780 Rb1 in sporadic colon cancer

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Background: Mutations in numerous genes, oncogenes, tumour suppressor and mismatch repair genes are involved in colorectal carcinogenesis. It is considered that one of the gatekeeper genes involved in this process is *Rb1*. The aim of our study was to evaluate changes in *Rb1* at different levels in the cell in order to elucidate its role in colon cancer.
Material and Methods: Primer specific PCR was used to evaluate the loss of heterozygosity at the two polymorphic loci in 140 samples of normal tissue and corresponding tumour tissue from sporadic colon cancer patients. Rb1 mRNA expression was analyzed in 50 pairs of normal and corresponding tumour tissue samples using real-time PCR method. Rb1 protein expression was analyzed on 50 tumour paraffin sections using immunohistochemistry.
Results: Heterozygosity was detected in 63% and 79% of analyzed samples and LOH was observed in 17% and 12% of informative samples for polymorphic markers Rb1.2 and Rb1.20 respectively. Expression of Rb1 mRNA was higher in moderate and poorly differentiated tumours and tumours classified as Dukes' C. Rb1 protein was immunohistochemically positive in 83% of examined tumour samples. The expression of pRb was found higher in moderate and poorly differentiated tumours and it positively correlated with Dukes stage.
Conclusions: Our results support the thesis that malignant transformation in colon tissue is a consequence of more than one genetic alteration and suggest that *Rb1* plays a role in this multistep process.

781 Crosstalk between retinoid and steroid regulation pathways in the control of seminoma cell proliferation

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Background: In human males, an increase in the incidence of testicular cancer and infertility have been observed in many industrialized countries over the last 30 years. Such male reproductive disorders have been attributed to the increase in concentration of endocrine disruptor compounds (EDCs) in the environment and food. Epidemiological, clinical and experimental studies have suggested that excessive exposure to xeno-estrogens during fetal/neonatal life can lead to reproductive disorders in adulthood.
Material and Methods: Using the unique pure seminoma cell line TCam2 as a model for the main form of testicular germ cell tumour, we measured proliferation rate, activation of transduction pathway and target genes expression after cell exposure to natural steroid, retinoic acid or to several related classes of EDCs.
Results: We demonstrated that proliferation rate varies upon steroid or retinoid treatments through the activation of non-specific steroid receptor (GPER) and EGFR-dependent transduction pathways. Moreover, we point out crosstalk between retinoid and steroid-dependent regulation pathways. Taken together, these results allow classifying this type of testicular germ cell tumour as a hormone-dependent one.
Conclusions: The data also shed light upon possible mechanisms which could trigger carcinoma in situ cell proliferation and development of a testicular germ cell tumour in post-pubertal males. Therefore, we propose to use the TCam2 cell line as bio-indicator in order to characterize the effects of emerging pollutant mixes at low doses not only *in vitro* but also *in vivo* using xenografted Nude mice.

782 An automated analysis protocol for research of KRAS/BRAF mutation detection for data generated on capillary electrophoresis instruments

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Biomarker research continues to be an important focus in oncology studies, including the role of KRAS and BRAF mutations in CRC and other EGFR-associated cancers. This has lead to increased research of these genes as possible predictive markers and targets for continued study.
With this increased interest comes a need for automation of data analysis and report generation to decrease bottlenecks in the research laboratory by reducing manual review time. This poster will present an automated workflow for detection of KRAS and BRAF mutations and concise report generation in sample data generated on capillary electrophoresis instruments using fragment analysis software tools. We will demonstrate how key features in the software, such as sample quality values, allele binning and report analysis, enable this workflow to be a significant improvement over visual scoring methods.
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783 Epigenetic and genetic programs of osteogenic mesenchymal stem cell differentiation: a genome-wide integrative approach

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During malignant transformation the finely tuned processes of differentiation and proliferation gets out of balance. Understanding the normal balance is central in both oncology and normal biology. We here aim to identify genetic and epigenetic programs that control stemness and govern normal bone formation during mesenchymal development. These programs will be compared to those identified in bone cancer, specifically osteosarcomas, in order to reveal cancer specific events.
To study normal bone development, we have generated an immortalized human mesenchymal stem cell line that can be differentiated to the osteogenic lineage, evidenced by upregulation of osteogenic specific genes, induction of alkaline phosphatase activity and calcium deposition. Using high-throughput technology we have identified a subset of mRNAs and miRNAs that are up- and downregulated during osteogenic differentiation. The CpG methylation status of more than 14,000 gene promoters, including more than 900 cancer-related genes and 144 methylation hot-spots in cancer, have been mapped. Another epigenetic layer of information is contained in the covalent modification of histone N-terminal tails. Using chromatin immunoprecipitation combined with next-generation sequencing technology (ChIP-Seq) we are mapping the genome-wide profiles of histone H3 tri-methylation of lysines K4, K9, K27 and K36 and acetylation K9 at various stages of differentiation. Using bioinformatic approaches the different layers of information will be integrated to reveal regulatory networks governing stemness and osteogenic differentiation. In parallel, a corresponding set of data are being generated from a large panel of ostesarcoma cell lines, xenografts and primary tumours. Ultimately, data from normal and cancer cells will be compared to identify a set of genes specifically changed in osteosarcomas with the epigenetic regulatory mechanisms underlying cancer specific deregulation. Finally, we aim to develop a molecular staging tool for ostesarcomas, based on their differentiation status in the mesenchymal developmental hierarchy.

784 Clinical applications of molecular profiling of colorectal cancer

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Introduction: Despite developments in diagnosis and treatment, 20% of colorectal cancer (CRC) patients present with metastatic disease and 30% of cases recur after curative surgery. Furthermore, the molecular factors involved in prognosis and response to therapy in CRC is poorly understood. Objectives of the study were to quantitatively examine the expression of target genes in colorectal cancer and to correlate their expression levels with clinico-pathological variables.
Methods: A detailed analysis of published CRC microarray data was performed to identify the most prominent genes. The selected genes were validated in fifty-two pairs of fresh colorectal tumour and associated normal tissue specimens by RQ-PCR using TaqMan® assays. Statistical analysis and correlation with clinicopathological data was performed using SPSS software.
Results: Expression levels of *CXCL12* ($p = 0.000$), *CDH17* ($P = 0.026$), *MUC2* ($p = 0.000$), *L-FABP* ($p = 0.000$) and *PDCD4* ($p = 0.000$) were down regulated and IL8 ($p = 0.000$) was upregulated in tumours compared to normal colorectal tissues. No significant differences were noted in expression of *CEACAM5, CXCR4, CXCR7, TGFB1, TGFBR1* and *TGFBR2*. Furthermore, we found significant associations of gene expression levels and clinicopathological variables such as survival, tumour size, grade, invasion and lymph node status.
Conclusion: We identified a comprehensive list of genes with highly differential expression patterns in colorectal cancer that could serve as molecular markers to complement existing histopathological factors in diagnosis, follow up and therapeutic strategies for individualised care of patients.

785 HGUE-C-1 a novel colon carcinoma cell line

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HGUE-C-1 cells were obtained from ascytic efussion of a 76-year old colon carcinoma male patient. Citology of ascytic efussion obtained after paracenthesis confimed colon adenocarcinoma origin. Chemotherapy was then started with capecitabine and irinotecan, After a second cycle of chemoherapy, patient was admitted to the hospital. During admission, two parecenthesis of 5000 and 54000 ml of malignant hematic ascytis with 72 h interval were performed.

HGUE-C-1 cells were obtained from peritoneal ascitis fluid by centrifugation followed by culture in DMEM supplemented with 20% FCS. Cell aliquots were frozen and preserved in liquid nitrogen, genomic DNA and total RNA were also isolated and preserved for further analysis.
To characterize HGUE-C-1 cells at the genomic level, we used genomic DNA and RNA isolated from the original ascitis recovered cells and from HGUE-C-1 cells after 2−3 passes in culture. Initially, we determine whether HGUE-C-1 cells show the microsatellite instability phenotype (MSI). Since this phenotype is characterized by widespread somatic alterations in length of nucleotide repeat sequences, we have used five quasimonomorphic mononucleotide repeats probes (BAT-26, BAT-25, NR-21, NR-22 and NR-24) to performance a pentaplex PCR followed by size determination in an automatic sequencer. The parental HGUE-C-1 cell line did not show MSI phenotype. To further prove this point, we isolated clonal populations of cells from the HGUE-C-1 cell line by extreme dilution after, 15 passes in culture. These clones were named HGUE-C-1A to I. None of the clones showed MSI phenotype.
KRAS, BRAF and TP53 mutations are quite common in colon carcinoma and they have been related to colon carcinogesis. HGUE-C-1 cells were analyzed for mutations on those three genes, using RT-PCR and sequencing. Our results demonstrate that HGUE-C-1 does not show mutations in KRAS, BRAF, or in theTP53 hot spot exons (exons 4, 6, 7, 9, 10 and 11).
HGUE-C-1 cells were also analyzed and compare with HT-29 cells, a well known colon carcinoma cell line by their sensitivity and resistance to different treatments that include: 5-fluoracyl (FU), Trichostatin A, SAHA, gefiitinib, erlotinib, sorafenib, rapamycin, 17-AGG (an HSP90 inhibitor), BEZ-235 (PI3K and m-Tor inhibitor), and AZD-6244 (a MEK inhibitor).
HGUE-C-1 cell shown resistance to 5-FU, AZD-6244 and partial resistance to 17-AGG.
HGUE-1 may be an interesting model to study colon carcinogenesis in situations were MSI phenotype, KRAS, BRAF and TP53 mutations are not involved.

786 Tumour markers and the coincidence of frame shift mutations in BRCA1 among south Indian familial breast cancer patients

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Background: Carcinoma of the breast is a major lethal cancer in females in the Indian population, on a par with lung and colon cancer. The familial breast cancer patients were studied for a linkage between the tumour markers and the type of mutations present in the patients within the south Indian population.
Methods: The blood samples were collected from two main cancer labs in and around the Coimbatore city along with their family history that comes in different stages of breast cancer. A total of 67 subjects had analyzed for the CEA and Ca15.3 levels which indicate the presence of mutations. Cases with increased level of protein makers are subjected to chromosomal aberrations by GTG banding and BRCA1 exon 2 analyses, with the single strand conformation polymorphism assay on genomic DNA amplified by polymerase chain reaction.
Results: The Ca15.3 and CEA levels showed a significant ($p < 0.05$) increase in mean value (35.3 ± 4.08 and 10.89 ± 1.04) when compared to the controls. In Exon 2 of BRCA1 gene analysis we found that the incidence of 185delAG mutations is frequent in most patients with stage III status. The percentage of deletions and that of translocations comes almost near to 82% and 80% respectively in the stage III patients.
Conclusions: Identification of the mutations present in the patients showed the level of tumour markers can be used as the credentials for these mutations. Though the levels aren't of much consequence, it is really reliable to get an early recognition of the mutations present in the patients. The conclusions suggest that any given populace should widen a mutation database for its series of breast cancer assortment.

787 An evaluation of prostate cancer gene 3 (PCA3) in patients with suspected prostate cancer

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Objectives: To identify the clinical relevance of PCA3 in patients with suspected prostate cancer.
Methods: Patients with suspected prostate cancer consented for digital rectal examination followed by collection of urine sample for PCA3 analysis using transcription-mediated amplification technology. Transrectal ultrasound prostate biopsies (TRUS) were subsequently obtained. Results of Prostate-specific antigen (PSA), PCA3 and TRUS biopsies were prospectively collected and analysed.
Results: From 1 August 2008 to 31 Jan 2009, 99 patients with suspected prostate cancer with mean age of 64 (range 38−76) had their urine samples collected for PCA3 analysis prior to their ultrasound guided prostate biopsies. Means (SD) of PSA and PCA3 were 52.7 (60.4) and 9.2 (5.7), respectively. At a PCA3 score cutpoint of 35, sensitivity was 64.9% and specificity was 64.7%. For serum PSA at the established cutpoint of 4.0 ng/ml sensitivity and specificity were 95.8% and 9.8%, respectively. ROC-AUC for PCA3 was 0.7.
Conclusion: The clinical evaluation of PCA3 has shown that the PCA3 score supplements PSA in diagnosis of prostate cancer. The addition of PCA3 during the process of diagnosis will not result a state of certainty for urologists. TRUS biopsy and management decisions might be better informed with PCA3 as an additional diagnostic tool.

788 Investigation of the differentially expressed C-FABP & FABP-pm in human prostate tissues and cell lines: histopathological and molecular biology study

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Introduction: Prostate cancer is the most commonly occurring from non-tobacco related cancers of man in the developed world. Our understanding of the molecular pathology of prostate cancer is currently very limited. At present, clinical therapy focuses on androgen blockage by physical or pharmaceutical castration. Previous work in our pathology laboratory has led to the identification of several genes whose elevated expression may contribute to the malignant progression of the prostate cancer cells. Tow of these genes are that coding for human cutaneous fatty acid binding protein (C-FABP) and membrane associated fatty acid binding protein (FABP-pm). The work described in this research is aimed to study further the possible role of C-FABP and FABP-pm on prostate cancer tumourigencity, to investigate whether these two FABPs modulate the malignant progression of prostate cancer cells in a coordinated manner and to explore the therapeutic possibilities by manipulating their expressions in prostate cancer cells.
Materials and Methods: Immunhistochemical staining for human prostate tissues comprised an archival set with follow-up data held within the diagnostic archive in the Department of Pathology, University of Liverpool, UK. Tissues were taken from 73 prostate adenocarcinoma patients with an average age of 73 years and from 33 benign prostatic hyperplasia (BPH) patients with an average age of 67.5 years who were treated by trans-urethral resection of prostate (TURP) in the Royal Liverpool University Hospital during the 8-years of 1995–2003. The 7 normal prostate tissues were taken from road accident victims with an average age of 48 years who did not have a history of prostatic disease. This study was approved by Liverpool Local Science Ethics Committee in accordance with the Medical Research Council guidelines. The PC3, DU145, PC3M, PC3M3, 22RV1, LNCaP-WT and LNCaP prostatic cancer cell lines with the non malignant cell line PNT2 were used for Cell Culture and Western blotting to analyse the cellular proteins. All cell lines were obtained from the storage of the Department of Pathology, University of Liverpool.
Results: Western blot results showed that the expression of C-FABP was significantly higher in androgen independent cell lines than that in androgen dependent cell lines whereas the expression of FABP-pm was significantly higher in androgen dependent cell lines than that observed in androgen independent cell lines. These results showed that C-FABP and FABP-pm express in opposite manner in prostate cancer progression. Immunhistochemical staining of an archival set of prostate cancer tissues partially supported this relationship between these two genes as levels of both nuclear and cytoplasmic C-FABP expression in carcinoma tissues were significantly higher than those in normal and PBH tissues whilst the FABP-pm expression in normal and BPH tissues were significantly higher than those in carcinoma tissues.
Conclusion: These results together seemed to suggest that the C-FABP and FABP-pm express in opposite manner in prostate cancer progression. These findings indicated that increased expression of C-FABP or decreased expression of FABP-pm maybe a valuable prognostic factor predicting the outcome in prostate cancer patients, and it may also prove to be an important target for designing effective strategies to treat the disease.

789 Mitochondrial apoptotic molecules and genistein

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There are two clasical pathways of apoptosis: the mitochondrial (intrinsic) and death receptors pathway (extrinsic). Although the extrinsic and the intrinsic pathways of apoptosis are capable of operating independently, accumulated evidences suggest that cross-talk between the two pathway exists in cells.
B-cell chronic lymphocytic leukemia (B-CLL) is a neoplasic disorder characterized by defective apoptosis. The major problem in the treatment of leukemia is the development of resistant leukemic cells to drugs and of antiapoptotic machinery.
In this study we investigate the effects of genistein (a soy flavonoid) on mitochondrial pathway of apoptosis using a leukemic cell line EHEB, derived from the peripheral blood of a B-CLL patient.