 30, 25, 20, 15, 10 and 5%. By visual inspection, the histogram quality with respect to resolution of G0/1 and G2/M peaks of a diploid stemline was assessed.
Results. As expected, the interpretability of DNA histograms deteriorated with decreasing sample sizes and higher CVs. For CVs of 15% and lower, a clearly bimodal peak pattern with well distinguishable G0/1 and

G2/M peaks were still seen at a sample size of 273 cells, which is our current average sample size with 3D CLSM DNA cytometry.
Conclusions. For unambiguous interpretation of DNA histograms obtained using 3D CLSM, a CV of at most 15% is tolerable at currently achievable sample sizes. To resolve smaller near diploid stemlines, a CV of 10% or better should be aimed at. With currently available 3D imaging technology, this CV is achievable.

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THE PLACE OF VIRTUAL MICROSCOPY IN QUALITY ASSURANCE PROGRAMMES

Richard Williams, John Skinner
St Vincent's Hospital, Victoria, Australia
Flinders Medical Centre, Adelaide, South Australia

rwil8475@bigpond.net.au

Traditionally, quality assurance programmes in Anatomical Pathology have consisted of slides of a case with a purportedly definitive diagnosis being circulated to individual laboratories and a summary of the collective results of the participating practitioners in the laboratories being assessed against the "right" answer. This requires cases from which multiple sections can be cut and stained showing the same features. The process assesses laboratory performance up to a point but de-emphasises the performance of the individual pathologists whose quality of work also needs to be assured. It is a fact that most reports are issued by individuals on behalf of the laboratories and the reports may not be validated in consultation with colleagues until perhaps after the event during audits. Any reasonable outside assessing authority would see the flaw in this approach and lawyers representing clients harmed by mistaken diagnoses already have.
In developing a reliable quality assurance programme digital images of a whole section can be used and circulated to individual participants, thus ensuring identical material is available to each observer. In addition to the digitized material, the assessment of an individuals approach to the material, using a probability system based on a reproducible belief network would enable individual pathologists to compare their approach to tissue analysis, with the approach of their peers. Such a tool or set of tools would help standardize the approach to diagnosis by mandating an assessment using a succession of tissue features and using the underlying belief network to make a decision on diagnosis. This allows individuals to test the repeatability of approach to diagnosis with themselves over time (internal benchmarking) and to compare their diagnostic opinion with that of their peers using the same standardized approach over time (external benchmarking) and, if required, with a group of "experts" assessing the same case and themselves internally assessed in the same way as a group. The background has been reliably tested in the some available systems and is both a learning tool a diagnostic adjunct and a self assessment tool.
The combination of the QAP approach with the use of an educational tool with built-in self evaluation reduces the pressure on the experienced practitioner teaching the neophyte in the traditional master-pupil approach presently used. It also reduces manpower needed to produce multiple copies of traditional stained sections and the variability in output and is a quantum leap forward in the production of a combined quality assurance and continuing professional development programme. The multiple advantages include efficient delivery of the programmes, maintenance of quality assurance, education for the individual pathologist, risk minimization to satisfy the requirements of medical indemnity insurers. It minimises the psychological stress aspect of compliance with the requirements of an external quality assurance bodies, being seen as an opportunity for continuing professional development rather than as a punitive and competitive exercise, the common perception of current QA programmes.

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THE APPLICATION OF A DIAGNOSTIC DECISION SUPPORT SYSTEM FOR USE IN URINARY PATHOLOGY FOR SCORING DIAGNOSTIC ACCURACY

Dr J. Diamond, Dr PW Hamilton, Dr G. Wright, Professor R. Williams
Biomedical Imaging and Informatics Research Group, Queen's University Belfast, UK
Department of Anatomical Pathology, Queensland Medical Laboratory, Brisbane, Queensland, Australia.
Department of Anatomical Pathology, St. Vincent's Hospital and the University of Melbourne, Victoria, Australia

Urinary cytology is a rapid and inexpensive technique used extensively in the diagnosis of disease. To remove diagnostic subjectivity, a diagnostic decision support system (DDSS) called InView© has been developed, based on a Bayesian belief network (BBN) for the

diagnosis of urine cytology (ThinPrep). In addition to acting as a DDSS, the system implements a computer-based training (CBT) system, providing a novel approach to breast cytology training. The system guides the trainee cytopathologist through the diagnostic process, allowing the user to grade each diagnostic feature using a set of on-screen reference images as visual clues. The trainee positions a slider on a spectrum relative to these images, reflecting the similarity between the reference image and the microscope image. From this, an evidence vector is generated, allowing the current diagnostic probability to be updated by the BBN. As the trainee assesses each clue, the evidence entered is compared with that of the expert through the use of a defined teaching file. This file records the relative severity of each clue and a tolerance band within which the trainee must position the slider. When all clues in the teaching case have been completed, the system informs the user of inaccuracies and offers the ability to reassess problematic features. Testing in this way shows how the trainee graded each clue in relation to the expert, but fall short of scoring the diagnosis.

To score the diagnosis the actual final diagnostic probability and the route taken must match. In achieving this we must compare the cumulative probability graph of the expert and the trainee. This is achieved by generating a metric which is the square of the difference between each graph. This metric increases as the trainee graph deviates. It is however more important to to state this measure as a normalised metric where 100% represents a perfect match and 0% representing the worst case deviation. After the assessment of the diagnostic case the trainee will be given their score in the assessment of individual clues will be given, indicating the occurance of interperative bias that they may be including. The system developed for this study represents one of the only completely digital training systems for pathology. All training material is digital, where diagnostic cases are provided by slide scans (via Aperio ScanScope). Assessment in pathology has always been subjective systems and metrics such as described can offer an objective quantifiable measure of diagnostic accuracy.

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QUANTITATIVE STUDY ON THE EXPRESSION OF THYROID TRANSCRIPTION FACTOR-1 IN LUNG CARCINOMA NUCLEUS

Xiaoyan Bai, Hong Shen
Department of Pathology, Nan Fang Medical University (or Southern Medical University), GuangZhou, 510515, China

Objective. To investigate the differences and the significance on the expression of TTF-1 in normal adult lung, embryonic lung and lung cancer, in different kings of lung carcinoma, and in primary lung cancers and metastasis lung cancer in lymph nodes. Method: Paraffin embedding and section. The intensity of TTF-1, positive unit (PU), were investigated by SP method of immunohistochemistry using monoclonal antibody to TTF-1 and were measured quantitatively by Leica Q500MC image analysis system.

Results. The TTF-1 PU in normal adult lung is significant bigger than that in embryonic lung ($P<0.001$). The TTF-1 PU in lung carcinoma is significant smaller than that in normal adult or embryonic lung ($P<0.001$). The TTF-1 PU in lung adenocarcinoma and small cell carcinoma is significant bigger than that in lung squamous cell carcinoma and large cell carcinoma ($P<0.001$). The TTF-1 PU in lung squamous cell carcinoma is significant bigger than that in large cell lung carcinoma ($P<0.001$). For the adenocarcinoma, squamous cell carcinoma and large cell carcinoma of lung, the TTF-1 PU in metastasis lymph nodes is significant bigger than that in primary cancers ($P<0.001$, $P<0.001$, and $P<0.05$). For the small cell carcinoma of lung, the TTF-1 PU in metastasis lymph nodes is very closed to that in primary cancers ($P>0.05$). The TTF-1 PU in lung carcinoma with lymph node metastasis is significant bigger than that with no lymph node metastasis ($P<0.001$). And the TTF-1 PU was not associated with lung cancer growth pattern, differentiation and patients sex ($P>0.05$). The TTF-1 PU in TNM stage □-□ is significant bigger than that in stage □($P<0.001$).

Conclusions. 1. the quantification of TTF-1 expressed in normal lung, embryonic lung and lung cancer are obvious different; 2.The quantification of expressed TTF-1 differ in different types of lung carcinomas. 3. The stronger the expression of TTF-1, the easier the lung carcinoma metastasizes. Lung carcinoma cells with strong expression of TTF-1 are important hallmark to the cells with distinct potential of metastasis.

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A DIAGNOSTIC DECISION SUPPORT SYSTEM FOR USE IN URINARY PATHOLOGY

Dr J Diamond, Prof. PW Hamilton, Dr G. Wright, Prof R. Williams
Biomedical Imaging and Informatics Research Group, Queen's University Belfast, UK
Department of Anatomical Pathology, Queensland Medical Laboratory, Brisbane, Queensland, Australia
Department of Anatomical Pathology, St. Vincent's Hospital and the University of Melbourne, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia

j.diamond@qub.ac.uk

Incidence of bladder cancer in the UK is approximately 12000 cases per annum with a resulting mortality rate of 5000 cases per year (Cancer Research UK). Diagnosing bladder cancer involves a combination of cytoscopy, biopsy, and voided urine cytology. The latter has been in use mostly as a screening test for recurrent neoplasms of the urinary tract. Yet, acceptance of urinary cytology as a useful clinical tool has been slow, both by clinicians and pathologists. Much of the apprehension is related to its lack of sensitivity in detecting low-grade superficial tumors: the cytological findings here are subtle and subjective.

To remove this subjectivity a Diagnostic Decision Support System (DDSS) called InView has been developed, based on a Bayesian Belief Network (BBN) for diagnosis in urine cytology. By pathologist definition a set of ten exhaustive diagnostic clues were established pertaining to diagnosis from urine cytology. Each clue was defined with an order number, with initially presented clues having the most impact on the ovewrall diagnosis. Clues were also defined using the state conditions familiar to pathologists.

The system guides the pathologist through the diagnostic process, allowing the grading of each diagnostic feature using a set of on-screen reference images as visual clues. The pathologist positions a slider on a spectrum relative to these images, reflecting the similarity between the reference image and the microscope image. From this, an evidence vector is generated, allowing the current diagnostic probability to be updated by the BBN. In addition to the final diagnostic probability is the ability of the system to map the diagnostic route taken by the pathologist, this is presented in the form of a Cumulative Probability Graph. This graph has proved important in conveying information to the pathologist on why the decision was made.

The system can be used in a number of ways. Firstly, it can be used to assist experienced cytopathologists in making difficult decisions, i.e. as a decision support system. While it is not expected that the system will be used for every case, it can provide assistance in particularly difficult cases. Secondly, it can be used to study in more detail the process of decision-making in urine cytology. The decision pathway is mapped numerically and can be statistically analysed to understand the contribution of certain observations to the final diagnostic decision. This quantitative approach to decision making provides objective and reproducible data that may contribute to improved diagnostic systems in the future. Thirdly the system provides a valuable training tool for juniors. Here junior cytopathologists can use the system to test their skills on stored cases of varying difficulty. The system compares their results with those of an expert, which have been previously stored in the system, indicating where inaccuracies in diagnosis have arisen. This provides a novel teaching aid for doctors and provides a consistent approach to training that is currently lacking.

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A MORPHOMETRIC STUDY OF ANGIOGENESIS IN HODGKIN'S LYMPHOMA USING IMAGE ANALYSIS

Dr Nikolaos Kavantzas, Dr Penelope Korkolopoulou, Dr Irini Thymara, Dr Theodoros P. Vassilakopoulos, Dr Maria K. Angelopoulou, Dr Styliani I. Kokoris, Dr Evangelia Dimitriadou, Dr Marina P. Siakantaris, Dr Konstantinos Anargyrou, Dr Panayiotis Panayiotidis
Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
First Department of Internal Medicine and Department of Haematology, School of Medicine, Laikon General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
Department of Histology and Embryology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

nkavan@cc.uoa.gr

Purpose. To evaluate various morphometric characteristics of microvessels, highlighted by means of anti-CD34 immunohistochemistry, in the lymph node of 286 patients with HL at diagnosis and investigate their relationship with several clinicopathologic parameters and prognosis.

Experimental Design. Microvessel density (MVD), total vascular area (TVA) and several size- and shape-related microvascular parameters were quantitated in the region of most intense vascularization using image analysis. *Results.* An increase in microvessel caliber parameters (namely area, perimeter, major axis length and minor axis length) and a decrease in MVD was noted with increasing stage. An inverse relationship was similarly recorded between MVD and the number of involved anatomic sites or LDH ratio. In univariate analysis, overall disease-specific survival was adversely affected by MVD and TVA, whereas inferior failure-free survival was associated with the presence of more flattened vessel sections. Multivariate analysis disclosed that the extent of angiogenesis (MVD/TVA), age and the number of involved anatomic sites independently affected overall survival. Accordingly, failure-free survival was independently linked to the shape of microvessels and albumin levels or the number of involved anatomic sites. *Conclusions.* Our data support the view that angiogenesis in HL provides independent prognostic information, requiring the concomitant evaluation of quantitative and qualitative aspects of microvascular network.

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QUANTIFYING GLOBAL METHYLATION STATUS OF BPH, PIN AND CANCER AND ITS RELATIONSHIP TO CHROMATIN PHENOTYPE

Dr CF Toland, R Montironi, P Keane, K Williamson, P Hamilton

c.toland@qub.ac.uk

Background. Our group has previously used chromatin texture analysis to quantify differences in chromatin phenotype in benign prostatic hyperplasia (BPH), prostate intraepithelial neoplasia (PIN) and invasive prostate cancer (PCa). Interestingly, this has shown a greater degree of chromatin disruption in PIN lesions in comparison to PCa and benign lesions. The underlying biological mechanisms that cause the changes in the distribution of chromatin in tumorigenesis are as yet unknown but are likely to be under epigenetic control. DNA methylation is an epigenetic modification within the chromatin structure that is associated with hyper condensed chromatin and gene inactivation. The aim of this study was to study the global nuclear distribution of DNA methylation using quantitative immunocytochemistry and determine its relationship to chromatin structure and the evolution of prostate cancer.
Materials and Methods. Tissue sections of 5µm thickness were cut from paraffin embedded specimens of radical prostatectomies and prostates removed at post mortem from University Of Ancona, Italy. Two serial tissue sections from each specimen were cut. One of the sections from each specimen was routinely stained using Haematoxylin and Eosin (H&E). The second tissue section was stained using immunohistochemistry to detect 5-methyl cytosine residues within the DNA. Lesions of normal prostate (NP) (n=5), Benign Prostatic Hyperplasia (BPH) (n=6), Prostatic Intraepithelial Neoplasia (PIN) (n=5), PIN (PINPCa) and prostate cancer (PCaPIN) on the same section (n=7) and prostate cancer (PCa) (n=1) were defined microscopically based on a combination of architectural and cytological findings. High resolution digital texture analysis was used to measure sixty texture features reflecting (1) the density and distribution of chromatin on H&E stained samples and (2) the density and distribution of anti-5mec immunostaining for nuclei segmented from specific lesions. Multivariate expression maps were used to simultaneously compare texture profiles across the different pathological lesions.
Results. Global hypermethylation was seen in premalignant PIN lesions whereas global hypomethylation was associated with PCa lesions. There were statistically significant differences in the density and distribution of DNA methylation and chromatin across the diagnostic groups of normal, BPH, PIN, PINPCa, PCaPIN, and PCa. While normal prostate and BPH showed a very similar chromatin phenotype, multivariate expression maps, demonstrated clear differences in the density and distribution of DNA methylation between these two benign groups. Conversely, the chromatin phenotype of PIN and PINPCa was very different and the amount however the distribution of DNA methylation was very similar. The PCa lesions were also phenotypically very different in chromatin patterns yet had similar methylation patterns.
Conclusions. Chromatin organisation and global methylation status show significant changes in malignant and premalignant prostate lesions. While there is an association between chromatin and methylation phenotype, chromatin reorganisation can occur without obvious global changes in methylation and vice versa. A combination of chromatin analysis and DNA methylation may distinguish between different prostatic lesions, when chromatin texture analysis suggests lesions may be morphologically very similar.

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3-D CHROMATIN TEXTURE ANALYSIS OF PROSTATE TISSUE USING CONFOCAL LASER SCANNING MICROSCOPY

Mr A. Huisman, Dr L.S. Ploeger, Dr H.F.J. Dullens, Mr H. Lammers, Dr G.N. Jonges, Dr N.M. Poulin, Prof W. Grizzle, Prof P.J. van Diest
Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands
Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA

a.huisman-4@lab.azu.nl

Introduction. An objective quantitative nuclear grade of prostate core needle biopsy may improve diagnosis making and prognosis assessment of prostate cancer and may result in the detection of relevant premalignant lesions in an earlier stage. One of the constituents of a quantitative nuclear grade is chromatin texture. This has been proven to be useful when measured in conventional 2-D prostate sections. However, the drawback of such sections is that nuclei are not present in their integrity.
Confocal laser scanning microscopy (CLSM) allows to perform such measurements in 3-D reconstructed nuclei. The aim of this project was therefore to develop a technique for accurate and reproducible measurement of chromatin texture using CLSM.
Material and methods. 19 features from four categories of 3D texture features were implemented: descriptive statistics for the gray-value distribution, features discriminating nuclear regions with low, medium and high amount of DNA, co-occurrence matrix features and fractal features.
These features were especially chosen for their potential power to detect nucleoli.
One prostate cancer specimen was stained with TO-PRO-3, a stoichiometric DNA binding fluorochrome. Fields containing normal and malignant nuclei were sampled, and 3-D confocal image stacks were recorded at x80 final magnification (x40 objective) at a wavelength of 633 nm with a Leica SP2 confocal microscope fitted with an acousto-optical beam splitter. After segmentation of 38 benign and 36 malignant nuclei the aforementioned texture features were computed. ROC analysis was used to discriminate between benign and malignant cells, where the cut-off values for the different texture feature values were varied. The area under the ROC curve (AUC) was used as a measure for the predictive performance (0.5-1) of a feature.
Results. Eleven of the nineteen texture features had predictive potential (AUC > 0.5). The five features with the highest univariate predictive performance were: normalized gray-value variance (AUC: 0.985), co-occurrence matrix entropy (AUC: 0.968), the volume of the low DNA area (AUC: 0.895), lacunarity (AUC: 0.893) and the ratio of low versus medium amount of DNA regions (AUC: 0.875).
Conclusion. 3D nuclear chromatin texture features measured by CLSM can accurately discriminate between benign and malignant prostate nuclei after staining with TO-PRO-3. This technology will be further tested on more clinical material to explore the potential with regard to detection of (pre)malignant changes in biopsies and prognosis assessment of prostate cancer.

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IMAGE ANALYSIS FOR STRUCTURAL GENOMICS IN NORMAL AND CANCER CELLS

Yuval Garini, Ian T. Young, Bart J. Vermolen, T. Chuang, Landon Wark, Sabine Mai
Quantitative Imaging Group, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands
Manitoba Institute of Cell Biology, CancerCare Manitoba, University of Manitoba, 675 McDermot Avenue, Winnipeg, MB, R3E 0V9, Canada

The organization of the interphase nucleus has been studied for decades and it is now well accepted that the position of chromosomes in the nucleus plays an important role in gene regulation and its alteration can be related to cancer. The three-dimensional (3D) structure of the genome in the interface nucleus can be studied in a sensitive way by fluorescence microscopy in combination with DNA specific probes.
A recent point of interest is the study of telomeres. They can be easily fluorescently-labeled and their 3D organization in the nucleus can be measured (e.g. by confocal microscopy).
Nevertheless, the acquired data forms an enormous data set that has to be analyzed and requires quantitative image analysis and appropriate algorithms. We developed novel tools for the quantitative analysis of the 3D structure of the genome in the nucleus (both telomeres [1] and chromosomes).
By studying the 3D organization of the telomeres in interface nuclei with the developed tools, we found that telomeres re-organize during the cell cycle [2]. In G0/G1

and S phase the telomeres are uniformly distributed in the nucleus, but in G2 they move into a disk-like volume. This organization changes in cancerous cases and telomeres tend to form aggregates.
The analysis method, algorithms and part of the results on normal and cancerous cells will be presented.

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3D ORGANISATION OF CHROMOSOME 11 CENTROMERES IN PROSTATE CANCER CELL LINES

Dr CF Toland, Dr Y Garini, Dr B Vermolen, Prof. PW Hamilton

c.toland@qub.ac.uk

Background. Digital chromatin texture analysis can detect quantitative differences in the chromatin organisation of two prostate cancer cell lines DU145 and PNT1A, metrics that are independent of cell cycle phase. While this throws some light on nuclear topology, the arrangement of specific chromosomes with nuclei and their rearrangement following the onset of trisomy remain unclear. FISH on 3D preserved nuclei allow us to identify alterations in the 3D organisation at the chromosome, centromere, telomere or gene locus. Centromeric FISH allow us to detect aneusomy in a cell line but also permits the study of locational changes in centromere position within the nucleus when analysed using 3-D visualisation and measurement software. This is an extremely important tool in understanding nuclear architecture and the impact that nuclear topology has on gene expression and cell function.
Materials and Methods. Prostate cancer cell lines DU145 and PNT1A were grown onto microscope slides and fixed using formalin and liquid nitrogen to allow the preservation of the 3D structure of the nucleus. Biotin labelled centromeric probe (Zymed) for chromosome 11 was used to carry out FISH, and subsequently were labelled with an FITC anti biotin secondary antibody (Sigma Aldrich). The nuclei were counterstained with propidium iodide. Confocal microscopy (Leica SP2) was used to obtain 256 x 256 pixel images of sections of nuclei approximately 0.12µm apart. Approximately 100 images were used to generate the image stacks ANALYZE 3-D software (AnalyzeDirect) was used to compile and render 3D images of the nuclei (red) and their centromeres (green). A variety of parameters were assessed for individual nuclei including (i) the 3D volume of the nucleus (ii) the surface area of the nucleus (iii) the centre of gravity of the nucleus (iv) the volume of the centromere and (v) the centre of gravity of the centromere. These were used to generate inter-centromeric distances and cenromere/nuclear centre distances.
Results. The PNT1A cell line is disomic for chromosome 11, DU145 is pleomorphic, displaying both disomy and trisomy for chromosome 11. The volume of the centromeres in the PNT1A is similar within and between nuclei (approx 1168 voxels). The volume is the same in the DU145 nuclei that display disomy. In trisomic nuclei, two of the centromeres share the same volume while one is approximately twice that size. The distance of the centromeres from each other in the PNT1A nuclei was variable. In the DU145 cell line, the two regular sized centromeres also shared the same distance from the nucleus centre while the third larger centromere was closer to the centre. The position of the three centromeres created an isosceles triangle. The giant centromere was positioned equidistant from the two normal size centromeres. Further algorithms are currently being applied which allow disomy and trisomy to be characterised by the areas and volumes of the triangles or pyramids they create with the centre of the nucleus. This is likely to provide new tools for characterising nuclear topology.
Conclusions. 3D reconstruction of FISH stained nuclei allow the position and volumetric quantitation of centromeres. These are distinctly different in disomic and trisomic cells with consistent patterns emerging. By applying the appropriate algorithms to the 3D images, the underlying nuclear chromatin organisation would be better understood. This may have implications in the understanding of nuclear topology and gene expression in cancer.

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EAMUS - INTERNET BASED PLATFORM FOR AUTOMATED QUANTITATIVE MEASUREMENTS IN IMMUNOHISTOCHEMISTRY

Dr Gian Kayser, Dr Dominik Radziszowski,
Mr Piotr Bzdyl, Prof Martin Werner, Prof Klaus Kayser
Department of Pathology, University Hospital Freiburg, Germany
UICC-TPCC, Charite, Berlin, Germany
AGH University of Science and Technology, Krakow, Poland

gian.kayser@uniklinik-freiburg.de

Background. Immunohistochemistry is a frequent technique applied in tissue-based diagnosis, and serves for quantitative assessment of nuclear (proliferation, hormone receptors) and membrane-bound (her2_neu) characteristics.
Theoretical considerations. Automated performance of quantitative immunohistochemistry requires algorithms, which take into account different reference spaces (nuclei, cytoplasm), measurement aims (stereologic, texture-related), and information-associated strategies (sampling performance) and active sampling to assure a background- and slide thickness - independent selection of a useful segmentation threshold. Quantitative analysis of membrane - and cytoplasm stained immunohistochemical images requires additional knowledge of average membrane/cytoplasm features (length, area, etc.).
Program features. An automated quantitative measurement of immunohistochemical images should separate clinical data (patient's identification, disease classification, etc.) from image features, the selection appropriate colour/saturation string, the assessment of the segmentation threshold, and an appropriate report with standardized denotation of images and patient's file (for example DICOM 3).
Results. An internet based, "open" program has been developed which fulfils the above mentioned features, and permits automated, self-controlled measurements of digitised images acquired from immunohistochemically stained slides including immunofluorescence. The program has been applied in several studies on various cancer sites. Several image features of clinical and prognostic significance have been evaluated. Most of them are related to textural features, such as staining intensity associated to cell type of nearest neighbouring cells, cluster formation, structural entropy and current of structural entropy. The system is available via an internet portal, and measure individual cases within 20 minutes the latest.

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STUDY ON THE DESCRIPTION AND TEST OF THREE DIMENSIONAL FORM FACTOR BASED ON THE PRINCIPLE AND METHODS OF STEREOLOGY

Hong Shen
Department of Pathology
Nan Fang Medical University (Originally First Military Medical University), Tonghe, Guangzhou, 510515, China

The concept named as sphere grade (Sg) or three dimensional form factor was defined to describe the degree that a three dimensional structure approach to sphere in shape. Two methods to describe and test the sphere grade were set up by the principle and methods of stereology. Method one, sphere grade based on surface (Sg1 or Sg,s): the surface area of sphere whose volume is equivalent to the particle is called "volume equivalent surface", written as "s_{ev}". And we defined that, the ratio of volume equivalent surface to the surface area of the particle itself (s) as sphere grade by surface, that is: Sg,s = s_{ev} / s. To test and calculate Sg,s, the formula for calculating Sg,s was deduced as:

$$\mathrm{Sg,s} = \sqrt[3]{36\pi \cdot Nv \cdot Vv^2} \Big/ Sv .$$

Method two, sphere grade based on volume (Sg2 or Sg,v): We called the volume of sphere whose surface area is equivalent to the particle as "surface equivalent volume", written as "v_{es}". And now we defined that the ratio of a particle volume to its surface equivalent volume, as sphere grade based on volume, that is: Sg,v = v / v_{es}. To test and calculate Sg,v, the formula to calculate Sg,v was deduced as:

$$\mathrm{Sg,v} = 6Vv\sqrt{\pi \cdot Nv} \Big/ \sqrt{Sv^3} .$$

In these formulas, Vv, Sv and Nv mean separately the volume density, surface density and numerical density. It should be noted that the containing space for volume density, surface density and numerical density must be same each other to calculate Sg,s or/and Sg,v based on Vv, Sv and Nv.

The Study was supported by NSFC (30271462).

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INVESTIGATION OF THE ROLE OF PERICYTES INFLUENCE OF PERICYTE DERIVED FACTORS ON THE GROWTH OF HUMAN MICROVASCULAR ENDOTHELIAL CELLS (HMEC-1)

Maire McIlroy, Martina O'Keeffe, Claire Ward, Martin O'Rourke[1], Tom Gardiner[2], T Robson[1], David Hirst[1], Stephanie McKeown
School of Biomedical Sciences, University of Ulster Coleraine
Deptartments of [1]Pharmacy and [2]Ophthalmology, Queen's University Belfast

It is well recognised that pericytes have a role in the development of normal microvessels. However their characterisation in the development of tumour microvessels is poorly documented. Initially we investigated the presence of pericytes in a series of bladder tumours as compared to normal bladder mucosa. Our evidence suggested that bladder tumours have limited pericyte coverage although tumours with more adequate coverage of pericytes had poorer progression free survival.

Prior to characterising the interaction between these two cell types in a tumour microenvironment we investigated the role of pericyte condition medium (PCM) in controlling the growth and gene expression of human microvascular endothelial cells (HMEC-1).

Pericyte conditioned medium (PCM) was used to investigate the influence of soluble pericytes secreted factors on the cellular characteristics and gene expression profiles of HMEC-1. PCM significantly inhibited HMEC-1 migration in an *in vitro* wound assay and retarded tubule formation of HMEC-1 cells grown on matrigel. A microarray screen for 10,000 genes was employed to investigate alterations in endothelial gene expression induced by a 6 hour exposure to PCM. As expected a number of genes were found to be differentially expressed following this treatment; when an arbitrary change of 2 was defined as the cut off. The most up regulated gene was PAI-1 with a 3.4 fold induction. Using Northern blot analysis cells exposed over a range of times from 0 – 24 hours showed a peak of PAI-1 expression in the 6 hour sample. When PAI-1 was over expressed in HMEC-1 cells there was greater cell killing, impaired cell migration and retardation of angiogenic branching. This supports the conclusion that up regulation of PAI-1 is at least partly responsible for the inhibition of endothelial cell function.

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THE 2'5'-OLIGOADENYLATE SYNTHETASE/RNASEL PATHWAY IS A NOVEL EFFECTOR OF BRCA1 AND INTERFERON-GAMMA MEDIATED APOPTOSIS

Alison M. Hosey, Paul B. Mullan, Niamh E. Buckley, Jennifer E. Quinn, Patrick G. Johnston, D.Paul Harkin

BRCA1 has been reported to have roles in DNA damage repair, cell cycle checkpoint control, transcriptional regulation and ubiquitination. We have previously demonstrated that BRCA1 is a potent activator of a subset of interferon regulated genes and that BRCA1 synergistically activated a number of these genes in the presence of IFN-γ, but not type I interferons. Here we report that one of these targets, 2,5 Oligoadenylate synthetase (2,5 OAS) is a mediator of BRCA1/ IFN-γ induced apoptosis. We show that the induction of 2,5 OAS in response to IFN-γ is BRCA1 and STAT1 dependent. Consistent with a role as a negative regulator of proliferation, transient transfection of 2,5 OAS into breast cancer cell lines results in decreased colony growth and apoptosis. Furthermore we show that IFN-γ induced apoptosis is dependent on functional BRCA1 and STAT1 and we demonstrate that IFN-γ induced apoptosis is dependent on 2,5 OAS induction. 2,5 OAS is the only known upstream regulator of RNaseL, a recently identified hereditary prostate tumour suppressor gene implicated in apoptosis. We propose that BRCA1 may be an upstream regulator of RNaseL, acting in concert with IFN-γ to transcriptionally activate 2,5 OAS, leading to the downstream activation of RNaseL and apoptosis.