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

S. Joseph[1], S. Sellappa[1], K. Keyan[1], S. Raman[2], S. Prathyumnan[1]. [1]*Karpagam University, Biotechnology, Coimbatore Tamilnadu, India,* [2]*Bharathiar University, Human Genetics Laboratory, Coimbatore Tamilnadu, India*

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

A.H.L. Wong[1], J. Gelister[2]. [1]*Countess of Chester Hospital, Trauma & Orthopaedics, Chester, United Kingdom,* [2]*Barnet Hospital, Urology, Barnet, United Kingdom*

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

M. Malki[1], S. Forootan[1], C. Foster[1], Y. Ke[1]. [1]*University of Liverpool, Pathology, Liverpool, United Kingdom*

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

D. Hotnog[1], V. Roman[1]. [1]*"Stefan S. Nicolau" Institute of Virology, Molecula Biology, Bucharest, Romania*

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.

In order to investigate the potential effect of genistein we have tested different steps in the mitochondrial apoptotic pathway that may be affected by genistein in B-CLL.
Genistein was found to have pro-apoptotic and antiproliferative efects in many cells types and in this particular cell line (EHEB) our results indicated that genistein, down regulate the expression of bcl-2 anti-apoptotic protein, upregulate the expression of bax proapoptotic protein and induce dissipation of the mitochondrial transmembrane potential.
All this data suggest that genistein is implicated in reestablished of a normal apoptotic process in leukemic cells and also that this agent may be used in chemoprevention or for new strategies of combined therapy for this type of leukemia.

790 Scribble deficiency: a novel model of prostate cancer

H. Pearson[1], R. Simon[2], A. Ryan[3], J. Pederson[3], P. Humbert[1]. [1]*Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia,* [2]*Department of Pathology, University Medical Centre Hamburg Eppendorf, Hamburg, Germany,* [3]*TissuPath Laboratories, Hawthorn, Victoria, Australia*

Prostate cancer is a heterogeneous and multifocal disease, which is currently the most commonly diagnosed male cancer in Australia. The adult prostate gland is a highly organised network of ducts composed of polarised layers of epithelial cells. Loss of polarity is a hallmark of epithelial cancer progression, suggesting that cell polarity mediators may play a crucial role in prostate tumourigenesis. The polarity regulator Scribble (Scrib) regulates several events that have been shown to be deregulated in epithelial cancers, including apical-basal cell polarity, proliferation, migration, apoptosis and stem cell maintenance [1]. Scribble mislocalisation and deregulated expression have been observed in both human colon adenocarcinoma [2] and mammary tumours [3]. This suggests that Scribble may be crucial for the homeostatic maintenance of other epithelial tissues by coordinating multiple biological processes and signalling pathways that underlie its tumour suppressive function. To address the role of Scribble within prostatic epithelium we have generated a cohort of Scribble heterozygous (Scrib+/−) transgenic mice, as Scribble null mice are neonatal lethal. Histological analysis of Scrib+/− male mice revealed a predisposition to prostate hyperplasia. These lesions display a marked increase in proliferation, androgen receptor expression and activated MAPK signalling. Taken together, this data indicates that Scribble plays a tumour suppressive role within the prostate and presents a direct mechanism for tumourigenesis, whereby Scribble loss instigates deregulation of both the androgen and MAPK signalling networks. By crossing Scribble floxed (Scribfl) mice to the PBCre transgenic line we have been able to specifically deplete Scribble within prostate epithelial cells. PBCre Scrib+/fl mice also displayed prostate hyperplasia indicating that the observed phenotype is cell intrinsic. Immunohistochemical analysis of a human prostate tissue microarray has validated this novel murine prostate cancer model, revealing a correlation between Scribble mislocalisation and advanced stages of prostate cancer. It is hoped further dissection of the molecular mechanisms underlying the development of prostate cancer in the context of Scribble loss will divulge innovative therapeutic routes of intervention in the clinic.

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Poster Session
Oncogenomics

791 An oligo microarray design for detection of known and putative oncogenic fusion transcripts

M. Løvf[1], G.O.S. Thomassen[1], G.E. Lind[1], R.A. Lothe[1], R.I. Skotheim[1]. [1]*Institute for Cancer Research and Oslo University Hospital, Department of Cancer Prevention and Centre for Cancer Biomedicine, Oslo, Norway*

Background: In a pilot study, we have validated a novel approach for fusion gene detection, using a custom made oligo microarray combining direct measurements of chimeric transcript junctions with shifted expression levels between sequences up-and downstream of the fusion break-points (Skotheim *et al*., Mol Cancer, 2009). We have now further developed this universal fusion gene detection tool to cover all known fusion genes and the design also include novel fusion transcripts identified from deep-sequencing studies (*e.g*. Maher *et al*., Nature, 2009) to serve as a high-throughput validation strategy.

Material and Method: A database including 556 fusion genes has been compiled by a combined literature survey and database integration. A Python script, using the exon sequences from all known fusion partners as one of the inputs, generates 599,000 oligos covering all theoretically possible exon-exon junctions between known fusion gene partners as well as all exons in the different genes. NimbleGen HD2 3-plex microarrays (max. 3 × 720 k oligos per slide) are used as platform for custom production of the fusion gene microarray. Also, a prototype automated scoring of all potential fusion transcripts has been developed. We are now utilising this universal assay to investigate the presence of fusion genes in a series of 67 cell lines from 15 different cancer types.
Results: In five out of ten leukaemia cell lines with known fusion gene status, the correct fusion transcript score as the number one hit among the 1,180,103 theoretical combinations per sample. These include *BCR-ABL1* (cell lines KU812 and K562), *TCF3-PBX1* (RCH-ACV and 697), and *MLL-MLLT3* (THP-1). Among the remaining 57 cell lines, we have found promising hits in several cancer types, including colorectal. An RT-PCR-based approach has been initiated to experimentally validate the presence of fusion genes in cancer types without previous fusion gene record.
Conclusions: We present here the 2nd generation of a universal microarray based assay for detection of oncogenic fusion transcripts. With this new and improved assay we are able to identify the correct fusion genes in several cell lines with known fusion gene status. Furthermore, promising hits are found in cancer types not previously known to carry fusion genes.

792 The cancer cell line project – systematic resequencing of known cancer genes in over 750 cancer cell lines

H. Davies[1], G. Bignell[1], M. Stratton[1], A. Futreal[1]. [1]*The Wellcome Trust Sanger Institute, Cancer Genome Project, Cambridgeshire, United Kingdom*

Cancer cell lines are used extensively as model systems in many areas of cancer research. An understanding of their genetic background allows for a more informed choice of cell lines for biological experiments and drug screening, and helps with the interpretation of the results.
At the Cancer Genome Project, the Cancer Cell Line Project is a large project set up to characterise a set of over 750 cancer cell lines. The cell lines in the set are derived from a wide variety of different cancers, with examples from all the major types. We have included many of the most commonly used cell lines, including the NCI60 set. As part of this project we have systematically screened the cell lines for mutations in a set of known cancer genes. Mutation screening was performed by capillary resequencing of PCR products covering all the coding exons of the genes. In addition, homozygous deletions of six tumour suppressor genes (CDKN2A, PTEN, RB1, STK11, MAP2K4 and SMAD4) were investigated by multiplex PCR and agarose gel analysis. Copy number data from SNP6 Genome Wide Affymetrix arrays is also available for the majority of the cell lines.
To date we have screened 58 known cancer genes for mutations. The results are released regularly on our COSMIC (catalogue of somatic mutations in cancer) web site (http://www.sanger.ac.uk/genetics/CGP/CellLines/). Over 1700 mutations have been released so far on the cancer cell line web pages. These mutations, classified as likely to be oncogenic, are sequence changes which have previously been shown as somatic mutations in cancer or are consistent with the position and type of mutations for a given cancer gene. An additional 2100 variants, also identified in the screen, are available to down load from the web site. The role in oncogenesis of these additional variants is considered tentative or unknown. Matched normal controls are not available for the vast majority of the cell lines. Therefore, the additional set of variants will include rare SNPs as well as passenger somatic mutations.
This ongoing project provides an extensive resource of genetic information on a large set of publically available cancer cell lines. The data can be utilised not only for own in house research projects but is freely available for public use. The data set increases the value of these cell lines as reagents for drug discovery and the evaluation of new therapies.

793 Integration of gene expression and DNA copy number changes in progressive vs. complete response ovarian cancer samples improves survival prediction

S. Shams[1], C. Parman[1]. [1]*BioDiscovery Inc., R&D, El Segundo CA, USA*

The Cancer Genome Atlas (TCGA) project has generated a very significant quantity of genomic data for different cancer types. The availability of such large amount of high quality data can be viewed as a goldmine for establishing better understanding of the biological processes in cancer, similarities and differences between cancer types and the complex interaction of various genetic and epigenetic changes in cancer. Here, we will describe our analysis of DNA copy number changes and mRNA expression changes in the Ovarian Cancer dataset. We obtained from the TCGA web site raw data for a total of 489 samples hybridized on 2-color oligo-arrays with 244 K probes per array. Raw log-ratio data representing DNA copy number values relative to a DNA pool of normal samples were used in the analysis. The raw data